INHALED AEROSOLISED PROSTACYCLIN AS A SELECTIVE PULMONARY VASODILATOR

PETER VERNON VAN HEERDEN

A dissertation submitted to the FACULTY OF MEDICINE ,
University of the Witwatersrand, Johannesburg,
in fulfilment of the requirements for the degree of
Doctor of Philosophy in the branch of Anaesthesia.

Johannesburg ,2000

This thesis is dedicated to

הַקָּדוֹשׁ בָּרוּךְ הוּא

for giving me the ability,

to my beloved parents for showing me the way

and to Fiona, Daniel, Adam and Liron, for their love and support and for travelling the road with me.

Preface and Acknowledgements

As a practicing intensive care specialist I am often confronted with the critically-ill patient receiving mechanical ventilation for respiratory failure. One cause of respiratory failure in the Intensive Care Unit (ICU) is the acute respiratory distress syndrome (ARDS). ARDS is caused by a wide range of pulmonary and extra-pulmonary factors. There is no "cure" for this condition and treatment remains supportive.

Until the 1980's the mainstay of treatment for the hypoxaemia associated with ARDS had been mechanical ventilation with supplemental oxygen and application of positive end-expiratory pressure (PEEP). During the provision of this supportive therapy the lungs would heal as the ARDS abated and resolved, leaving survivors with relatively normal lung function. Approximately fifty percent of patients with severe ARDS would die. A proportion of patients would also suffer complications of the supportive therapy they had received, principally pulmonary oxygen toxicity (from the high inspired concentrations of oxygen required) and volu- or barotrauma (due to the modes of mechanical ventilation prevalent at that time).

In the 1980's nitric oxide (NO) gas was introduced as an adjuvant modality for the treatment of severe hypoxaemia associated with ARDS. NO rapidly gained currency in the Intensive Care world, with an estimated tens of thousands of applications per year currently. NO is a selective pulmonary vasodilator (SPV), which improves ventilation and perfusion matching by affecting pulmonary vessels only in ventilated regions of the lung. Use of NO allows the use of lower inspired concentrations of oxygen for the treatment of hypoxaemia. As such, it may theoretically limit the risk of pulmonary oxygen toxicity. However, NO is a difficult agent to administer, with a requirement for specialised delivery and monitoring equipment for its safe use. Environmental contamination with NO and its higher oxides is another concern. Delivery and monitoring equipment are expensive and bulky, not ideal for the patient in the operating room nor during transport. So, although NO represented an advance in the supportive treatment of patients with ARDS, it has a number of drawbacks.

In 1993 a case series appeared in The Lancet describing a new application of an agent, prostacyclin, previously discovered in 1978. This involved the administration of aerosolised prostacyclin as a SPV to patients who were hypoxaemic due to ARDS (Walmrath D. et al. Aerosolised prostacyclin in adult respiratory distress syndrome. The Lancet 1993;342:961-2). The administration of what I was to call inhaled aerosolised prostacyclin (IAP) requires only very basic equipment, a standard jet nebuliser, and no specific monitoring equipment. Also, the initial report indicated good efficacy.

This fired my imagination! NO had provided the means of controlling the pulmonary circulation without a deterioration in oxygenation, and indeed gas exchange improved during NO administration. Would IAP offer the same advantages as NO as a SPV in patients with severe ARDS? If so, then IAP therapy might be a way of reducing pulmonary oxygen toxicity in patients with severe ARDS. At the time of commencement and planning of the studies described in this thesis, there was scant information regarding the use of IAP as a SPV. No comparison had been undertaken between IAP and the then gold-standard SPV, nitric oxide. Safety of IAP therapy had not been addressed, nor had dose-response relationships been determined. All of these are investigated and reported in this thesis. I decided to undertake the investigational work in the group of patients at most risk of adverse effects related to the supportive therapy they would receive – i.e. patients with severe ARDS. The work would be carried out in the ICU at Sir Charles Gairdner Hospital in Perth, Western Australia. This is an 18 bed general ICU admitting approximately 1,400 patients per year.

The investigational work for this thesis was only made possible with the support and advice of a number of people, to whom I wish to express my appreciation and gratitude. My sincere thanks go to –

- Prof. Charles Feldman, Dr. Peter Cameron and Prof. David Morrell, my supervisors, for their support, advice and encou agement,
- Ms. Brigit Roberts for her superb organisational skills,
- Dr. Richard Parsons and Mr. Max Bulsara of the Biostatistical Consulting Service of the Dept. of Public Health of the University of Western Australia for their statistical advice in the planning and analysis of the studies reported in this thesis,
- Dr. Anne Barden and Mr. Nick Michalopoulos for laboratory support,
- Dr. P. Caterina, Mr. Pierre Filion and Dr. Dominic Spagnolo for their assistance with histological studies,
- Dr. Neville Gibbs, for instructing me in the use of the thrombelastograph,
- Sir Charles Gairdner Hospital for use of clinical and laboratory facilities and financial support via the Research Fund,
- My colleagues in the Dept. of Intensive Care and
- My patients, for allowing me to study them, at a time when they were most vulnerable.

The research undertaken for this thesis has resulted in a number of publications, all of which were among the first on the topic of IAP as a selective pulmonary vasodilator.

These publications include –

van Heerden PV, Barden A, Michalopoulos N,Bulsara M, Roberts BL.
Dose-response to inhaled aerosolized prostacyclin for hypoxaemia due to ARDS.
Chest 2000;117:819-27.

van Heerden PV, Caterina P, Filion P, Spagnolo DV, Gibbs NM.
Pulmonary toxicity of inhaled aerosolised prostacyclin (IAP) therapy– an observational study.
Anaesthesia and Intensive Care 2000;28:161-6.

Blythe D, van Heerden PV, Power BM.
Pulmonary hypertension and selective pulmonary vasodilators in acute lung injury.
Anaesthesia and Intensive Care 1998;26:26-39.

van Heerden PV.
Systemic levels of 6-keto-prostaglandin $F_{1\alpha}$ following administration of inhaled aerosolized prostacyclin.
Anaesthesia and Intensive Care 1997;25:701-3.

van Heerden PV.
The efficacy of inhaled aerosolised prostacyclin as a selective pulmonary vasodilator.
Chest 1998;114:265S.

van Heerden PV, Gibbs NM, Michalopoulos N.
Effect of low concentrations of prostacyclin on platelet function *in vitro*.
Anaesthesia and Intensive Care 1997;25:343-6.

van Heerden PV .
The pulmonary and haematological toxicity of inhaled aerosolised prostacyclin.
Critical Care 1997;1 (supplement 1):28-9.

van Heerden PV, Power BM, Leonard RC.
Delivery of inhaled aerosolized prostacyclin.
Anaesthesia and Intensive Care 1996;24:624-5.

van Heerden PV , Blythe D, Webb SA.
Inhaled aerosolized prostacyclin and nitric oxide as selective pulmonary vasodilators in ARDS – a pilot study.
Anaesthesia and Intensive Care 1996;24:564-8.

van Heerden PV , Webb SA, Hee G, Corkeron M, Thompson WR..
Inhaled aerosolized prostacyclin as a selective pulmonary vasodilator for the treatment of severe hypoxaemia.
Anaesthesia and Intensive Care 1996;24:87-90.

The research has also received significant recognition when presented at Intensive Care meetings in Australia, Canada and Belgium, including –

- The Matt Spence Medal (1997) from the Australian and New Zealand Intensive Care Society and
- The ACCP Young Investigator Award (1998) from the American College of Chest Physicians.

This has been a fascinating endeavour for me, and one which I hope will advance the care of critically-ill patients at least in some small measure. This work is a step towards a better understanding of the control of the pulmonary circulation, where the issues are not merely pressure and flow, but also the effect manipulation of the pulmonary circulation may have on gas exchange.

Abstract

This thesis examines the premise that inhaled aerosolised prostacyclin (IAP) is a safe and efficacious agent for use as a selective pulmonary vasodilator (SPV) in patients with severe acute respiratory distress syndrome (ARDS).

Initially, three tests of nebuliser function were undertaken to support the use of this device for the delivery of aerosols in subsequent studies described in this thesis. The findings were –

- both jet nebuliser brands (MistyNeb and Sidestream) used in the studies described in this thesis produced a large proportion (approximately 50 – 80%) of aerosolised particles in the respirable range (1 – 5 microns). The Sidestream device was the more efficient of the two tested,
- aerosolised particles were deposited widely within the lung of a test subject as assessed by ventilation scan and
- the maximum rate of nebulisation by the Sidestream jet nebuliser was less than 15 ml/hr.

Next, a pilot study using nitric oxide (NO) 10 parts per million (ppm) and IAP 50 nanograms per kilogram per minute (ng/kg/min) was done, comparing the two agents as SPV's in five patients with hypoxaemia secondary to ARDS. Neither SPV resulted in systemic haemodynamic changes, indicating true pulmonary selectivity. IAP improved oxygenation to a degree comparable to NO as measured by the ratio of arterial partial pressure of oxygen to inspired oxygen concentration (PaO_2/FiO_2), shunt fraction and alveolar to arterial oxygen difference or gradient ($A\text{-}aDO_2$). Neither agent had a significant effect on mean pulmonary artery pressure (MPAP).

Thereafter pigs (mass up to 25 kg) were exposed to aerosolised normal saline, the control group (C group), aerosolised alkaline glycine diluent (D group) or IAP in a concentration of 10 micrograms/millilitre in glycine buffer (P group) for up to 8 hours. Pigs in the D and P groups developed mild acute sterile tracheitis, involving the superficial layers of the trachea (shown histologically and ultrastructurally). The D group also showed inflammatory changes in the bronchioles. These findings suggest caution in the use of high volumes of aersolised alkaline glycine diluent during IAP therapy.

A study was then carried out to determine the efficacy of and dose-response relationships to IAP, when used as a SPV in patients with severe hypoxaemia due to the ARDS. This was an unblinded interventional prospective clinical study in a university tertiary referral centre, general intensive care unit. Nine adult patients with severe ARDS (as defined by a lung injury score ≥ 2.5)

were enrolled. All patients received IAP over the dose range 0 to 50 ng/kg/min. The IAP was delivered via a jet nebuliser placed in the ventilator circuit. Dose increments were 10 ng/kg/min every 30 min. Cardiovascular parameters, indices of oxygenation and shunt fraction were measured or calculated at each dose interval, as were platelet aggregation and systemic levels of prostacyclin metabolite. A generalised linear regression model was used to determine a dose effect of IAP on the measured parameters. Wilcoxon ranked sums test for related measures was used to compare the effects of various doses of IAP.

IAP acted as a SPV, with a statistically significant dose-related improvement in PaO_2/FiO_2 ($p = 0.003$) and $A\text{-}aDO_2$ ($p = 0.01$). Systemic prostacyclin metabolite levels increased significantly with delivered IAP dose ($p = 0.001$). There was no significant dose effect on systemic or pulmonary arterial pressures, nor on platelet function, as determined by platelet aggregation in response to challenge with adenosine diphosphate. The findings indicated that IAP is an efficacious SPV, with marked dose-related improvement in oxygenation and with no demonstrable effect on systemic arterial pressures over the dose range 0 – 50 ng/kg/min. Despite significant systemic levels of prostacyclin metabolite, there was no demonstrable platelet function defect.

In order to further clarify the possible antiplatelet effect of systemic prostacyclin metabolites observed during IAP therapy, an *in vitro* study was performed. Platelet aggregation in response to adenosine diphosphate (ADP) and collagen, as well as measurement of the maximum amplitude by thrombelastography, was undertaken *in vitro* using venous blood from eight healthy subjects exposed to extremely low concentrations of prostacyclin (0, 10, 100 and 500 pg/ml). A significant anti-platelet effect was demonstrated.

Conclusions –

Following completion of the above studies the candidate concluded that –

- IAP is safe and efficacious when used as a SPV
- A dose-response to IAP exists with regard to indices of oxygenation
- Caution is required with regard to potential pulmonary inflammation with use of high volumes of alkaline glycine buffer when IAP therapy is administered
- An anti-platelet effect is theoretically possible during IAP therapy, but could not be demonstrated *in vivo*.

Table of Contents

List of figures

List of tables

Abbreviations

$A\text{-}aDO_2$	alveolar-arterial oxygen partial pressure gradient or difference
ADP	*adenosine diphosphate*
ALI	acute lung injury
AMP	adenosine monophosphate
ARDS	acute respiratory distress syndrome
BAL	bronchoalveolar lavage
°C	degrees centigrade
cAMP	cyclic adenosine monophosphate
CaO_2	oxygen content of arterial blood
CcO_2	oxygen content of pulmonary capillary blood
CI	cardiac index
cm H_20	centimetres of water pressure
CO	cardiac output
cpm	counts per minute
CvO_2	oxygen content of mixed venous blood
CVP	central venous pressure
DO_2	oxygen delivery
FiO_2	fractional inspired oxygen concentration

g	force of gravity
GMP	guanylate monophosphate
HPV	hypoxic pulmonary vasoconstriction
HR	heart rate
IAP	inhaled aerosolised prostacyclin
IABP	invasive arterial blood pressure
ICU	intensive care unit
INR	international normalised ratio
IPPV	intermittent positive pressure ventilation
ITLC	instant thin layer chromatography
kPa	kiloPascals
Kg	kilogram
L/min	litres per minute
$L/min/m^2$	litres per minute per square metre
LAP	left atrial pressure
LIS	lung injury score
LPA	laser particle analyser
mm	millimetre

M	molar
MAP	mean arterial pressure
metHb	methaemoglobin concentration
ml	millilitres
ml/hr	millilitres per hour
MMD	mass median diameter
mmHg	millimetres of mercury
MPAP	mean pulmonary arterial pressure
µg	microgram
µl	microlitre
µm	micron
µmol	micromole
µmol/L	micromole per litre
NO	nitric oxide
PA	pulmonary artery
PAP	pulmonary arterial pressure
$PaCO_2$	arterial partial pressure of carbon dioxide
P_AO_2	alveolar partial pressure of oxygen
PaO_2	arterial partial pressure of oxygen

P_VO_2	mixed venous partial pressure of oxygen
PaO2/FiO2	ratio of partial pressure of arterial oxygen to inspired oxygen concentration measured in millimetres of mercury (mmHg)
P_{BAR}	barometric pressure
PCWP	pulmonary capillary wedge pressure
PGI_2	prostacyclin/epoprostenol
pg/ml	picograms per millilitre
PHT	pulmonary hypertension
PMN	polymorphonuclear neutrophil
ppb	parts per billion
PPP	platelet poor plasma
PPH	primary pulmonary hypertension
PPHN	persistent pulmonary hypertension of the newborn
ppm	parts per million
PRP	platelet rich plasma
PTE	pulmonary thromboembolism
PVR	pulmonary vascular resistance
Qs/Qt	shunt fraction
Rf	retardation factor

rpm	revolutions per minute
RV	right ventricle
SaO_2	% oxygen saturation of arterial haemoglobin
SD	standard deviation
SEM	standard error of the mean
Shunt	% shunt fraction
SpO_2	% oxygen saturation of haemoglobin as measured by a finger probe
SPV	selective pulmonary vasodilator
SvO_2	% oxygen saturation of mixed venous haemoglobin
TEG	thrombelastograph
Vm	minute ventilation
V/Q ratio	ventilation/perfusion ratio
Vt	tidal volume
w/v	weight for volume
%w/v	percentage weight for volume
6 -KPGF$_{1\alpha}$	6 -keto prostaglandin F_1 alpha

Chapter 1

1.0 INTRODUCTION AND LITERATURE REVIEW

The use of selective pulmonary vasodilators (SPV) has recently advanced the therapeutic options available to the clinician dealing with pulmonary conditions such as the acute respiratory distress syndrome (ARDS). Hypoxaemia and pulmonary hypertension (PHT) are features of ARDS.[1-3] This chapter provides an overview of the pulmonary circulation, abnormalities of the pulmonary circulation and the potential role of SPV's in the therapy of hypoxaemia and PHT.

1.1 The Pulmonary Circulation.

The normal pulmonary circulation is a low-resistance, high flow system able to accommodate passage of the cardiac output (CO) at typical pressures of 20/12 millimetres of mercury (mmHg) [4]. Pulmonary arterial pressure normally increases with age and altitude [4].

Pulmonary hypertension (PHT) is defined as a mean pulmonary artery pressure (MPAP) of greater than 20mmHg [5]. PHT may be **passive**, secondary to increased pulmonary capillary pressure, which, in turn, may be due to elevated left atrial pressure (LAP). Alternatively, PHT may be an **active** phenomenon, when the primary pathological process resides at the precapillary or capillary levels.

When discussing pulmonary hypertensive states, pulmonary vascular resistance (PVR) may be used to describe the degree of obstruction to the passage of blood via the pulmonary circulation. PVR is defined as -

$$PVR = (P_{pa} - P_{la}) / Q$$

where P_{pa} = pulmonary artery pressure, P_{la} = left atrial pressure, and Q = cardiac output.

However, "resistance" is a concept derived from the fluid dynamics of laminar, non-pulsatile flow of a Newtonian liquid in channels with a constant radius. Blood is not a Newtonian fluid, blood flow is pulsatile and may be turbulent, and the pulmonary vasculature does not have a constant radius. The use of PVR is therefore a simplification and is subject to error. The use of PVR as a descriptor may be justifiable, as the errors become less significant as PVR increases. The changes seen in pulmonary hypertensive states are of such magnitude that the assumptions made in determining PVR become less significant. Nevertheless, estimates of PVR should always be interpreted in the light of supporting clinical information.

In order for PHT to develop, a number of homeostatic mechanisms in the pulmonary circulation have to be overridden. These homeostatic mechanisms include -

- great **distensibility** of the pulmonary vasculature due to a large amount of elastic tissue and a relatively small amount of smooth muscle in the pulmonary arteries and arterioles [4].
- **recruitment** of alveolar capillaries when pulmonary blood flow increases, thereby limiting pressure rises [6]. As pulmonary arterial pressure rises, so the opening pressure of more and more alveolar capillaries is overcome, allowing flow through these usually or intermittently closed vessels.

In addition to these homeostatic mechanisms the pulmonary circulation is controlled by -

- **Hypoxia**, which causes pulmonary vasoconstriction, so-called hypoxic pulmonary vasoconstriction (HPV). HPV is mediated both by the alveolar partial pressure of oxygen (PAO_2) and by the mixed venous partial pressure of oxygen (P_vO_2) [7].
- **Hypercapnia**, which exerts a mild pulmonary vasoconstrictor effect.
- **Autonomic innervation** – in the pulmonary circulation α-adrenergic stimulation causes vasoconstriction and β-adrenergic and parasympathetic stimulation causes vasodilation.
- **Lung volume** - lung inflation is thought to have a dual effect, expanding large vessels by radial traction while compressing smaller vessels. It has been shown that the vessels in

collapsed alveoli do not constitute a mechanical obstruction to flow [8]. Lung expansion, within physiological limits, probably does not greatly influence pulmonary blood flow.

- **Gravity** - perfusion is distributed down a vertical gradient in the lung, reflecting the balance between intra-alveolar pressure and the distending vascular pressure [9]. Consequently there is a spread of ventilation/perfusion (V/Q) ratios through the lungs, ranging from about 0.5 at the base (i.e. relative shunt) to 2 at the apex (i.e. relative dead space) in healthy subjects. Posture also affects the pulmonary circulation, pulmonary blood volume and pressure both being reduced in the erect position compared with the supine.

When pulmonary arterial pressures are elevated by one or more of the above controlling factors, the compensatory mechanisms of vessel distension and recruitment may be attenuated. Increased pulmonary blood flow may then result in PHT. In this setting, the right ventricle (RV) may not be able to maintain flow against this elevated afterload and may fail [10].

Most PHT is **secondary** to chronic pulmonary or cardiovascular disease. **Primary** PHT (PPH) is a disorder in which PAP is elevated in the absence of any demonstrable cause and accounts for a small proportion of cases. Common causes of PHT are listed in table 1.1.

Pathophysiological mechanisms that contribute to the development of PHT are hypoxia via the mechanism of HPV, loss of, or mechanical distortion of vessels or obstruction of vessels by thrombus [4].

1.2 Primary pulmonary hypertension (PPH)

PPH is a disorder where no aetiology for the PHT is evident. It occurs most commonly in women between the ages of 20 and 50 years. The condition is rare, prognosis is poor, and diagnosis is made by exclusion of other causes [4,11]. The pathophysiological mechanism appears to be an abnormal proliferative response in the small muscular arteries in response to an unknown stimulus [12]. Histological examination shows characteristic changes in both media and intima, as well as evidence of inflammation [10,12,13].

Table 1.1

Causes of pulmonary hypertension.

I.	Primary pulmonary hypertension
II.	Secondary pulmonary hypertension
1.	Parenchymal disease - Chronic airflow limitation - Fibrotic and granulomatous disorders - upper airway obstruction
2.	Vascular disease - Thromboembolus - Vasculitis - Congenital heart disease - Portal hypertension - Granulomatous pulmonary hypertension - Sickle cell disease - Toxin-induced disease - Human immunodeficiency virus (HIV) infection - Acute Respiratory Distress Syndrome (ARDS)
3.	Ventilatory disease - Kyphoscoliosis - Neuromuscular junction disorders - Restrictive pleural disorders - Sleep apnoea
4.	Vascular obstruction - Mediastinal tumour - Aneurysms - Granulomata - Mediastinal Fibrosis - Pulmonary embolus
5.	Venous hypertension - Left ventricular failure - Pulmonary veno-occlusive disease

1.3 Secondary pulmonary hypertension

1.3.1 PHT in cardiac disease

Cardiac disease causes PHT by both passive and active means. In passive PHT, such as in the case of pulmonary venous hypertension (the usual cause being left ventricular failure), longstanding venous hypertension results from an increased upstream pressure. Higher pulmonary arterial pressures result in order to maintain the pressure gradient which maintains adequate pulmonary blood flow. In this setting pulmonary arterial intimal proliferation and vascular remodelling may follow the above sequence of events. Perivascular fibrosis may also occur with sustained irreversible pulmonary arterial hypertension resulting [5,10].

Cardiac disease *per se* can also result in active PHT. Disorders characterised by left-to-right shunting such as atrial or ventricular septal defects, and patent ductus arteriosus may also result in pulmonary arterial intimal thickening and fibrosis, with smooth muscle hypertrophy. These features become irreversible, and flow becomes bidirectional or right-to-left. The resultant hypoxia compounds the PHT. Severe PHT with shunt reversal constitutes the Eisenmenger syndrome. Chronic distension of vessels in the setting of left-to-right shunting may itself act as a stimulus to vasoconstriction [10].

Acute increases in pulmonary blood flow are more likely to produce PHT than more gradual increases in flow due to the intercession of the homeostatic mechanisms described above [10]. Emboli originating from the systemic venous system or the right side of the heart, such as may occur in congestive heart failure, may also contribute to the development of PHT, by mechanical occlusion of pulmonary vessels.

1.3.2 PHT in pulmonary thromboembolic disease

Pulmonary thromboembolism (PTE) is a common cause of PHT. The PTE syndromes can be classified according to which segment of the pulmonary vasculature is affected: small, intermediate or large arteries [4].

The commonest occurrence is occlusion of pulmonary arteries of intermediate size by embolus. Such emboli generally originate from lower limb vessels and are often recurrent and multiple. PHT arises acutely, both from loss of cross-sectional area of the pulmonary vasculature, and from HPV secondary to the hypoxaemia caused by V/Q mismatch.

Hypoxaemia is also contributed to by a number of other processes. Shunting is demonstrable in some patients and may occur from opening of a patent foramen ovale and intracardiac right-to-left shunting as right atrial pressure rises due to pulmonary hypertension. Atelectasis from impaired surfactant production may cause areas of low V/Q ratio and contribute to shunting and V/Q mismatch. The fall in cardiac output (CO) which occurs with most pulmonary emboli can result in areas of high V/Q ratio and increased dead space which may also contribute to hypoxaemia. The role of humoral influences remains controversial. Thromboxane release from activated platelets at the site of embolism probably contributes directly to vasoconstriction and the development of PHT. The majority of survivors of massive, proximal vessel PTE survive because of clot resolution, in which case chronic PHT does not usually occur [14].

1.3.3 The pulmonary circulation in respiratory failure

There are several mechanisms by which respiratory disorders elicit PHT [15]. For example, fibrotic or inflammatory disorders cause microvascular narrowing, leading ultimately to occlusion of vessels. Cross-sectional area available for flow is reduced, and, at the same time, remaining vessels are less distensible.

In airflow limitation, V/Q mismatch causes hypoxaemia and reflex vasoconstriction. In asthma and emphysema, vessels are compressed by hyperexpanded lungs and in emphysema, destruction of parenchyma leads to concomitant loss of vessels. In hypoventilation syndromes, chronic hypoxia causes chronic pulmonary vasoconstriction, and eventually, fixed pulmonary hypertension [16].

Alveolar hypoxia is a common feature of many respiratory disorders, and a major contributor to development of PHT [15,17]. Alveolar hypoxia occurs as a result of alveolar hypoventilation, ventilation/perfusion mismatch with increased physiological dead space or intrapulmonary shunting. Alveolar hypoxia produces a vasoconstrictor effect – the so-called *hypoxic pulmonary vasoconstriction* (HPV) [18]. HPV has the beneficial effect of diverting blood flow away from regions where the oxygen tension is low and thereby optimising V/Q matching [19]. The vasoconstrictor response to hypoxia occurs mainly in small arterioles of about 200μm diameter [20].

There are two major theories as to how alveolar hypoxia may bring about pulmonary vasoconstriction [18]. Firstly, alveolar hypoxia may cause release of a vasoconstrictor substance. No such substance has been identified to date. The response is probably mediated by decreased production of nitric oxide (NO) [21-23]. The second possibility is that hypoxia may stimulate cellular metabolism in vascular smooth muscle in such a way as to influence excitation-contraction coupling and directly cause vasoconstriction [24]. The two mechanisms are not mutually exclusive.

Other mechanisms also contribute to PHT in the chronic forms of respiratory failure [15] such as increased blood viscosity due to polycythaemia, and PTE. Several of these mechanisms usually operate concurrently in chronic respiratory disease. HPV is often the major contributor to PHT in acute respiratory failure [25].

1.3.4 The pulmonary circulation and acute lung injury (ALI)

Acute respiratory failure is commonly seen in the critically ill patient and results from a diffuse injury to lung parenchyma [26]. The spectrum of disease that results is called acute lung injury (ALI), of which ARDS is the most severe form [3]. The main diagnostic features of ARDS as determined by the American-European consensus conference on ARDS [3] are –

- hypoxaemia as defined by a PaO_2/FiO_2 ratio of less than or equal to 200 millimetres of mercury (mmHg).
- bilateral alveolar pulmonary infiltrates on chest radiology
- acute onset
- no evidence of left atrial hypertension or a pulmonary capillary wedge pressure (PCWP) of less than or equal to 18 mmHg.

These diagnostic features are applied in the patient who has been exposed to one of the wide range of precipitants of ARDS. These precipitants include direct lung injury, such as aspiration of gastric contents, pulmonary infection or pulmonary contusion or indirect precipitants such as sepsis, pancreatitis or severe non-thoracic trauma.

The above four ARDS criteria define the more severe end of the spectrum of ALI. ALI is described as a syndrome of inflammation and increased endothelial permeability within the lung that is associated with a constellation of clinical, radiological and physiological abnormalities that cannot be explained by, but may co-exist with left atrial or pulmonary capillary hypertension [3]. The above explains why all patients with ARDS have ALI, but not all patients with ALI have a process severe enough to be defined as ARDS. Only patients with severe ARDS as defined by the Murray lung injury score (LIS) [27] were studied by the candidate, as the use of an investigational agent such as prostacyclin to reduce oxygen requirements is justifiable in this group of critically ill patients. The Murray LIS is discussed further in Chapter 3 and the components of the score are tabulated in table 3.1.

From the definition above, it is evident that hypoxaemia is a major feature of ALI and ARDS, while pulmonary hypertension may or may not be present [28]. The hypoxaemia is multifactorial in origin and causes include –

- **atelectasis**, with right to left shunting of blood in the pulmonary circulation, as it passes through pulmonary vessels which supply collapsed and therefore unventilated areas of the lung.
- **occlusion** of pulmonary vessels with microthrombi and platelet and white cell emboli (micro-emboli). This may be a major feature in ARDS due to pulmonary fat embolism.
- pulmonary interstitial **oedema** (reduced lung compliance and therefore reduced ventilation) or alveolar oedema (increased distance for perfusion of gases between alveolus and pulmonary capillary).

The earliest abnormality of ALI is increased endothelial permeability, unless the precipitating cause is an injury to the alveolar epithelium. The neutrophil is suspected to be central to the response. The accumulation of neutrophils in the pulmonary circulation is an early feature of the process. ARDS may, however, occur in the neutropenic patient. It may be that tissue macrophages are also capable of initiating the process and certain cytokines and endotoxins may be capable of causing membrane damage directly.

Interstitial pulmonary oedema soon accumulates to the point where the lymphatics are overwhelmed and alveolar oedema occurs (the exudative phase) [29]. Early in the course of disease, type I alveolar epithelial cells are reduced in number, parenchymal elasticity is reduced and the basement membrane is exposed. Lung compliance falls and atelectasis ensues. Surfactant abnormalities compound the process of alveolar collapse and flooding. Later, type II alveolar epithelial cells proliferate across the damaged basement membrane in an attempt at healing. Given an intact basement membrane and in the absence of ongoing insults, this would

lead to regeneration of type I cells (the proliferative phase). In some patients a dysfunctional response is seen which results in fibrosis, as shown by the work of Meduri and others [30,31]. It is this sequence of events which causes the refractory hypoxaemia characteristic of ALI [20,32,33].

However, the above are not the only mechanisms operating to produce hypoxaemia. At least some of the hypoxaemia in ALI is due to oedema-independent alterations in V/Q matching. Shunting of blood from injured vascular beds to relatively normal ones (HPV) is a critical protective mechanism in the normal or in the regionally injured lung [20]. HPV is particularly evident in the acute situation when acidaemia due to metabolic acidosis and hypercarbia, both of which potentiate pulmonary vasoconstriction, are often present.

In the diffusely-injured lung, the protective vasoconstrictor response can create an inappropriate widespread increase in vasomotor tone and increase pulmonary arterial pressures significantly [34]. The stimulus for the enhanced HPV appears to be not only hypoxaemia, but also release of vasoactive mediators, such as platelet-activating factor (PAF), tumour necrosis factor (TNF), interleukins 1,6 and 8 and thromboxane A_2 [25,35-37]. Microvascular thrombosis is another feature of ALI, which may contribute to PHT.

1.3.5 The role of the endothelium in pulmonary hypertension

Many of the mechanisms operating in the genesis of PHT, including mechanical and hydrostatic factors, are mediated via the endothelium (particularly in secondary PHT), by means of substances such as substance P [38], NO[39,40] ,endothelin[41] and cytokines such as interleukin -1 and interleukin - 6[42]. Welsh and co-workers have demonstrated unique changes in pulmonary vasculature in response to hypoxia [43]. These changes include enhanced cellular proliferation and the generation of inositol 1,4,5-triphosphate in response to hypoxia and serum or platelet-derived growth factor. These changes could not be demonstrated in systemic (mesenteric) vessels. The pulmonary circulation, in particular fibroblasts, appears to exhibit changes in response to hypoxia

and mitogens resulting in remodelling of all three layers of pulmonary vessels. Sustained hypoxia results in acute changes due to the HPV response in the short term, followed by remodelling in the longer term. Consequently, the effect of therapeutic efforts, such as the use of SPV drugs, would be more effective before remodelling occurred.

Christman and co-authors [44], among others, have shown that both primary and secondary PHT are associated with an imbalance between vasodilator substances (such as prostacyclin) and vasoconstrictor substances (such as thromboxane). These substances are either produced by the endothelium or are modulated by the endothelium. Platelets also appear to play an important role in the production of and response to vasoconstrictor substances such as thromboxane A_2. Platelet aggregation occurs within the lungs of patients with ALI/ARDS [45] and the increased production of thromboxane A_2 is thought to play a role in the PHT associated with ALI. Therapy aimed at improving the balance between vasoconstrictor substances (e.g. blocking production of thromboxane A_2) and vasodilators at the level of the endothelium (e.g. administering prostacyclin or NO) would therefore appear to have a sound basis.

PHT, both primary and secondary, is associated with the secretion of vasoactive substances from pulmonary neuroendocrine cells [45]. These neuroendocrine cells proliferate in pulmonary hypertensive states, releasing a variety of peptides, including serotonin. Serotonin is stored in "dense granules" in endothelial cells and platelets. Serotonin has several actions within the pulmonary circulation, including [45] –

- endothelial disruption and platelet stimulation
- opening of junctions between endothelial cells resulting in oedema
- pulmonary vasoconstriction (opposite to the effect it has in the systemic circulation), intensified by hypoxaemia
- hypertrophy and hyperplasia of smooth muscle cells (via protein kinase C and cyclic nucleotides)

- accelerating structural and functional changes due to the interplay between inflammation, thrombosis, fibrinolysis, vasospasm and vascular remodelling.

Serotonin has been implicated in the PHT associated with pulmonary embolism, ARDS, shock and hypoxic disorders [45]. The effects of serotonin are mediated via several classes of receptors, particularly the S1 and S2 receptors in pulmonary arteries. The S2 receptor antagonist, ketanserin, has been extensively studied as a pulmonary vasodilator [45]. Interestingly, NO has several actions which directly oppose those of serotonin (e.g. reduced platelet aggregation and inhibition of vasospasm and remodelling). NO deficiency is also seen as a factor in appetite suppressant-induced PHT [46].

PHT is therefore best considered not as a single cause-effect model, but rather as an imbalance between vasodilation/vasoconstriction, mitogenesis/cytostasis and thrombosis/fibrinolysis[45]. Substances such as serotonin, NO, prostacyclin and endothelin[41] all play a role in the complex balance required to maintain pulmonary normotension. Genetic, environmental, nutritional, gender-related and pathological process factors will all affect the interplay.

Addressing the imbalance between vasoconstriction/vasodilation by the administration of NO or prostacyclin clearly plays only a partial role in the treatment of PHT.

1.4 Management of Hypoxaemia and PHT Associated with Acute Lung Injury

It has long been known that RV systolic function is impaired in patients with PHT secondary to chronic lung disease, and, more recently, in patients with ALI. Impaired RV function has been demonstrated using both thermodilution techniques and radionuclide assessment. PHT is not usually severe in the patient with ALI, but some patients develop right ventricular failure and the prognosis correlates with the degree of increase in PVR. Treatment of PHT, if present, in the patient with ALI is worthwhile, as lowering pulmonary arterial pressures (PAP) and the effective

pulmonary capillary pressure improves RV function and possibly promotes resolution of pulmonary oedema [47,48].

Until recently, the mainstays of treatment of PHT in ALI have been supportive, including maintenance of oxygenation and the use of systemic vasodilator therapy. Phlebotomy as a therapeutic tool has fallen into disuse. Attempts at improving pulmonary circulation with cardiotonic agents (digoxin) [49] and anticoagulation (warfarin) have not met with marked success. However, prophylactic anticoagulation has been advocated for patients with chronic PHT [50].

1.4.1 Oxygen therapy and mechanical ventilation

Because hypoxaemia is a major contributor to both acute and chronic PHT, correction of hypoxaemia is an important component of management. However, patients with ALI gain only partial relief of hypoxaemia from delivery of high fractions of inspired oxygen (FiO_2) [34]. This is because the main problem is one of intrapulmonary shunt and venous admixture [51].The specific effect of correcting hypoxaemia on haemodynamic performance is difficult to define in isolation. In early ALI, most of the increase in PVR can be abolished by perfusing the lungs with oxygenated blood, suggesting that HPV is responsible for the PHT [52]. Later, when histological changes of fibrosis and vascular remodelling have occurred, the defect is not readily reversible. Accordingly, manipulation of haemodynamic and respiratory variables to achieve optimal oxygenation is crucial early in the management of ALI, before the oxygen transport problem is of sufficient duration to bring about fixed PHT.

The use of high inspired concentrations of oxygen to maintain systemic oxygenation may have pathological consequences in the lung (among them, perpetuation of the ALI and pulmonary fibrosis in the longer term). Techniques which would improve oxygenation without using high inspired concentrations of oxygen would therefore be worth pursuing. One such technique is the use of SPV drugs – as discussed below and the main topic of this thesis.

Another recent area of therapeutic intervention for hypoxaemia has been the manipulation of lung volumes. There are areas of the lung which are collapsed in patients with ALI which are potentially recruitable. Once re-expanded, these areas of the lung would no longer contribute to right-to-left shunting and there would be a consequent improvement in oxygenation. Three ventilatory techniques that have shown promise are –

- the so-called "open lung" approach described by Amato and co-workers [53-55], where a recruitment manoeuvre (application of relatively high airway pressure, 30 – 40 centimetres of water pressure (cmH_2O)) is carried out before the application of positive end-expiratory pressure (PEEP) (e.g. 15 - 20 cmH_2O), sufficient to maintain the "open" status of the re-expanded lung (i.e. to prevent de-recruitment).
- placing hypoxaemic patients in the prone position to reduce so-called "compression atelectasis" in the dependent areas of the lung, thereby allowing re-expansion of potentially recruitable lung units [56] and
- the use of recruitment techniques, where patients are placed in the prone position and high PEEP is applied for up to 90 seconds to recruit potentially-expandable lung units [57,58], before resuming normal mechanical ventilation.

In addition to improvement in oxygenation, expansion of lung units also opens collapsed larger pulmonary vessels by radial traction.

All of these techniques have been shown to improve oxygenation in the short-term. Long-term effects and effects on outcome (on morbidity and mortality) are less clear [58]. As ALI/ARDS is not a homogenous condition, with many precipitating factors and various degrees of severity, it is likely that future research will better refine the use of therapies for the hypoxaemia associated with ALI/ARDS. It appears likely that SPV's will be proven more useful in primary ARDS, where recruitment techniques are less beneficial, while lung-expansion techniques may be more useful in other types of ALI/ARDS.

1.4.2 Systemic, non-selective vasodilator therapy

Although **intravenous** vasodilator therapy may lead to haemodynamic and symptomatic improvement in patients with chronic PHT, the effect is not universal, may not be sustained and there may be significant side-effects [5,10]. Therapy must therefore be individualised and patient response monitored carefully. Various agents have been used as vasodilators in both primary and secondary PHT. These include α-adrenergic antagonists [59], β-adrenergic agonists [60], diazoxide [61], hydralazine [62], nitrates (including NO), angiotensin-converting enzyme inhibitors [63], calcium channel blockers [64,65], prostaglandins , phentolamine [66], and adenosine [67,68].

The **ideal pulmonary vasodilator** would decrease RV afterload while increasing CO and systemic oxygen delivery. To achieve these goals a substantial decrease in PAP would need to occur while stroke volume (SV) and systemic mean arterial pressure (MAP) remained unchanged. This combination of haemodynamic goals has proven difficult to achieve with systemically administered vasodilator agents. In patients with PPH undergoing right heart studies and vasodilator testing prior to long-term therapy, the "ideal" response is seen in only 25-30% of patients [69]. However, when achieved, the "ideal" response is associated with regression of right heart abnormalities [70] and improved survival.[71]

Although **intravenous vasodilators** can decrease PHT when administered systemically, their clinical usefulness is limited by their non-selectivity. There is a twofold requirement for selectivity before agents can be classed as **selective pulmonary vasodilators (see below).** Agents should be selective only for the pulmonary circulation (as opposed to the systemic circulation). In the case of **inhaled agents,** they should also be selective for well-ventilated alveoli as opposed to poorly-ventilated alveoli, thereby avoiding increased right-to-left shunt, venous admixture and worsening oxygenation. Several trials of **intravenously infused** agents (including agents infused directly into the pulmonary artery) have demonstrated that reductions in PAP can usually only be gained at the expense of systemic hypotension, with the concomitant risks of RV ischaemia and

heart failure [72-78]. Furthermore, increased flow to the areas of poor ventilation worsened V/Q mismatch. The use of systemic vasodilators in ALI has therefore largely been abandoned. An exception is adenosine. Adenosine is a synthetic purine nucleoside. Several studies describe its use as a pulmonary vasodilator in PPH [67,68].

Selective pulmonary vasodilators (SPV) delivered by the inhaled route are only able to exert their selective effect if they are deposited/delivered to the ventilated areas of the lung only. This would require their administration as a gas or aerosol in inhaled gas being delivered to the lung either spontaneously or via a mechanical ventilator. Also, the duration of action of the SPV has to be short to ensure selectivity of the agent. A short half-life will prevent action within the pulmonary circulation in areas of low V/Q ration (worsening right to left shunting). It will also minimise the effect of the SPV within the systemic circulation. Non-selective action within the systemic circulation could reduce MAP and possibly RV perfusion. Pulmonary selectivity is discussed in more detail below.

1.4.3 Selective pulmonary vasodilators (SPV)

1.4.3.1 Nitric oxide (NO)

Although currently not licensed for medical therapeutic use in most countries, over 14,000 research papers have been written on NO in the last five years alone and it has become widely accepted for use as a SPV in ALI.

NO is a gaseous, endothelium-derived smooth muscle relaxant which plays a key role in maintaining a basal level of systemic and pulmonary vascular relaxation [79], as well as acting as an inflammatory mediator, a signaling molecule and a mediator of host defences [80-86]. NO activates guanylate cyclase [87] and stimulates production of cyclic GMP (c-GMP). In smooth muscle, c-GMP activates a protein kinase which reduces intracellular calcium concentrations, decreasing muscle contractility. Exogenous NO dilates the pulmonary vasculature when inhaled at low

concentration.[23,88] It is rapidly inactivated by binding to haemoglobin in the pulmonary circulation and is therefore devoid of systemic effect [88]. CO and oxygen delivery are not increased. Also, because it is delivered to ventilated lung units, it has the effect of increasing blood flow in proportion to ventilation [89]. The dual requirements for selectivity are thereby fulfilled.

NO has many other actions which include (see table 1.2) –

- a bronchodilator effect, which has been demonstrated in animal studies and in humans with methacholine-induced bronchospasm, although the effect may not be as marked in man [90].
- neurotransmission in both central and peripheral nervous systems [91].
- a role in macrophage toxicity [82].
- regulation of leucocyte adhesion [92], platelet aggregation [82] and smooth muscle proliferation [93].

Inhaled NO has been shown to be an effective SPV in several clinical settings. The first report of its use to decrease PAP was in 1988, when Higgenbottam and co-workers reported that inhalation of NO decreased PAP in patients with PPH [94]. The idea was subsequently developed using a number of experimental models [23,88,95-97]. PHT was reversed without adverse systemic consequences in the majority of these models, confirming its potential as a SPV.

Table 1.2

Some important pharmacological actions of Nitric Oxide (NO)[80]

Site/clinical setting	***Effect***
Blood vessels	Vasodilation [79] via cGMP (endogenous and exogenous administration[23,88])
Heart	Modulates response to beta adrenergics [85]
Smooth muscle	Bronchodilation [90], regulates smooth muscle proliferation[93]
Platelets	Reduces aggregation [80,82]
Central nervous system	Neurotransmitter [91], important in memory [81]
White blood cells	Mediator of host defences [80,86], inhibits adhesion[85,92], role in inflammation [81]
Macrophages	Role in macrophage toxicity [82]
Mast cells	Modulates histamine release [80]
Shock	Vasodilation [79], maintain gut epithelial integrity [85]
Sepsis	Inflammatory mediator, signalling molecule[80,86]

Note: This table is not an exhaustive list of actions of NO, but allows for easier comparison with the effects of prostacyclin listed in Table 1.3.

Similar results have been obtained in humans. Rossaint et al.[89] reported decreased PAP and improved oxygenation secondary to improved V/Q matching in ten patients with ALI. No adverse consequences were seen. Confirmation of Rossaint's findings have been reported by Payen [98], and other groups [99-101]. Of great interest is the observation that NO results in improved PAO_2 and reduced PA pressures, while CO is unaffected. Intravenous prostacyclin, in contrast, reduces PA pressures and increases oxygen delivery, principally by increasing CO. The reasons for these differences in actions are not entirely clear. The focus of NO research has now shifted to establishing dose-response relationships, investigating potential toxicity of NO and determining the effect on outcome of NO when used in conditions such as ARDS [102-104].

The initial work on NO had been done with doses ranging from 1 to 128 parts per million (ppm)[100,101]. More recently, using doses of between 60 and 230 parts per billion (ppb) Gerlach et al.[103] demonstrated a significant improvement in oxygenation and decrease in shunt fraction without change in pulmonary resistance. Others have also noted that the doses of NO required to improve oxygenation are much lower than the doses required to reduce PAP [99,101]. This suggests that the dose-response curve for pulmonary artery pressure may not be the same as that for oxygenation, and that the beneficial effect of NO on pulmonary perfusion is due to a redistribution effect, rather than pure enhancement of flow. In most cases, in adults, concentrations of 5-40ppm are sufficient to lower PAP [105]. Concentrations as low as those used by Gerlach approach the level found in room air (usually around 10 ppb, although levels in expired air can reach 250ppb).[105]

The response to NO is not entirely predictable. Several studies have now documented groups of critically ill patients with ALI who have not responded to low-dose NO (<40ppm)[89,104]. The mechanism of this variability in response to NO is not known, although the degree of initial PHT appears to correlate with the likelihood of response, those patients with the most marked PHT being most likely to benefit [104].

Withdrawal of NO therapy has also proven to be problematic in patients with ALI and PHT. Sudden worsening of PHT and consequent hypoxaemia has been reported on cessation of therapy [104,106]. This is an unpredictable phenomenon and the mechanism is not known. Possibilities include suppression of endogenous NO production by exogenous administration (product inhibition), or release of a vasoconstrictor substance.

The toxicity of NO remains an area of active research. NO reacts with oxygen to form strong oxidants with as yet incompletely understood effects on normal and pathological cellular metabolism. The rate of oxidation is dependent on the oxygen concentration and the square of the NO concentration [107]. At high concentrations, NO causes systemic methaemoglobinaemia and has caused death [108]. At lower concentrations, the avid binding to haemoglobin may protect the systemic circulation from toxicity, but the pulmonary circulation and alveoli remain at risk.

The clinical significance of reduced platelet adhesion following NO administration is not clear [102]. There are isolated reports of coagulation problems with NO in clinical use [109]. Most toxicological studies have not addressed the issue of long-term NO administration, or the potential for accumulation over prolonged periods of therapy. There are studies reporting NO administration to infants for periods of up to several weeks [110]. and to adults with PPH in a pulsed fashion over periods of up to nine months, without adverse effect [111].

However, the higher oxides of nitrogen are known to be toxic substances. Nitrogen dioxide (NO_2) has considerable pulmonary toxicity, causing pneumonitis and pulmonary oedema, and has been reported as a cause of cytotoxicity and death in experimental animals [112,113]. NO_2 is metabolised to nitrous and nitric acids in aqueous solution, and a number of other higher nitrates and nitrites are formed with as yet unknown effects [114]. Metabolism of NO to NO_2 is therefore a potential hazard, and NO and NO_2 monitoring are mandatory during use of NO, to ensure that recommended

ambient levels of NO are not exceeded [115]. NO itself has been implicated in the exacerbation of ALI in certain conditions, such as after aspiration of acid gastric contents[116]. Indirectly, suppression of NO production in such states as septic shock and ARDS may reduce morbidity and mortality, alluding to "toxicity" attributable to NO [117].

Apart from potential toxicity, there are other problems with administration of NO. These include the delivery system required, which must be constructed of stainless steel to overcome the corrosive effects of the NO. The inspired concentrations of NO and it's higher oxides also need to be constantly monitored - requiring intricate administration and monitoring equipment. This equipment has a high capital cost, is complex to set up and operate and is not readily portable. Although NO gas itself is currently not expensive, the cost of administration is significant.

NO, although an efficacious SPV[42,118], is not ideal in many respects (some of them discussed above), prompting and justifying ongoing research by the candidate and others into alternative SPV's. In particular, recent large outcome studies, such as the studies by Dellinger and co-workers [119] and Lundin and co-workers[120], have shown that although NO improved oxygenation, this improvement was only transient (hours) and did not result in reduction in either morbidity or mortality. Although this data detracts from the utility of NO as a treatment for ALI/ARDS, it should be seen in the context of many therapies used daily in Critical Care Medicine which have not been shown to improve outcome. Also, SPV therapy may be a useful short-term holding measure or as a bridge to other treatments, such as extracorporeal membrane oxygenation, in the terminally hypoxaemic patient. As ALI is a heterogeneous process, it is possible that subsets of patients may yet be shown to benefit from SPV therapy.

1.4.3.2 *Prostacyclin*

Prostacyclin (PGI_2) (figure 1.1) is a member of the prostaglandin family of lipid mediators derived from arachidonic acid and is a potent vasodilator. It is the main product of arachidonic acid metabolism in all vascular tissues studied so far [121]. Endothelial cells produce most PGI_2, but it is also synthesised by smooth muscle cells[121,122]. PGI_2 is the most potent known endogenous inhibitor of platelet aggregation [123].

These effects are achieved by activation of intracellular adenylate cyclase, which is initiated when PGI_2 binds to a specific cell-surface receptor [124,125]. Adenylate cyclase catalyses production of cyclic adenosine monophosphate (cAMP) which activates a protein kinase, and decreases intracellular calcium levels. PGI_2 is therefore a potent vasodilator in all vascular beds including that of the pulmonary circulation [124,126,127].

The physiological role of prostacyclin is to maintain endothelial non-reactivity to platelets by inhibiting thrombus formation (while maintaining the capacity for vessel wall repair) [121], and to maintain a low vascular resistance in beds such as the pulmonary circulation [128], where its secretion is increased by hypoxia [129]. It also stimulates endothelial release of NO [130]. Disorders of PGI_2 production have been implicated in PHT although the significance of these observations remains uncertain [131,132]. Other pharmacological actions of PGI_2 are listed in table1.3.

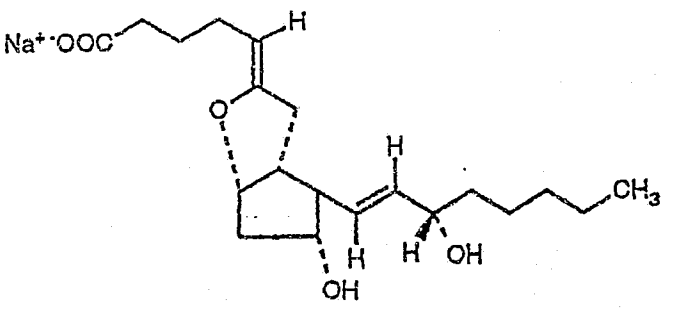


Figure 1.1 **Chemical structure of prostacyclin.**

Table 1.3

Pharmacological actions of prostacyclin [28].

Site/Clinical setting	Effect
Blood vessels	Vasodilatation irrespective of size or location of the vessel.
Platelets	Inhibits platelets. Prevents thrombus formation and disperses existing aggregates. Prevents platelet adhesion to endothelium.
White blood cells	Prevents adhesion to vessel walls. Reduces white cell activation.
Shock	Enhanced production and release during shock states. Cytoprotective. Preservation of organ function.
Sepsis	Enhanced production and release during septic states. Cytoprotective. Preservation of organ function.
Reperfusion injury	Cytoprotective and membrane-stabilising function.
Splanchnic circulation	Preserves and enhances blood flow.

PGI_2 has a short half-life (2-3 min) in animal test systems and few side-effects [28,126,133,134]. PGI_2 has a pharmacological half life of 6 min. in human blood *in vitro* at 37 °C [28,135]. Catabolism of PGI_2 begins with its spontaneous hydrolysis in plasma to its major metabolite, 6-keto prostaglandin $F_{1\alpha}$ (6-ketoPGF$_{1\alpha}$)[136,137]. Another named metabolite is produced enzymatically – 6,15-diketo-13,14-dihydro-prostaglandin $F_{1\alpha}$[138]. These compounds have pharmacological activity orders of magnitude less than prostacyclin.

There is further extensive metabolism of PGI_2, mainly in the liver, and inactive compounds are excreted in the urine (82%) and stool (4%) [139-141]. PGI_2 is not inactivated in the pulmonary circulation. The major PGI_2 metabolites may be measured using standard radio-immunoassay techniques (6-keto prostaglandin $F_{1\alpha}$, H-RIA-Kit, Advanced Magnetic, Cambridge, Mass, USA).

PGI_2 was first used intravenously (IV) as a pulmonary vasodilator in 1978 [124]. Following administration by the IV route, PGI_2 is effective in animal models of PHT [136,142], and in humans with conditions such as PPH [143,144], ARDS [47,48,76], heart failure [145], peripheral vascular disease [146], sepsis [147], and following heart transplant [148]. PGI_2 has also been given by the IV route to assess the response of established PHT to vasodilator therapy [62].

In PPH, a beneficial effect has been shown in several trials [143,144,149]. Studies in cardiac failure have failed to demonstrate reduced mortality [145], but there may be a place for the drug given IV in the acutely decompensating patient in whom an agent with a short half-life, minimal toxicity and no tendency to develop tolerance is required. Similarly, PGI_2 finds a role in pulmonary hypertensive crisis following cardiac surgery [150].

In the setting of ALI, Radermacher et al.[47] demonstrated a decrease in PAP in patients with ARDS, associated with a fall in systemic pressure and a rise in CO. Overall, there was little net

effect on oxygenation, as there was a marked deterioration in V/Q matching. The same group subsequently demonstrated improved RV function with PGI_2 [48]. There was a lack of a beneficial effect on oxygenation when PGI_2 was used intravenously – due to its non-selectivity for the pulmonary circulation, or for pulmonary vessels supplying well-ventilated alveoli only when delivered by the intravenous route. Intravenous PGI_2 and its stable analogue iloprost continue to be used intravenously for conditions such as Raynaud's disease. These treatments remain very expensive (tens of thousands of Rands per annum per patient). Oral beraprost, another stable PGI_2 analogue, has been used experimentally for PHT in rats [42].

The earliest report of the use of PGI_2 via the inhaled route (inhaled aerosolised prostacyclin - IAP) in normal human volunteers appeared in 1978 [124]. A canine model in which administration of IAP led to a reversal of HPV and a decrease in PAP without systemic consequences was described in 1993 [151]. A case series of three patients with severe ARDS reported favourable effects on mean pulmonary arterial pressure (MPAP) and improved oxygenation during IAP therapy, again without systemic effect [152]. The prostacyclin was delivered into the inspired gas of subjects by means of an aerosol produced by either an ultrasonic or gas-driven jet nebuliser. This equipment is cheap, readily-available and portable. This publication by Walmrath et al was one of only four publications on this topic (the use of aerosolised prostacyclin) at the time of commencement of the investigational work described in this thesis [77,123,151,152]. The success of IAP as a SPV in the Walmrath series [152] and the known pharmacological properties of prostacyclin motivated this candidate's research in this field. These early studies confirmed the potency of aerosolised prostacyclin (active in nanogram doses). At the time of commencement of the research described in this thesis there was scant information regarding the safety of IAP, dose-response studies did not exist and IAP had not been formally compared to NO as a SPV. All of these areas proved fruitful ground for investigation by the candidate. In the description of each study in successive chapters the candidate refers to work by other authors on the same or related topics, published at about the same time the candidate's papers were published.

The advent of NO, and more recently IAP provided clinicians, for the first time, with an opportunity to effectively treat the hypoxaemia and PHT characteristic of ALI, without major systemic adverse effect. NO (and potentially IAP) acts as a selective pulmonary vasodilator, and its administration results in beneficial effects on haemodynamic performance and on oxygenation. SPV's represent a major advance in the control of the pulmonary circulation, without adversely affecting gas exchange by altering V/Q ratios in the lung. Previous use of intravenously-administered agents had the dual drawback of worsening venous admixture in the lung and causing systemic hypotension. SPV's allow for precise control of pulmonary pressures, while improving oxygenation. This is a therapeutic goal unattainable prior to the advent of NO. While control of the systemic circulation has been extensively studied, the pulmonary circulation has been largely neglected. SPV therapy has changed that outlook.

The main advantages of IAP over NO for use as a SPV would appear to be ease of administration (cheap equipment, no monitoring required) and lack of toxicity in reports to date. It has otherwise not been extensively investigated. More knowledge about the use of IAP would conceivably also open the therapeutic field to similar agents that could be delivered as an aerosol. As such, investigation of IAP could well prove to be the bridge between NO and a new range of aerosolised SPV's.

Chapter 2

2.0 AIMS

The aim of this thesis was to determine whether inhaled aerosolised prostacyclin (IAP) is a SAFE and EFFICACIOUS agent for use as a selective pulmonary vasodilator (SPV) in patients with severe acute respiratory distress syndrome (ARDS).

2.1 Determining the Safety of Aerosolised Prostacyclin as a SPV in ARDS

2.1.1 Prostacylin

Prostacyclin was chosen as an agent for investigation as it has the pharmacological properties which make it a potentially useful SPV as discussed in Chapter 1. These properties should ensure selectivity in terms of potent action within the circulation in which it is administered, the pulmonary circulation in this instance, without spillover into the systemic circulation. Also, when administered as an aerosol, it should be deposited only in ventilated alveoli, improving V/Q matching.

In determining the safety and efficacy of prostacyclin as a SPV the candidate wished to address -

- the potential toxicity of the high pH diluent in which prostacyclin is dissolved in terms of pulmonary inflammation
- the pulmonary selectivity of aerosolised prostacyclin i.e. demonstrate lack of effect on the systemic circulation, while improving oxygenation
- the possibility of an antiplatelet effect of IAP therapy
- the possible dose-response relationship between inhaled dose of IAP and effect on indices of oxygenation.

2.1.2 Rationale for the study of prostacyclin in patients with severe ARDS

Patients with severe ARDS as defined by the Murray LIS [27] (discussed further in Chapter 3) were chosen for the study of IAP as a SPV by the candidate for the following reasons. The discussion of ARDS in Chapter 1 makes it evident that hypoxaemia is a major feature of ARDS, while pulmonary hypertension may or may not be present [28]. Aerosolised prostacyclin would therefore appear to be an attractive agent for use in the patient with severe ARDS. It would theoretically act as a SPV and reduce hypoxaemia, obviating the need for high inspired concentrations of oxygen (FiO_2) in the therapy of these patients. A lower FiO_2 would assist in avoiding the toxic consequences of oxygen administration (such as absorption atelectasis, ALI, retinal vessel pathology and cerebral oxygen toxicity). If PHT were present the patient would also benefit from a reduction in pulmonary artery pressure. A reduction in pulmonary arterial pressure, however, would not be the major focus of either the therapy or the research into the use of IAP as a SPV.

The patient with severe ARDS could, theoretically, also benefit from the other properties of prostacyclin listed in Table 1.3. These properties include reduced platelet and white cell adhesion in the pulmonary circulation, reducing the tendency to microthrombi and ongoing inflammation at the interface between the alveolus and pulmonary circulation.

2.2 Outline of Studies

In order to pursue the objective of this thesis a number of studies were performed with the following specific aims.

2.2.1 Bench study and Animal study – "Validation of Nebuliser Function" - (Chapter 4).

Aims –

1) To determine or infer the site of deposition of IAP within the lung by –

- measuring the **size of the particles** of aerosolised prostacyclin produced by a jet nebuliser using a laser particle analyser, and
- *administering radiolabelled diluent (in which IAP is delivered) to a piglet and performing a* nuclear medicine ventilation scan to determine site of deposition of diluent particles within the lung.

2) To test the nebulising efficiency of the MedicAid Sidestream nebuliser under standardised conditions.

2.2.2 Clinical study – "Pilot Study Comparing the Efficacy of the Selective Pulmonary *Vasodilators Inhaled Aerosolised Prostacyclin (IAP) and Nitric Oxide* (NO)" - *(Chapter 5).*

Aim –

To compare the **efficacy of IAP and nitric oxide** (the current gold-standard) as selective pulmonary vasodilators in a pilot study in patients with severe ARDS, to determine whether IAP is an efficacious agent in this setting, worthy of further study.

2.2.3 Animal study – "The Pulmonary Toxicity of Inhaled Aerosolised Prostacyclin Therapy" - (Chapter 6).

Aim –

To explore the potential **pulmonary toxic** consequences of delivering prostacyclin directly into the lung. The major concerns being the high alkalinity of the buffer (pH = 10.5) in which the prostacyclin is dissolved prior to administration.

2.2.4 Clinical study – "The Dose-Response Relationship Between Inhaled Aerosolised Prostacyclin and Indices of Oxygenation" - (Chapter 7).

Aims –

1) To confirm a dose-response relationship for IAP in patients with ARDS in terms of improvement in indices of oxygenation.

2) To confirm the effects of IAP on the pulmonary circulation and the lack of effects on the systemic circulation (i.e. selectivity) in patients with ARDS.

3) To determine the systemic concentrations (serum arterial levels) of prostacyclin/prostacyclin metabolite achieved during drug administration via the inhalational route.

4) To measure the effect on platelet function of the systemic plasma concentrations of prostacylin metabolite achieved by IAP *in vivo* (platelet aggregation studies).

2.2.5 *In vitro* study – "*In Vitro* Assessment of the Effect of Extremely Low Concentrations of Prostacyclin and Prostacyclin Metabolites on Platelet Function" - (Chapter 8).

Aim –

To carry out an *in vitro* assessment of the effect of prostacyclin on coagulation (specifically platelet function). using thrombelastography and platelet function tests.

These studies are described in detail in the subsequent sections. Figure 2.1 provides an outline of the construction of the thesis.

Chapter 1 -

Literature review.

Putting the work in perspective.

Chapter 2 -

Aims.

Defining the purpose of the work.

Chapter 3 -General methods and materials.

A description of the methods and materials common to all the studies in the thesis

Chapter 4 -

Validation of nebuliser function.

Validating the drug delivery system used for delivery of aerosolised prostacyclin

Chapter 5 -

Pilot study comparing IAP with NO.

Determining the value of further research into the use of IAP.

Chapter 6 -

The pulmonary toxicity of IAP therapy.

Animal study to confirm safety of IAP therapy.

Chapter 7 -

The dose-response relationship between IAP and indices of oxygenation.

Determining the efficacy of IAP therapy.

Chapter 8 -

In vitro assessment of the effect of extremely low concentrations of prostacyclin and prostacyclin metabolites on platelet function.

An additional *in vitro* safety study.

Chapter 9 -

Conclusions

Figure 2.1 Outline of the Construction of the Thesis

Chapter 3

3.0 GENERAL METHODS AND MATERIALS

This section describes the methods and materials used in the subsequent studies described in this thesis, which are common to those studies. Methods and materials which are specific to an individual study or that differ from the general description given here will be described in more detail in each study where appropriate.

3.1 Ethics Approval

Ethics approval for all the work carried out and described in this thesis was obtained from –

1) The Committee for Research on Human Subjects (Medical) of the University of the Witwatersrand, Johannesburg (clearance certificates M970352, M970353, M970354), for all work involving human subjects.
2) The Human Rights Committee of the University of Western Australia, which is the institutional ethics committee for the Sir Charles Gairdner Hospital, Perth, Western Australia, where the studies were carried out, for all research involving human subjects.
3) The Clinical Drug Trials Committee of the Sir Charles Gairdner Hospital, Perth, Western Australia for all work involving administration of drugs to human subjects.
4) The Animal Experimentation Ethics Committee of the University of Western Australia for all work involving animals.

3.2 Location

All experimental work described was undertaken at the Queen Elizabeth II Medical Centre, Perth, Western Australia, which includes the Department of Intensive Care of the Sir Charles Gairdner Hospital and the Animal Laboratory of the University Department of Surgery.

3.3 Supervisors

This research was supervised by –

1) Prof. Charles Feldman, Department of Pulmonology, Johannesburg Hospital.
2) Dr. Peter Cameron, Department of Intensive Care, Sir Charles Gairdner Hospital.
3) Prof. David Morrell, Department of Anaesthesia, Johannesburg Hospital.

3.4 Physiological Monitoring and Measurements

3.4.1 Human subjects

During the administration of nitric oxide (NO) and/or inhaled aerosolised prostacyclin (IAP) to human subjects, the studies were conducted as described below. The majority of these procedures represent standard Intensive Care management of the critically ill patient and were not additionally invasive or occasioned only by the research.

3.4.1.1 Patient population

Inclusion criteria -

All patients included in clinical trials were adults (16 years and older) who had severe acute respiratory distress syndrome (ARDS), as defined by the American-European consensus conference on ARDS [3] and the Lung Injury Score (LIS) as described by Murray and co-workers (table 3.1) [27]. A LIS of equal to or greater than 2 (in the study described in Chapter 5) or 2.5 (in the study described in Chapter 7) was required for patient inclusion. Basic demographic information for each patient was recorded, including age, gender, acute physiological and chronic health evaluation (APACHE II) score [153], ICU length of stay, day of ICU stay on which study occurred and outcome (alive or dead). Written informed consent was obtained by the candidate in all instances from all patient's/human subjects or their legal surrogate, in the manner prescribed by the ethics committees listed above.

Table 3.1

Lung Injury Score [27].

Parameter	Score
Chest x ray score	
- no alveolar consolidation	0
- 1 quadrant alveolar consolidation	1
- 2 quadrant alveolar consolidation	2
- 3 quadrant alveolar consolidation	3
- 4 quadrant alveolar consolidation	4
Hypoxaemia score	
- $PaO_2/FiO_2 \geq 300$	0
- PaO_2/FiO_2 225-299	1
- PaO_2/FiO_2 175-224	2
- PaO_2/FiO_2 100-174	3
- $PaO_2/FiO_2 \leq 100$	4
Compliance (ml/cmH_2O)	
- ≥ 80	0
- 60-79	1
- 40-59	2
- 20-39	3
- ≤ 19	4
Positive end expiratory pressure (cmH_2O)	
- ≤ 5	0
- 6-8	1
- 9-11	2
- 12-14	3
- ≥ 15	4

Final score is obtained by dividing the aggregate sum by the number of components used.
Score –

- no injury	0
- mild to moderate injury	0.1 – 2.5
- severe injury	> 2.5

Exclusion criteria -

Patients were excluded if consent was not available, if they were haemodynamically unstable (requiring frequent fluid infusions or changes in the rate of inotrope or vasopressor infusions), were unable to remain supine or without nursing interventions during the study period or if they were at risk of intracerebral haemorrhage (severe head injury or bleeding diathesis as defined by an International Normalised Ratio (INR) equal to or greater than 1.5 or an activated partial thromboplastin time (APTT) of equal to or greater than 45 seconds on routine daily testing).

3.4.1.2 Mechanical ventilation.

All subjects were in-patients in the Department of Intensive Care at the Sir Charles Gairdner Hospital (SCGH). Subjects were all receiving mechanical ventilation (Siemens Servo 900C mechanical ventilator, Siemens Elema, Sweden) via endotracheal tube or tracheotomy tube at the time of study enrolment. Sedative agents (titrated infusions of morphine sulphate, David Bull Laboratories, Victoria, Australia and midazolam, Roche Products Ltd., NSW, Australia) and muscle relaxants (intermittent IV doses of vecuronium bromide, Organon Teknika Pty., Ltd., NSW, Australia, titrated to effect) were administered, per unit protocol, to enable patients to tolerate tracheal intubation and mechanical ventilation.

Inspired oxygen concentration (FiO_2) was titrated to produce an arterial partial pressure of oxygen (PaO_2) of at least 50 millimetres of mercury (mmHg). All subjects were receiving a FiO_2 of 0.5 or greater at the time of study enrolment. No lung recruitment manoeuvres were carried out prior to patient enrolment or during the conduct of the study. PEEP was not standardised, but rather was titrated to PaO_2 and ranged from 2.5 – 10 cmH_2O (see table 7.1). Minute ventilation (V_m) was adjusted to maintain arterial partial pressure of carbon dioxide ($PaCO_2$) within the physiological range (35 – 45 mmHg) where the pulmonary pathology permitted. Peak inflation pressure was maintained at or below 40 centimetres of water pressure (cmH_2O) in all instances (allowing tidal

volumes (V_t) of approximately 8 – 10 millilitres per kilogram (ml/kg)). All patients were ventilated with a ratio of inspiration to expiration of 1:1. Ventilator parameters were not altered during the study period.

Blood gas measurements were carried out intermittently as required by individual study protocol using blood drawn from the radial arterial line ("arterial") and/or the right atrial port of the pulmonary artery catheter ("mixed venous"). One millilitre of blood was drawn into a blood gas syringe containing 50 units of zinc heparin (Martell Medical, USA) and analysed within 5 minutes on an ABL 520 (Radiometer, Denmark) blood gas analyser. Measured and derived variables obtained from the blood gas measurements are listed in table 3.2. All results were calculated at a corrected patient temperature of 37 degrees centigrade (°C).

Patients were maintained in the supine position, with a maximum "head-up" elevation of 15 degrees, throughout the study period (maximum time of 4 hours). During this time no nursing interventions (e.g. washing, dressing changes, airway suctioning), drug administration or intravenous fluid bolus administrations were permitted. Constant infusions of sedative agents, vasopressors, inotropes and maintenance fluids present prior to enrolment were continued but not altered during the study period. Intermittent intravenous doses of muscle relaxants were administered as required during the study period.

Table 3.2

Blood gas measurements and calculations.

Parameter	Measurement or calculation by ABL 520 blood gas analyser	Units
PaO_2, PvO_2	measured	mmHg
$PaCO_2$, $PvCO_2$	measured	mmHg
SaO_2, SvO_2	measured	%
$PaCO_2$, $PVCO_2$	measured	mmHg
$A-aDO_2$	calculated: $A-aDO_2$ $=PAO_2 - PaO_2$ $=((FiO_2 \times P_{bar}) - P_{H2O}) - PaO_2$	mmHg
CcO_2	calculated: $CcO_2 = ([Hb] \times 1.39) + P_AO_2 \times 0.0031$	ml/100ml blood
CaO_2	calculated: $CaO_2 = 1.39 \times SaO_2 \times [Hb] + 0.0031 \times PaO_2$	ml/100ml blood
CvO_2	calculated: $CvO_2 = 1.39 \times SvO_2 \times [Hb] + 0.0031 \times PvO_2$	ml/100ml blood
% shunt	calculated: $Qs/Qt = CcO_2 - CaO_2/CcO_2 - CvO_2$	%
DO_2	calculated: $DO_2 = CO \times CaO_2$	ml/min
VO_2	calculated: $VO_2 = CO(CaO_2 - CvO_2)$	ml/min

Key –

PO_2 -	partial pressure of oxygen - a = arterial, v = mixed venous, A = alveolar
PCO_2 -	partial pressure of carbon dioxide - a = arterial, v = mixed venous
SO_2 -	percentage saturation of haemoglobin - a = arterial, v = mixed venous
$A-aDO_2$ -	alveolar-arterial oxygen partial pressure difference or gradient
FiO_2 -	fractional inspired concentration of oxygen
P_{bar} -	barometric pressure (measured by ABL 520, approx. 756 mmHg in Perth)
$P\ H_2O$ -	partial pressure of water vapour at 37 ° C
[Hb] -	haemoglobin concentration
C_xO_2 -	oxygen content - a = arterial, v = mixed venous, c = pulmonary capillary
% shunt -	% of deoxygenated blood entering the arterial circulation (right to left shunt)
Q_s/Q_t	% of deoxygenated blood entering the arterial circulation (right to left shunt)
DO_2 -	oxygen delivery
VO_2 -	oxygen consumption

3.4.1.3 Physiological monitoring

All subjects enrolled in the studies had the following physiological monitoring –

Pressures –

1) Invasive arterial blood pressure (IABP) (systolic, diastolic and mean pressures) – a 20 gauge cannula was present in the left or right radial artery of the patient and connected to a physiological monitor, (Siemens Sirecust 1261, Siemens Medical Electronics, USA) via a fluid-filled pressure transducing system (Abbott Critical Care Systems, Ireland).
2) Central venous pressure (CVP) (mean pressure) was measured using the monitor described in 1) above, connected to a 16 gauge central venous catheter placed in the superior vena cava via either the internal jugular or subclavian routes.
3) Pulmonary arterial (PA) pressures (systolic, diastolic and mean pressures) were measured using the monitor described in (1) above connected to the distal lumen of a 5-lumen 7.5 French gauge balloon-tipped, flow-directed pulmonary artery (PA) catheter (Arrow, model AH-05050-H, Arrow International, USA) placed in either the left or right pulmonary artery via the internal jugular or subclavian venous routes.
4) Pulmonary capillary wedge pressure (PCWP) was measured following inflation of the balloon of the catheter described in (4) above with not more than 1.5 ml of air, with the trace then "frozen" on the monitor screen and the measurement made at the end of patient expiration. PCWP was measured immediately before measuring the cardiac output (CO) in each instance.

All pressures were related to a zero point defined by the mid-axillary line, with the patient in the supine position, corresponding approximately to the level of the mitral valve. The correct placement of the central venous catheter (in the superior vena cava) and the pulmonary artery catheter (in the right or left pulmonary artery in the mid- or lower zone of the lung), was verified radiographically (supine, antero-posterior chest x ray) before measurements were commenced.

All transducers were levelled and zeroed prior to commencement of monitoring. All pressure waves were displayed continuously on the monitor during the study period.

Cardiac output --

CO was measured using the thermodilution technique. Three 10ml boluses of cold sterile 5% dextrose water were injected into the subject's right atrium, via the PA catheter, randomly during the respiratory cycle. The CO was calculated from the detected change in temperature at the thermistor in the pulmonary artery by the internal algorithm of the monitor described above (utilising the Stewart-Hamilton equation). The three measurements (provided the values were within 10% of each other) were averaged and saved. If the measurements were not within 10% of each other, the three CO measurements were repeated. CO was measured intermittently at defined time periods as required by each study protocol.

Cardiac indices –

The Sirecust 1261 monitor (Siemens Sirecust 1261, Siemens Medical Electronics, USA) automatically calculated the cardiac indices listed in table 3.3 from the measured pressures and CO.

Temperature –

Temperature was measured by the PA catheter thermistor located in the pulmonary artery and displayed continuously on the monitor.

Pulse oximetry –

Continuous pulse oximetry (SpO_2) as measured by a finger probe, utilising red and infrared light absorption methodology, and displayed by the Sirecust 1261 monitor (Siemens Sirecust 1261, Siemens Medical Electronics, USA) was employed in all patients.

Table 3.3

Cardiac indices calculated by the Sirecust 1261 monitor.

Parameter	Calculation	Units
Cardiac index (CI)	CI = CO/ BSA	L/min/m^2
Systemic vascular resistance (SVR)	SVR = (MAP – CVP)÷CO	dyne.s/cm^5
Systemic vascular resistance index (SVRI)	SVRI = SVR X BSA	dyne.s/cm^5/m^2
Pulmonary vascular resistance (PVR)	PVR = (MPAP – PCWP)÷CO	dyne.s/cm^5
Pulmonary vascular resistance index (PVRI)	PVRI = PVR X BSA	dyne.s/cm^5/m^2

Key –

CO	cardiac output
BSA	body surface area (calculated by the Sirecust 1261 monitor from internal nomogram using patient height and mass)
MAP	mean arterial pressure
MPAP	mean pulmonary arterial pressure
PCWP	pulmonary capillary wedge pressure
LAP	left atrial pressure

3.4.1.4 Haemoglobin concentration

The haemoglobin concentration for each subject as measured routinely by the Haematology Laboratory at 6 am daily was used for calculations. If bleeding or blood transfusions had occurred since this measurement, a new measurement was done prior to commencing the study.

3.4.1.5 Platelet aggregation studies

Blood samples were obtained from an antecubital vein with a 21G winged infusion set (Surflo, Terumo Medical Corp., Elkton, MD., USA) or from the radial arterial line into a vacuum tube blood collection system (Vacutainer ™). Each sample was collected into a siliconised glass tube containing 1 part 3.8% sodium citrate per 9 parts blood. Platelet aggregation studies were carried out on these samples within 10 min. if possible or batched and performed within two hours while the samples were gently agitated on an agitation table.

The samples were centrifuged at 150 times the force of gravity (g) for 15 minutes to produce platelet rich plasma (PRP) and at 1500 g for 20 minutes to obtain platelet poor plasma (PPP). The PRP was diluted with PPP to a final platelet count of approximately 250 x 10^9/L. Platelet aggregation was performed using a Daiichi Monitor IV aggregation recorder (Helena Laboratories, Beaumont, Texas, USA). Platelet aggregation was induced with various agonists, as described in the subsequent studies, such as adenosine diphosphate (ADP) sodium salt in buffer (Sigma Chemicals, St Louis, USA) in final concentrations of 1 μmol/L or 8 μmol/L or Reagent Horm collagen (Paxton Scientific Pty Ltd, Victor Harbour, Australia) in a final concentration of 10 μmol/L. The maximal rate of aggregation (slope) and the extent of aggregation (maximal and after 4 minutes) were determined by the degree of light transmission thought the sample. Figure 3.1 depicts a typical trace obtained from the aggregometer.

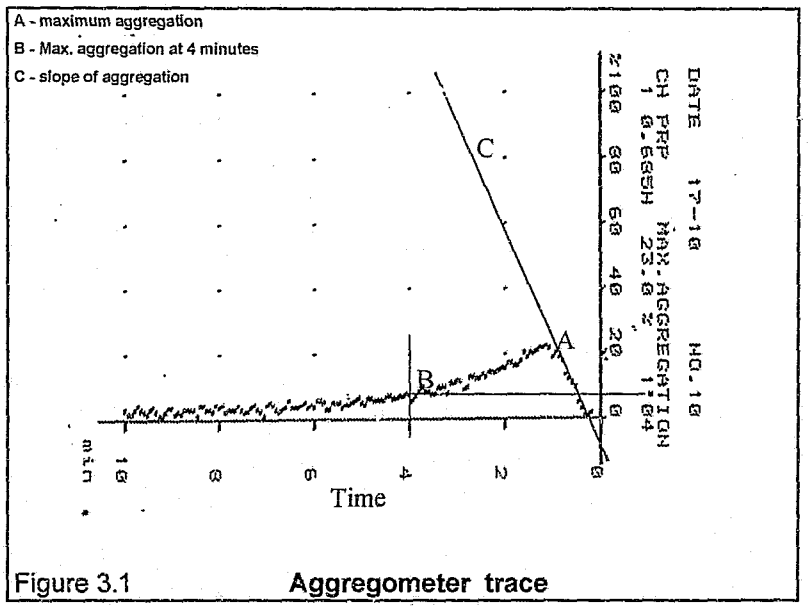


Figure 3.1 **Aggregometer trace**

3.4.1.6 Thrombelastography (TEG)

One or two computerised TEG machines were used as required, providing up to four channels simultaneously. Calibration of the maximum amplitude (MA) was checked using calibration pins (Haemoscope Corp., Skokie, IL., USA). Blood (0.35 ml) was added to disposable plastic cups in each channel. Venous blood from an antecubital vein or blood drawn from the radial arterial line was collected in a plastic syringe prior to testing in the TEG. A drop of olive oil was placed on the surface of each sample to reduce interference with test results due to evaporation. The reaction time (R), coagulation time (K) and the maximum amplitude (MA) of each TEG run were recorded. Normal values for human native whole blood measured in plastic cups are shown in brackets and these times are defined as and signify the following (see figure 3.2):

R time (23.6 +/- 4.8 mm) - the time from when the sample is put in the TEG until the first sign of clot formation. Anticoagulants and factor deficiencies prolong this time.

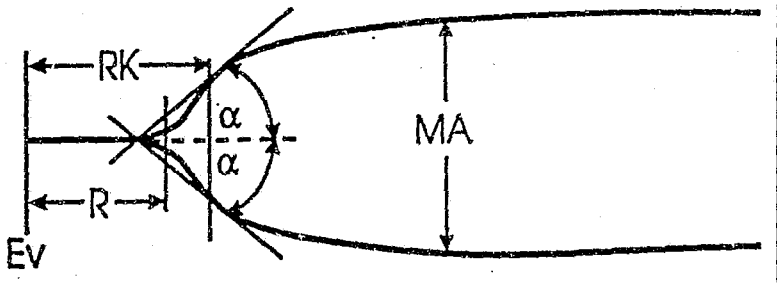
K time (10.6+/- 2.8 mm) - the time from the R (beginning of clot formation) to a fixed level of clot firmness is reached (20 mm of amplitude of the TEG trace). This is equivalent to the time RK minus R (fig. 3.2). Antiplatelet agents prolong K.

MA (54.0+/-5.6 mm) - indicates the maximum amplitude (signifying strength or stiffness) of the developed clot. Clot strength is imparted by the fibrin component (modest contribution) and by platelet function. Antiplatelet effect is demonstrated as a reduced MA.

α angle (60.2+/- 6.7 degrees) - indicates the rate of clot growth.

3.4.1.7 Result recording

All results were recorded manually on worksheets and then entered into a database (Excel, Microsoft Corp., USA) the same day.



Ev - event marker R - reactive time RK - clot time
K - RK minus R α - rate of clot growth
MA - maximum amplitude

Thrombelastography (TEG) trace.

Figure 3.2

3.4.2 Animal subjects

Large white/landrace cross piglets with a mass of up to 25 kilograms (kg) were used as the animal model in two of the studies (see Chapters 4 and 6) described in this thesis. These were healthy male and female animals bred specifically for research purposes. The Animal Experimentation Ethics Committee (AEEC) of the University of Western Australia, in whose facilities the experiments occurred, approved the protocols for animal research. These approvals (of the AEEC) were acceptable to the Postgraduate Education Committee of the Faculty of Health Sciences of the University of the Witwatersrand, Johannesburg.

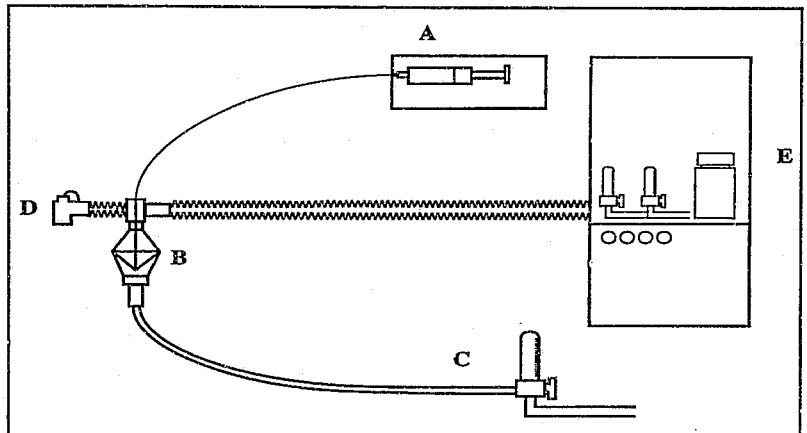
3.4.2.1 Location

All research involving animals was carried out in the Animal House of the University Department of Surgery of the University of Western Australia, on the Queen Elizabeth II Medical Centre campus, Perth, Western Australia. The animals were under the care of trained animal-handling personnel until the animals were anaesthetised. Once anaesthetised, the candidate was responsible for all care of the animals.

3.4.2.2 Anaesthesia

The piglets were anaesthetised by inhalational induction with halothane 3 - 5% in oxygen. Intubation of the trachea with a 6 mm cuffed endotracheal tube was performed without the use of muscle relaxants. Anaesthesia was maintained with halothane (0.5 - 1%) in oxygen-enriched air (FiO_2 0.4 - 0.5) and intravenous pentobarbitone infused at 8 mg/kg/hr by an infusion pump (IMED Gemini PC-1, Imed Corp., USA) via an intravenous line placed in the femoral vein.

The piglets were allowed to breath spontaneously (via the endotracheal tube connected to an anaesthesia delivery circuit – see figure 3.3), the effectiveness of which was confirmed by intermittent arterial blood gas measurements ($P_aCO_2 < 50$ mmHg).



A - syringe pump B - jet nebuliser C - nebuliser driving gas
D - catheter mount E - anaesthesia machine

Figure 3.3 **Aerosol delivery system for piglets.**

At the end of the study period (lasting up to 8 hours) the piglets were euthanased with a lethal intravenous injection of pentobarbitone. Death was defined as absence of respiration and heartbeat, as determined clinically.

3.4.2.3 Physiological monitoring

Monitoring of the animals included continuous IABP recording via a fluid-filled transducer system (Abbott Critical Care, Ireland) and recorded on a paper strip recorder (Grass, model 7D polygraph, Grass Intrument Co., Quincy, Mass., USA), continuous pulse oximetry (SpO_2) (Invivo model 4500, Invivo Lab., USA) and hourly rectal temperature measurements using an electronic thermometer (Filac model F-1500, Sherwood Medical, USA). The purpose of this monitoring was to detect any physiological variation that might adversely impact upon the histological testing which was to occur after the animals had been euthanased. Close physiological monitoring would detect for and allow correction of hypotension, hypoxaemia, hypoventilation, metabolic acidosis and malignant hyperthermia. A stable physiological environment during aerosol therapy was thereby ensured.

Normal saline solution (0.9%) was infused as hydration fluid via a femoral venous catheter at 2 ml/kg/hr, using a volumetric infusion pump (IVAC, Baxter Healthcare Corp., California, USA). The piglets were turned from side to side every two hours during the study period to minimise gravitational effects, such as pulmonary atelectasis.

3.5 Drug Therapy

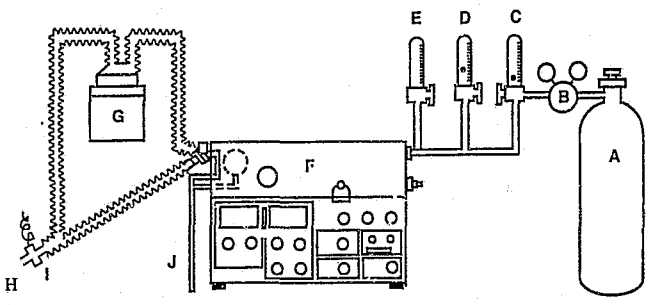
Two selective pulmonary vasodilators (SPV) were studied – nitric oxide (NO) and inhaled aerosolised prostacyclin (IAP).

3.5.1 Nitric oxide (NO) therapy

NO gas was delivered to humans only (see Chapter 5). NO was supplied in cylinders by Air Liquide, Western Australia in a concentration of 1 000 part per million (ppm) in nitrogen (N_2). NO was administered to subjects in a concentration of 10 ppm throughout the respiratory cycle, by diluting the concentrated NO (1 000 ppm) in the inspired gas being delivered to the subject by the *mechanical ventilator (see mechanical ventilation above)*. A flow rate of NO in nitrogen of approximately one hundredth the ventilator driving gas flow rate (oxygen and air being supplied to the mechanical ventilator as depicted in figure 3.4) was administered into the driving gas (i.e.1 000 ppm ÷ 100 = 10 ppm final concentration). The flow of concentrated NO into the inspired gas mixture was governed by a low-flow nitrogen gas rotameter (60 – 600 cm^3/min) made of stainless steel (to avoid corrosion by the NO).

The final concentration of NO actually being delivered to the patient was measured at the catheter mount connection (i.e. as near as possible to the patient airway as practical) by means of a NO analyser (PrinterNO$_x$, Micromedical, U.K.), utilising an electrochemical method. A concentration of 10 +/- 3 ppm was delivered to all patients in the relevant study (see Chapter 5).

In addition to the NO concentration being delivered to the subject, the PrinterNO$_x$ was also used to measure the concentration of the most prevalent toxic higher oxide of NO, namely NO_2. In no instance did the delivered concentration of NO_2 exceed 0.5 ppm. NO and NO_2 concentrations were monitored continuously during NO administration. Methaemoglobin (metHb) concentrations were measured oximetrically by the ABL 520 blood gas analyser, to determine any systemic toxic effect of the NO administration (i.e. to avoid concentrations of metHb $\geq$ 5%).



A - nitric oxide 1,000 ppm in nitrogen
B - pressure reducing valve
C - nitric oxide rotameter
D - air rotameter
E - oxygen rotameter
F - mechanical ventilator
G - humidifier
H - catheter mount connection
I - NO and NO_2 analyser
J - scavenging system

Figure 3.4 **Nitric Oxide (NO) delivery system.**

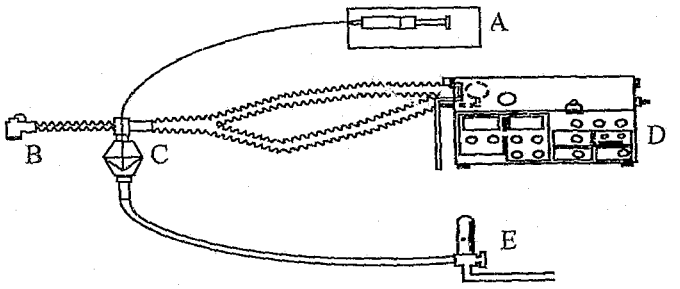
3.5.2 Aerosol production and delivery

Prostacyclin aerosol –

The synthetic analogue of prostacyclin (epoprostenol sodium, Flolan, GlaxoWellcome, Boronia, Victoria, Australia) was supplied in vials of 500 micrograms (μg). This was diluted in a 0.188% w/v solution of glycine in saline (0.147%w/v), prior to administration – the "diluent". The diluent is alkaline with a pH of 10.5 (+/- 0.3). In this thesis the terms prostacyclin (or PGI_2) and epoprostenol are used interchangeably.

Inhaled aerosolised prostacylin (IAP) was delivered by jet nebuliser into the inspired gas being inhaled by the subject, as depicted in figures 3.3 (for animals) and 3.5 (for humans). The jet nebuliser was placed as close as possible to the catheter mount connection, to minimise loss of aerosol in the exhaled gas and as rain-out in the ventilator circuit tubing. Rain-out was further minimised by heating and humidifying the inspired gas (Fisher and Paykel model 328 water bath humidifier, Fisher and Paykel Healthcare, Australia). The humidifier was set at a temperature of 40 °C, as per unit protocol. Animal subjects spontaneously breathed dry gas, delivered by the anaesthesia machine (figure 3.3).

Two brands of jet nebulisers were used. The Airlife Mistyneb nebuliser (Baxter Healthcare Corp., USA), was used in the study described in Chapter 5. The Medic-Aid Sidestream nebuliser (Medic-Aid Ltd., U.K.) was used in the other studies. The particle sizes produced by these two brands of nebuliser when driven by a 6 L/min flow of driving gas are shown in table 3.4. The FiO_2 of the nebuliser driving gas (mixed air and oxygen) was the same as that being delivered by the mechanical ventilator, so that nebuliser driving gas did not change the FiO_2 of gas delivered to the subject. The nebuliser driving gas was delivered at a pressure of 420 kPa, via a flowmeter at 6 l/min. However, the mechanical ventilator compensated for the additional gas flow into the ventilator circuit, so that PEEP, mean and peak airway pressures were unaltered. The exhaled V_m



A - Syringe driver pump with syringe of prostacyclin solution
B -Catheter mount
C - Jet nebuliser
D - Mechanical ventilator
E - Nebuliser driving gas

Figure 3.5. **Inhaled aerosolized prostacyclin delivery system.**

Table 3.4

Particle sizes produced by the MistyNeb and Sidestream jet nebulisers.

	MMD(μm)	% Particles less than 5.17μm
Normal saline		
MistyNeb	5.1	51
Sidestream	3.2	79.9
Glycine diluent		
MistyNeb	5.4	47.1
Sidestream	3.4	77.1

Key -
MMD - mass median diameter
μm - micron

Note: The figures quoted in this table were determined by a single measurement of particle size by laser particle analyser for each solution (normal saline and glycine diluent) in each nebuliser (Mistyneb and Sidestream) – see Chapter 4 for details. These are not manufacturers' data.

registered by the pneumotachograph of the ventilator, however, included the additional gas flow provided by the nebuliser driving gas. The pressure of the driving gas had no appreciable effect on airway pressure in the spontaneously breathing piglets (Chapter 6), because of the semi-open nature of the anaesthesia circuit (fig. 3.3). Validation of the performance of the nebulisers is discussed in detail in Chapter 4.

The concentration of prostacyclin solution (μg/ml) delivered into the nebulising bowl of the jet nebuliser for aerosolisation was determined by adding varying volumes of the diluent to the vial of epoprostenol as follows –

Step 1.

10 ml of diluent was added to the a vial of epoprostenol - this gave a concentration of 50 μg/ml. 5 ml of this solution was drawn off into a 20 ml Terumo syringe (Terumo Corp., Japan) and **labelled as "50".**

Step 2.

20 ml of diluent was added to the remaining 5 ml of 50 μg/ml solution in the vial – this gave a concentration of 10 μg /ml. 10 ml of this solution was drawn off into a 20 ml Terumo syringe and **labelled as "10".**

Step 3.

A second vial of epoprostenol was used. 12.5 ml of diluent was added to the 500 μg of epoprostenol in the vial – this gave a concentration of 40 μg/ml. 5 ml of this dilution was drawn off into a 20 ml Terumo syringe and **labelled as "40".**

Step 4.

7.5 ml of diluent was added to the vial used in step 3 – this gave a concentration of 20 μg/ml. 5 ml of this solution was drawn off into a 20 ml Terumo syringe and **labelled as "20".**

Step 5.

A third vial of epoprostenol was used for this dilution. 16.6 ml of diluent was added to the epoprostenol. This gave a concentration of 30 μg/ml. 5 ml of this solution was drawn off into a 20 ml Terumo syringe and **labelled as "30".**

In this way syringes of known fixed concentrations of prostacyclin were produced for use in the various studies - labelled 10, 20, 30, 40, 50 μg/ml as appropriate, to indicate the concentration of prostacyclin solution in the syringe. The appropriate syringe of prostacylin solution was then loaded into a syringe-driver pump (Terufusion model STC 523, Terumo Corp., Japan) and pumped into the nebulising bowl of the jet nebuliser at a constant rate during the study period. The rate of administration of solution into the nebuliser bowl was determined by the subject's mass. The rate at which to run the syringe pump in ml/hr was calculated as follows –

(Subject mass in kg X 60) ÷ 1,000

e.g. for a 70 kg subject – (70x 60) ÷ 1,000 = 4,200 ÷ 1,000 = 4,2 ml/hr.

The desired dose could then be varied according to study protocol by using the syringe of the appropriate solution e.g. to deliver 50 nanograms per kilogram per minute (ng/kg/min) to a 70 kg subject, the syringe labelled 50 (as described above) would be loaded in the syringe pump and run at 4.2 ml/hr into the nebulising bowl of the jet nebuliser – as depicted in figures 3.3 and 3.5.

For human subjects the aim was to reach a steady state at each dose of IAP, without remaining at that dose for such a prolonged period that confounding physiological changes were able to interfere. Each dose of IAP was therefore delivered for 30 minutes only, before changing dose.

In the animal study (Chapter 6) the aim was to determine the toxicity of the alkaline diluent, and the 10μg/ml solution of prostacyclin (or the pure diluent) were used. The solutions were delivered into the tracheobronchial tree at the maximum volume achievable by the jet nebuliser being driven by a 6 L/min flow of gas (i.e. 12 – 15 ml/hr of solution aerosolised – see Chapter 4).

The dose of prostacylin actually delivered to the subject's lungs by the above system is not exact and inconsistencies and problems with aerosol delivery are discussed in Chapter 4.

Normal saline aerosol –

Normal saline aerosol was used as a control in the study described in Chapter 6. Normal saline solution (0.9%w/v) aerosol was delivered to the nebuliser bowl in the same manner as described for animal subjects above.

3.5.3 Measurement of 6-keto prostaglandin F1 α levels

Prostacyclin is an ephemeral substance with a biological half-life of 2 – 3 minutes. It is rapidly hydrolysed to its major metabolite 6-keto prostaglandin F1 α (6-ketoPGF1α) *in vitro* and *in vivo*[137-139,154,155]. This metabolite is not inert, but has an activity (vasodilatation and platelet inhibition) much less than the parent compound [154]. 6-ketoPGF1α is however more stable in the circulation than the parent compound (half-life of 18-29 minutes [154]). Its level is measurable in serum as an estimate of the degree of absorption of the parent compound (from the respiratory tract into the

circulation). Any 6-ketoPGF1α detected in the serum would be contributed to by hydrolysis of both endogenous prostacyclin and exogenous (administered) prostacylin. Of interest therefore is the change in serum levels of 6-ketoPGF1α with increasing mass of prostacyclin delivered to the respiratory tract as aerosol. The studies described in Chapters 7 and 8 take note of the maximum serum levels of 6-ketoPGF1α when determining the antiplatelet effect such levels might produce.

Blood taken for the measurement of 6-ketoPGF1α serum levels was drawn from the arterial line. Seven ml of blood was drawn from the arterial line using a plastic syringe and immediately injected slowly into a 10 ml blood collection tube, via a 19 gauge needle. The siliconised glass tubes had previously been prepared by adding 500 international units of sodium heparin (0.5 ml volume) to prevent coagulation and 36 μg of acetyl salicylic acid to inhibit cyclo-oxygenase activity (and thereby the continued production of endogenous prostacyclin) in the glass tube. These samples were placed in a centrifuge within 5 minutes of collection and spun at 4 000 rpm for 10 minutes. The serum was then decanted by pipette into plastic storage tubes, which were stored at – 70°C, until analysis.

Analysis of serum levels of 6-ketoPGF1α was by radioimmuoassay (RIA) as follows. ^{3}H 6-ketoPGF1α (5000cpm) was added to plasma (1 ml) for estimation of recovery. The sample was extracted with 2 volumes of acetone. After centrifugation at 2000 g for 10 min. the neutral lipids were extracted into 2 volumes of hexane. The sample was again centrifuged, the hexane layer removed and the pH of the sample adjusted to 4. The sample was then extracted with two volumes of chloroform, centrifuged at 2000 g for 10 min. and the aqueous phase discarded. The organic phase containing 6-ketoPGF1α was taken to dryness in a centrifugal evaporator and reconstituted in methanol.

The 6-ketoPGF1α sample was separated from other prostanoids using thin layer chromatography and a solvent system containing chloroform:methanol:acetic acid:water (135:75:24:150). The 6-ketoPGF1α band was identified by running a standard containing ^{3}H 6-ketoPGF1α. The bands corresponding to 6-ketoPGF1α were scraped and the 6-ketoPGF1α was extracted into 0.1M phosphate buffered saline at pH 7.5 (1 ml). The sample was centrifuged and the supernatant divided for estimation of recovery and quantitation of 6-ketoPGF1α by radioimmunoassay (RIA).

The RIA utilised a ^{125}I Histamine 6-ketoPGF1α label antibody and standards ranging from 0 – 200 pg/ml. The bound 6-ketoPGF1α was separated by the addition of dextran coated charcoal and the bound fraction was counted in a γ counter. The recovery of ^{3}H 6-ketoPGF1α through the procedure was about 45% and the within and between assay variations were 5 and 12% respectively.

3.6 Statistical analysis

Statistical methods are described in detail where appropriate in each successive chapter. Advice regarding the planning and analyses of statistics for each study was obtained from Dr. Richard Parsons and Mr. Max Bulsara of the Biostatistical Consulting Service of the Dept. of Public Health of the University of Western Australia.

Chapter 4

4.0 VALIDATION OF NEBULISER FUNCTION

The following is a description of the methods used by the candidate to assess the adequacy of drug delivery by jet nebuliser with regard to delivery of aerosolised prostacyclin. Several published studies have described significant variation in nebuliser function, depending on the drug nebulised, the nebuliser gas flow rate and the brand of nebuliser used[156-162]. Three tests of nebuliser function were carried out.

4.1 Measurement of Particle Size

Only particles in the respirable range, 1 to 5 microns in diameter, are deposited in the tracheobroncial tree. Smaller particles are exhaled without being deposited in the lung, while larger particles rain-out or precipitate within either the ventilator tubing or the larger airways. In both these instances the drug is unavailable to produce an effect within the lung. It has been estimated that only a small proportion of particles produced by a jet nebuliser are deposited within the lung when delivered by mechanical ventilation[156,157,159,161,163]. The actual mass of aerosolised drug presented to the ventilated lungs may be calculated by gravimetric methods i.e. measuring the mass of the nebuliser before and after nebulisation and determining the mass of liquid aerosolised by the device[157]. This technique accounts for liquid nebulised into the inhaled gas, but not necessarily deposited in the lung. Another technique relies on radiolabelling of the nebulised substance to determine deposition within the lung (this is described in more detail below).

The equipment set-up for delivery of aerosolised prostacyclin to animals (figure 3.3) and to humans (figure 3.5) provides for the production of aerosol by a jet nebuliser being driven by 6 litres/min (L/min) of oxygen-enriched air at a pressure of 420 kPa. The particles thus produced are delivered into the inspiratory gas. The amount of drug that is deposited as precipitant within

the delivery tubing and in the larger airways is unknown, nor is the amount of drug exhaled and lost in the expiratory gas.

4.1.1 Aim

The aim of the first test was to measure the size of particles produced by the jet nebuliser for delivery into the inspiratory gas.

4.1.2 Method

Two brands of jet nebuliser were tested by the candidate; the Airlife Mistyneb nebuliser (Baxter Healthcare Corp., USA), (used in the study described in Chapter 5) and the Medic-Aid Sidestream nebuliser (Medic-Aid Ltd., U.K.) (used in the other studies). Each nebuliser was primed with a 2.5 millilitre (ml) volume of either normal saline (0.9% w/v) or the more viscous glycine buffer in which prostacyclin was dissolved prior to delivery, a 0.188%w/v solution of glycine in saline (0.147%w/v) (GlaxoWellcome, Boronia, Victoria, Australia). The jet nebulisers were then driven with 6 l/min of dry medical grade air and the particles so produced measured by a laser particle analyser (Malvern Master Sizer Laser Particle Analyser, model MS20, Malvern Instruments, Worcs., U.K.). A single measurement of particle size by laser particle analyser was carried out for each solution (normal saline and glycine buffer diluent) in each nebuliser (Mistyneb and Sidestream). A single measurement was deemed satisfactory in view of the high accuracy and low variability in results provided by the laser particle analyser.

The laser particle analyser (LPA) utilises laser light diffraction to measure particle size. The particles pass through a laser beam and light scattered by the particles is collected over a range of angles in the forward direction. The angular intensity distribution of the scattered light varies strongly with particle size over a wide range. The laser beam is expanded and collimated and is positioned in front of a lens. A photosensitive detector is placed in the focal plane of the lens. The

focal length of the lens, the dimensions of the detector and the laser light wavelength determine the particle size range which can be measured.

The portion of the laser beam unscattered by particles is focused in the same plane as the detector and is allowed to pass out of the system via an aperture in the detector. Annular detector rings around the central axis of the incident laser on the detector record the intensity of laser light scattered by the particles over a range of angles. The degree of laser light scatter depends on particle size. The distribution of intensity of the scattered light is analysed by a computer to yield the particle size distribution. The features of the LPA are illustrated in figure 4.1.

4.1.3 Results

The mass median diameter (MMD) of the particles produced by each nebuliser and the percentage of particles with a diameter of less than 5.17 microns (the maximum respirable particle size) measured by the above technique were (these are the same figures shown in table 3.4, repeated here for convenience) –

	MMD	**% particles less than 5.17 micron diameter**
Nebulised solution		
Normal saline		
MistyNeb	5.1	51
Sidestream	3.2	79.9
Glycine buffer		
MistyNeb	5.4	47.1
Sidestream	3.4	77.1

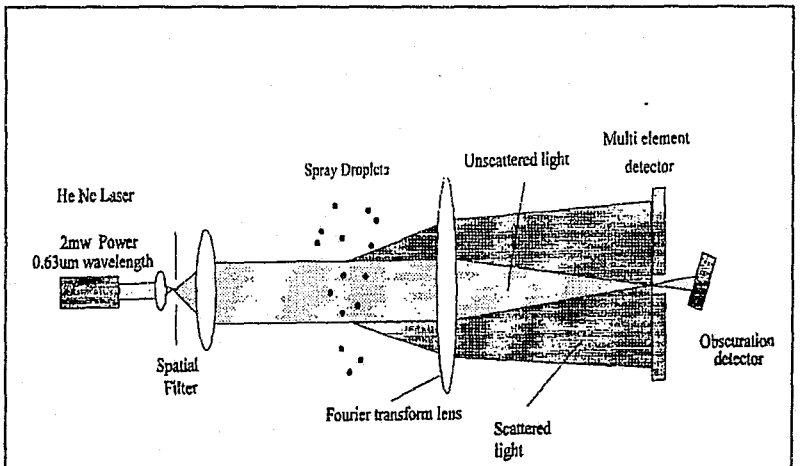


Figure 4.1 **Principles of the laser particle analyser**

4.1.4 Conclusion

The Sidestream brand of nebuliser produced a higher percentage of particles within the respirable range than the MistyNeb brand when used in the manner described above [164]. Also, even though the glycine buffer is more viscous than normal saline and theoretically more difficult to nebulise, the Sidestream device was able to produce a high percentage of particles in the respirable range. The candidate concluded that the Sidestream device is the preferred device for delivery of aerosolised prostacyclin (which is dissolved in the glycine buffer).

Note: Only the MistyNeb device was available for use by the candidate during the conduct of the study described in Chapter 5. The Sidestream device was used for subsequent studies.

4.2 Aerosol deposition in the distal airway

The jet nebulisers tested above produced large proportions of aerosol particles within the respirable range of 1 – 5 microns. However, it was still not known where these particles were being deposited within the lung. Distal deposition of the aerosolised prostacyclin particles within the lungs is important, if the full potential of prostacyclin as a selective pulmonary vasodilator is to be exploited.

4.2.1 Aim

The aim of the second test of nebuliser function was to determine the site of deposition of aerosolised particles when delivered by the systems described in Chapter 3 above (figures 3.3 and 3.5).

4.2.2 Method

Technetium labelled DTPA (^{99m}Tc-DTPA) was dissolved in glycine buffer (0.188% w/v solution of glycine in saline, 0.147%w/v) (GlaxoWellcome, Boronia, Victoria, Australia). This solution was then aerosolised and delivered to the lungs of an anaesthetised piglet (15 kg) using a Sidestream jet nebuliser (see Chapter 3 for details of anaesthesia and monitoring of piglet and nebuliser set-up).

The solution to be nebulised was made up as follows –

A vial of lyophilised DTPA (Pentastan RM5, Australian Isotopes) was reconstituted in 2 ml ^{99m}Tc-sodium pertechnetate. The labelling efficiency was determined to be greater than 95% using Instant Thin layer Chromatography (ITLC) strips (ITLC Baker-flex silica gel 1B) with a solvent of 85% methanol. In this system the labelled compound remains at the origin, while unlabelled free pertechnetate has an retardation factor (R_f) of 1.0. One millilitre of the ^{99m}Tc-DTPA was transferred to a sterile vial containing 1 ml of the glycine buffer, mixed and incubated at room temperature for 3 hours. This solution was tested for any breakdown of the labelled DTPA using the ITLC method. The solution remained stable as shown by a greater than 95% label after the 3 hour incubation period.

Once adequate labelling and stability of the solution had been established as above, 2 ml of the labelled DTPA was dissolved in a vial (50 ml) of glycine buffer, immediately prior to nebulising the solution using the system shown in figure 3.5. This is the same system that was subsequently used to deliver aerosolised prostacyclin to human subjects (described in detail in Chapter 3). A γ camera was then used to detect radioactivity over the lungs of the anaesthetised and intubated piglet, receiving mechanical ventilation (tidal volumes 10 ml/kg, frequency 10/minute, FiO_2 0.4). Radioactivity was measured over the anterior, posterior and lateral aspects of the lungs by means of a gamma camera.

The dose of radiation which the test subject was exposed to by this technique did not exceed the dose to which an adult human would be exposed during performance of a diagnostic nuclear medicine ventilation scan (100 – 200 megaBecquerel) . Nevertheless, the candidate preferred to carry out this test on an animal subject, rather than a healthy human subject.

4.2.3 Results

Figures 4.2 and 4.3 show the rapid uptake of radiolabelled aerosol by the lungs of the piglet. Figure 4.4 indicates the widespread deposition of the aerosol within both lung fields as shown from the anterior, posterior and lateral aspects. The very high concentration of radioactivity over the trachea and central airways was noted. Figure 4.5 shows the even distribution of radioactivity over both lungs (53-54% over the right lung and 46-47% over the left lung). Figure 4.6 shows the even distribution of radioactivity within each lung – 50.6% (posterior view) – 66.7% (anterior view) over the right inner lung and 33.3% (anterior view) – 49.4% (posterior view) over the right outer lung. Similar values are present for the left lung (see figure 4.6).

It was noted that (figure 4.3) it takes approximately 5 minutes for even and distal deposition of aerosol in both lungs. This would then be the approximate time expected for aerosol delivery to reach a steady state at its delivery point and exert its desired effect. This information, together with the known half-life of active agent (e.g. 2-3 min. for prostacyclin), is required to plan dosing intervals. For this reason, dosing intervals of 30 minutes were chosen for the studies described in Chapters 5 and 7. This interval would allow for adequate deposition of the active drug in the lung and for the physiological variables to reach a steady state, but not be long enough for other (extraneous) factors to significantly affect findings.

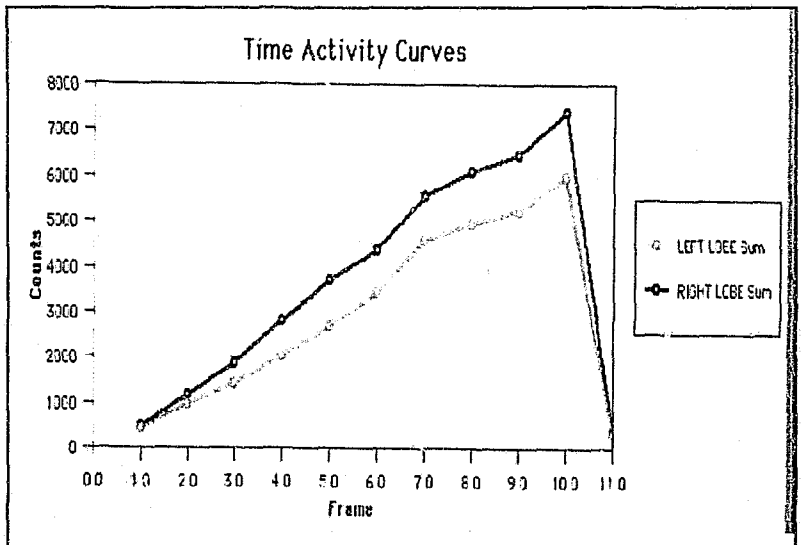


Figure 4.2 **Time Activity Curves**

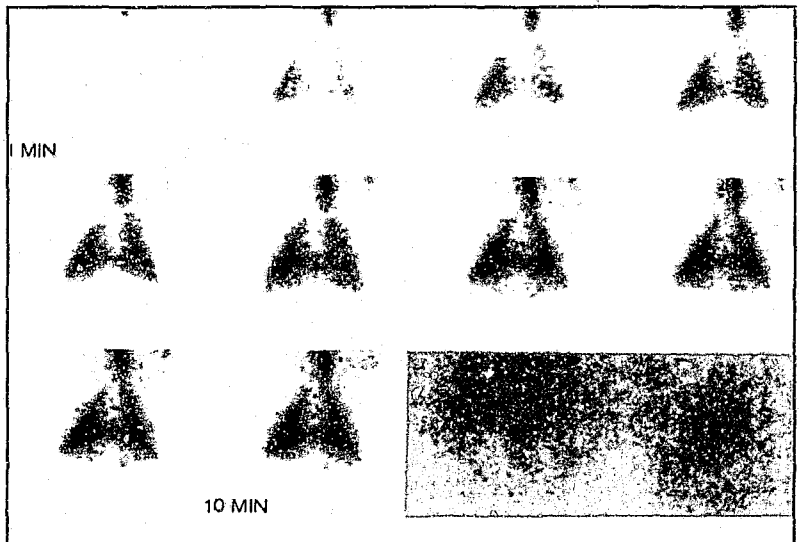


Figure 4.3 **Uptake phase during ventilation scan in a piglet**

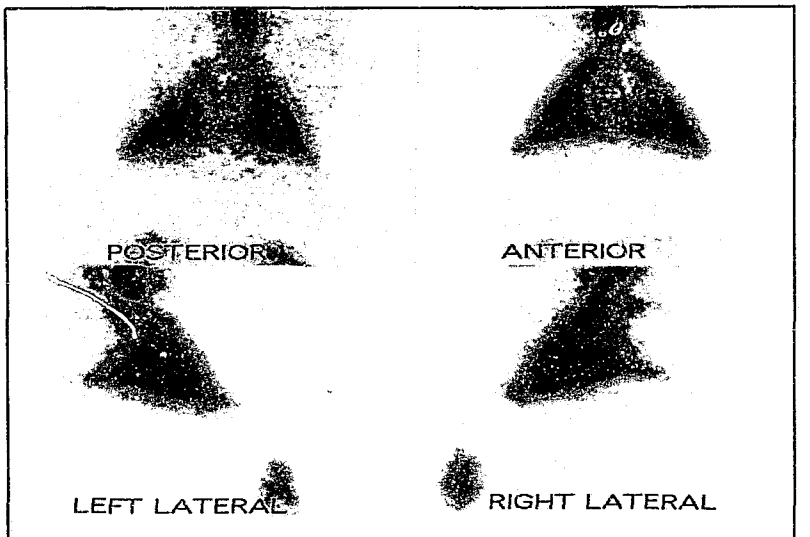


Figure 4.4 **Ventilation scan in a piglet.**

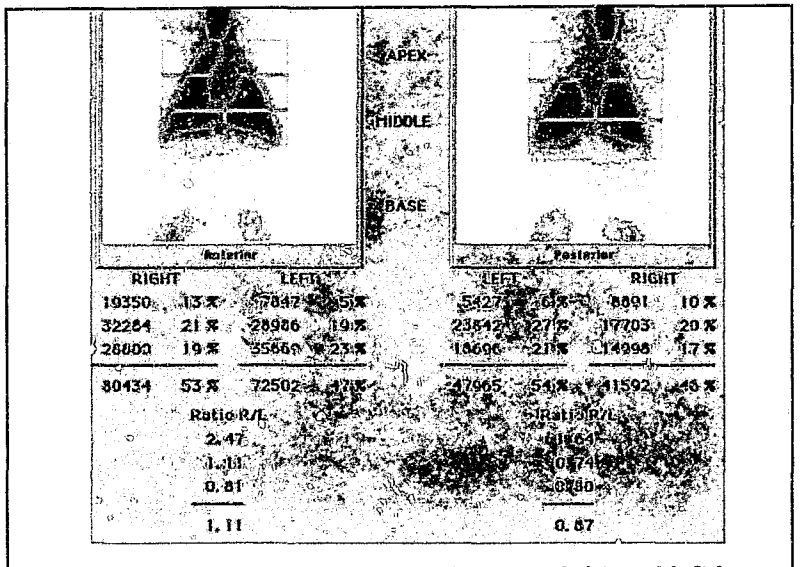
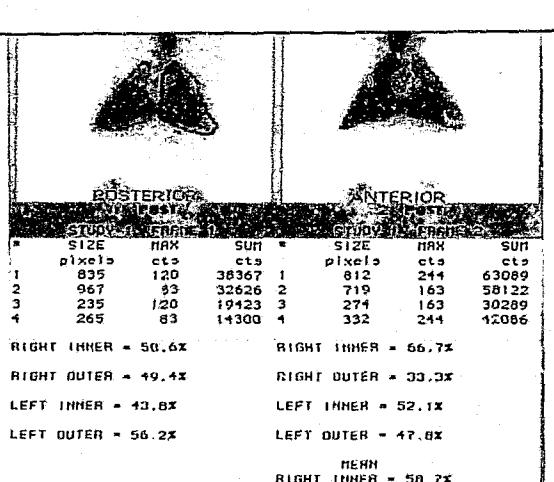


Figure 4.5 **Distribution of radioactivity over right and left lungs.**



Distribution of radioactivity

Figure 4.6 **(inner vs. outer) in both lungs.**

4.2.4 Conclusion

This ventilation scan of radio-labelled DTPA dissolved in glycine buffer confirmed widespread deposition of aerosol within both lungs of a piglet which received aerosol using the delivery system which would subsequently be used for human subjects. These findings supported the test of nebuliser function described in 4.1 above. Respirable aerosol particles were produced by the nebuliser and these were widely and distally deposited within the lungs. There was some rain-out of particles in the trachea and large airways as shown by the high level of radioactivity at this site (figure 4.4)

Limitations of this test included –

- The actual glycine buffer was not radiolabelled by the technique described here and indeed this was not technically feasible. The physiccchemical properties of radiolabelled DTPA dissolved in glycine buffer may be different from radiolabelled glycine. Indeed, it is possible that the act of radiolabelling the diluent nad some effect on the final deposition of the particles within the lung.
- The airways into which the aerosol was delivered in this model were not exactly the same size as would be encountered in an adult human in terms of length and diameter. However, these differences would not be great. Also, use of this model avoided the ethical considerations inherent in administering radio-active substances to humans for experimental purposes.

In view of these limitations this study was not definitive, but strongly supported the findings of the test described in 4.1, where the candidate was able to show that a large proportion of particles produced by the tested nebulisers was within the respirable range. Subsequent studies (Chapters 5 and 7) demonstrated convincingly that active agent was deposited in the lungs, strongly supporting the conclusions of the tests carried out in this chapter.

4.3 Nebuliser efficiency bench test.

A bench test was then undertaken to determine the maximum rate of nebulisation by the MedicAid Sidestream jet nebuliser (details in 4.1.2 above).

4.3.1 Aim

The aim of this test was to determine the maximum rate at which the MedicAid Sidestream jet nebuliser was able to aerosolise normal saline solution and glycine buffer solution in which prostacyclin would be dissolved during administration of IAP therapy.

4.3.2 Method

A bench test was performed as follows. A MedicAid Sidestream jet nebuliser was clamped in an upright position. Twelve L/min of dry air flowed continuously via a t-tube placed across the open top end of the nebuliser to simulate the flow of gas during mechanical ventilation. The nebuliser was driven by 6 L/min of medical grade dry air at 420 kPa. Normal saline solution or glycine buffer solution (described above) were pumped continuously/instilled into the nebulising bowl of the jet nebuliser (as described for the inhaled aerosolised prostacyclin delivery system – figure 3.5). Normal saline solution was pumped at fixed rates of 5, 10, 15 or 20 ml/hour via syringe driver pump (Terufusion, Terumo Corp., Japan) for exactly 60 minutes. At the end of the 60 minute period the residual volume of solution remaining in the nebulising bowl was measured, to the closest 0.2 ml, by aspirating the contents of the bowl into a 5 ml Terumo syringe (Terumo Corp., Japan). Each measurement was repeated a series of 5 times, each time using a different jet nebuliser (of the same brand), but the same syringe driver pump and syringes. The method was repeated for glycine buffer solution, but only at instillation rates of 5, 10 and 15 ml/hr (due to the high residual volume obtained when 20 ml/hr of normal saline solution was instilled).

4.3.3 Results

4.3.4 Table 4.1 shows the mean residual volumes retrieved from the nebuliser bowls for each volume of solution infused into the bowl over each 60 minute test period.

Table 4.1

Mean residual volumes recovered from the nebuliser bowl at various rates of instillation of solution into the nebuliser bowl.

Rate of instillation (ml/hr)	Solution	Mean residual volume (ml) (+/- SD)	
20	N. saline	5	(0.6)
15	N. saline	2.4	(0.4)
10	N. saline	0.8	(0.4)
5	N. saline	0.2	(0.2)
15	Glycine	1.6	(0.4)
10	Glycine	1.0	(0.2)
5	Glycine	0.2	(0.2)

Key –

N. saline - normal saline solution

ml/hr - millilitres per hour

ml - millilitres

SD - standard deviation

Glycine - glycine buffer diluent

4.3.4 Conclusion

The maximum rate that the Sidestream jet nebuliser was able to aerosolise solution under the above test conditions was 15 ml/hr. At this rate there was still an appreciable amount of accumulation of fluid in the nebulising bowl over the test period – theoretically 2.4 ml/hr of normal saline solution or 1.6 ml of glycine buffer solution for each 1 hour period of operation. This accumulation of residual fluid in the nebulising bowl would further reduce nebuliser efficiency with time. These findings must be interpreted with regard for the differences from the system used to deliver aerosolised prostacyclin to humans (figure 3.5) and piglets (figure 3.3), namely –

- in this test of nebuliser function the gas simulating minute ventilation was both dry and flowed continuously, both factors which may influence the rate of nebulisation. This was unlike the situations described in 3.5.2 where the ventilation was cyclical and the inspired gas was humidified,
- in this test the aerosol was released into non-pressurised carrier gas, unlike the situation in 3.5.2 where the ventilator circuit was pressurised both during inspiration (mean airway pressure) and during expiration (positive end-expiratory pressure).

In view of these differences from the setting where aerosolised prostacyclin was delivered to human subjects, it is possible that this test overestimates the efficiency of the Sidestream jet nebuliser. Therefore, with due regard to these findings, the candidate adopted the technique of delivery of aerosolised prostacyclin described in Chapter 3. This method allows for the delivery of up to 50 ng/kg/min to an adult, while requiring the nebulisation of less than 10 ml/hr of solution (in the range where residual volumes in the nebulising bowl as assessed by this test were small).

4.4 Summary

This Chapter describes three tests of nebuliser function undertaken to support the candidate's use of this device for the delivery of aerosols in the research described in this thesis.

The findings were -

- 4.1 - both jet nebuliser brands (MistyNeb and Sidestream) used in the studies described in this thesis produced a large proportion (approximately 50 – 80%) of aerosolised particles in the respirable range (1 – 5 microns). The Sidestream device was the more efficient of the two tested.
- 4.2 - the aerosolised particles were deposited widely within the lung of an animal subject as assessed by ventilation scan.
- 4.3 - the maximum rate of efficient nebulisation by the Sidestream jet nebuliser was less than 15 ml/hr (i.e. a maximum fluid flux of less than 0.2 ml/min).

Chapter 5

5.0 PILOT STUDY COMPARING THE EFFICACY OF THE SELECTIVE PULMONARY VASODILATORS INHALED AEROSOLISED PROSTACYCLIN (IAP) AND NITRIC OXIDE (NO).

5.1 Introduction

Administration of selective pulmonary vasodilators by the inhaled route will theoretically reduce shunt fraction (Q_s/Q_t) coupled with a reduction in pulmonary vasucular resistance (PVR) and possibly a reduction in mean pulmonary arterial pressure (MPAP). If the agent used is a true SPV the above effects are accomplished without systemic hypotension and result in an improvement of ventilation perfusion (V/Q) matching in the lung. The net effect is an improvement in oxygenation and reduction in pulmonary arterial pressure (PAP).

As discussed in Chapter 1 the two agents which have recently gained currency as SPV's are nitric oxide (NO) [80,82,83,100,103,105,165] and inhaled aerosolised prostacyclin (IAP) [47,151,152,166-169]. NO was the first agent available and has been shown unequivocally to improve oxygenation in very low concentrations [105,165] and to reduce PAP at higher doses [80,82,83] in patients with ARDS.

The first use of IAP was described in 1984, when it was administered to five volunteers [77]. Further work with IAP, including a small series in dogs [151] and in humans [152] appeared in 1993. Subsequent reports of IAP usage have been sparse, but include a series of 12 patients with pneumonia [167], isolated case reports [3,169,170], correspondence [166] and some animal research[168]. There have been two animal trials comparing the effects of NO and IAP in the same subjects [151,168]. These trials showed the efficacy of IAP to be comparable to NO. Pappert and co-workers reported a case series of three children with ARDS where IAP was compared to NO [171]. At

commencement of the study described in this chapter, there had been no adult comparative trials of NO versus IAP.

The current study was devised to compare the effects of (previously-proven) clinically useful doses of NO (10 ppm) [103,105] and IAP (50 ng/kg/min) [152,167] in patients with hypoxaemia due to ARDS. This pilot study, if the results were favourable, would justify the further investigation of IAP for hypoxaemia in patients with severe ARDS.

5.2 Materials and Methods

Institutional Ethics and Clinical Drug Trials Committee approval were obtained (see Chapter 3) and informed consent sought prior to enrolling patients in the study.

5.2.1 Inclusion/Exclusion Criteria

Patients considered for study enrolment were required to be mechanically ventilated in the ICU with an accompanying diagnosis of ARDS as defined by the American-European Consensus Conference on ARDS [3], with a LIS (table 3.1) [27] of greater than 2. Patients with cardiogenic pulmonary oedema (PCWP $\geq$ 18 mmHg) or pneumonia (bacteria on bronchoalveolar lavage (BAL) performed the day prior to enrolment) were excluded. Patients within the first 24 hours of admission to ICU or who were haemodynamically unstable, requiring frequent changes in inotrope and vasopressor requirements, were not deemed suitable for the trial. No intravenous volume replacement therapy, changes in inotrope/vasopressor infusion rates, changes in V_m or FiO_2 were permitted during the two-hour study period. If changes in any of these parameters were required the patient was excluded from the trial.

5.2.2 Study design

Patients meeting the above criteria were studied for a two-hour period, to minimise other temporal variables. Each patient was exposed to 30 minutes of either NO (10 ppm) or IAP (50 ng/kg/min) in random order. Each treatment period was followed by a 60 minute "rest" period to allow for the elimination of the study agent. The offset time of IAP, the longer acting of the two SPV's is only 10 minutes [151]. The patient were then exposed to the other study agent for 30 minutes (i.e. within-patient controlled crossover design). Patients were maintained in the supine position throughout the study period.

NO, 10 ppm, was delivered via the mechanical ventilator using the technique described in Chapter 3 (figure 3.4). IAP (50 ng/kg/min) was administered using the set-up depicted in figure 3.5. The inspired gas in both sections of the trial was warmed and humidified, as described in Chapter 3. The nebuliser bowl was primed with 2 ml of the epoprostenol solution prior to commencing the constant infusion that would ultimately deliver a dose of 50 ng/kg/min to the nebuliser bowl for aerosolisation into the inspired gas. It was not possible to measure the actual mass of IAP deposited in alveoli or the fraction vented in the expired gases. Se Chapter 4 for a discussion on nebuliser performance.

5.2.3 Measurements

On entry to the trial all patients were monitored as described in detail in Chapter 3. Blood samples were drawn from the arterial and PA catheters, as appropriate, immediately prior to and at the end of each 30 minute exposure to the SPV (either NO 10 ppm or IAP 50 ng/kg/min) and immediately analysed for -

- ABG and mixed venous blood gases and
- arterial metHb levels, utilising the co-oximeter of the blood gas analyser.

Arterial and pulmonary artery pressures and CO were measured at the same intervals – i.e.) immediately prior to exposure to the SPV and at termination of exposure to the SPV (after 30 minutes). A-aDO_2 and Q_s/Q_t were calculated using standard equations (table 3.2).

5.2.4 Statistical analysis

The paired data from the five patients entered into this pilot study were analysed using Wilcoxon matched-pairs signed ranks tests. A p value of < 0.05 was considered significant.

5.3 Results

Patient demographic data are shown in table 5.1. Raw data for this pilot study are listed in Appendix A and depicted in figures 5.1 – 5.3. It is evident from the raw data that the contribution by individual subjects to the significance of the overall results presented below varies and should be considered in the context of a small pilot study.

MAP, CO, HR, CVP and PCWP did not change significantly with exposure to either of the SPV's (see table 5.2). MetHb levels were obtained for four of the five patients. There was no significant increase in metHb levels after exposure to either SPV (table 5.2).

MPAP reduced after exposure to IAP in four of the five patients, while exposure to NO resulted in a decrease in MPAP in three patients and an increase in two patients (figure 5.1). None of these changes reached statistical significance (table 5.2), although the effect of IAP on MPAP approached statistical significance ($p = 0.07$). Q_s/Q_t (% shunt) reduced in response to IAP in all patients ($p = 0.04$) (table 5.2 and fig. 5.2) and to NO in three patients ($p = 0.42$) (table 5.2 and fig. 5.2). A rise in Q_s/Q_t occurred in two patients who received NO (figure 5.2). The pre-exposure values (i.e. pre NO versus pre IAP) and the post-exposure values (i.e. post NO versus post IAP) for all the variables listed in table 5.2 were not significantly different.

Table 5.1

Patient demographic data.

Patient no.	Age	Gender	APACHE II	LIS	Primary Diagnosis
1	22	male	14	3	Pulmonary contusion
2	46	female	19	2.25	End-stage liver disease
3	22	male	9	2.75	Endocarditis
4	29	female	26	3.5	Alcoholic liver disease
5	70	female	26	2.75	Pulmonary contusion

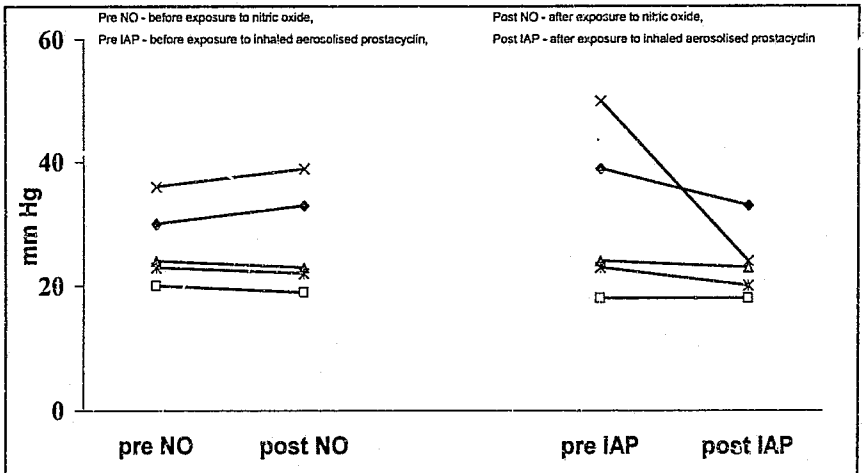
Key –

Age - patient age in years

APACHE II - APACHE II score [153] on day of study enrolment

LIS - Murray lung injury score [27]

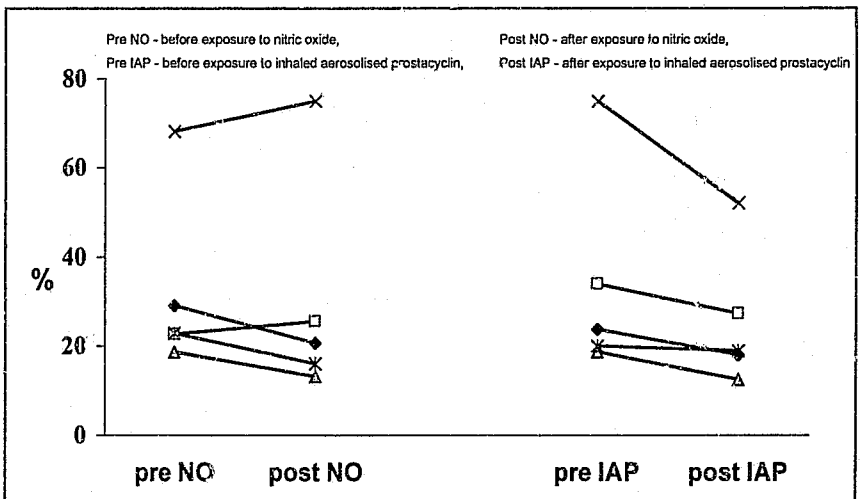
Primary diagnosis - reason for Intensive Care admission



Mean Pulmonary Arterial Pressure

(individual subject values)

Figure 5.1



Shunt fraction (Q_s/Q_t) data

(individual subject values)

Figure 5.2

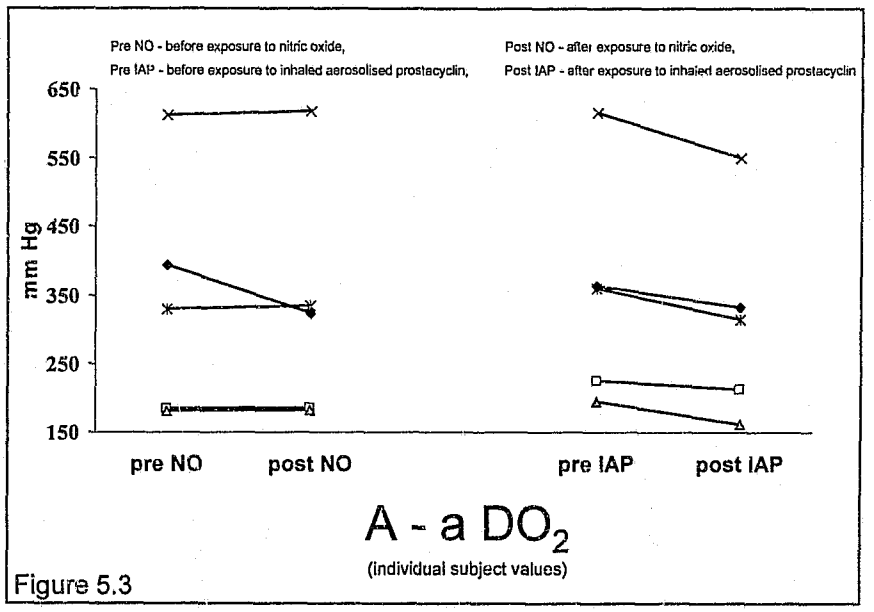

Pre NO - before exposure to nitric oxide,
Pre IAP - before exposure to inhaled aerosolised prostacyclin,
Post NO - after exposure to nitric oxide,
Post IAP - after exposure to inhaled aerosolised prostacyclin
650
550
450
350
250
150
mm Hg
pre NO
post NO
pre IAP
post IAP
A - a DO2
(individual subject values)

Figure 5.3

Table 5.2

P values for comparison of measurements between NO and IAP groups (Wilcoxon Matched-Pairs Signed Ranks Tests).

Variable	Pre NO/Post NO	Pre IAP/Post IAP	Pre NO/Pre IAP	Post NO/Post IAP
PaO_2/FiO_2	0.22	**0.04**	0.22	0.89
Q_s/Q_t	0.42	**0.04**	0.69	0.69
A-aDO_2	0.50	**0.04**	0.35	0.50
$PaCO_2$	0.08	0.69	0.50	0.89
MPAP	0.68	**0.07**	0.29	0.11
metHb	0.36	0.10	0.14	0.66
HR	0.14	0.10	0.10	0.72
MAP	1.0	0.85	0.08	0.08
CO	0.50	0.36	1.0	0.28
PCWP	0.69	0.26	0.46	0.34
CVP	0.07	0.71	0.18	0.28

Key –

PaO_2/FiO_2	-ratio of partial pressure of arterial oxygenation to inspired oxygen concentration
Q_s/Q_t	-shunt fraction
A-aDO_2	-alveolar-arterial oxygen partial pressure gradient
PaCO2	-arterial partial pressure of carbon dioxide
MPAP	-mean pulmonary artery pressure
metHb	-methaemoglobin concentration
HR	-heart rate
MAP	-mean arterial pressure
CO	-cardiac output
PCWP	-pulmonary capillary wedge pressure
CVP	-central venous pressure
pre NO/post NO	-pre exposure to NO versus post exposure to NO
pre IAP/post IAP	-pre exposure to IAP versus post exposure to IAP
pre NO/pre IAP	-pre exposure to NO versus pre exposure to IAP
post NO/post IAP	-post exposure to NO versus post exposure to IAP

A-aDO_2 reduced in all patients exposed to IAP ($p = 0.04$) (table 5.2) and in three patients exposed to NO ($p = 0.50$) (table 5.2). See also figure 5.3 and table 5.2. The increase in PaO_2/FiO_2 post IAP therapy was statistically significant ($p = 0.04$) (table 5.2).

5.4 Discussion

This study design controlled for all extraneous variables. Only the SPV to which the subject was exposed was varied. The time period for exposure was short, thereby further limiting extraneous effect to due temporal changes in disease process, administration of interfering medications and fluids or medical and nursing procedures. Any changes observed could then reasonably be attributed to the SPV administered, making this pilot study a powerful model for comparison of the effects of NO versus IAP, despite the small sample size. Agents were applied in random order to the five patients. However, the sample size is too small to determine whether there was a bias evident due to the order in which the agents had been delivered. Both agents (NO and IAP) have very short half-lives and the 60 minute rest period between agents should have been sufficient to prevent "hangover effect" of one agent from interfering with the effect of the subsequent agent.

In the five patients described above there were no systemic haemodynamic consequences of exposure to either NO or IAP. There was a statistically-significant reduction in MPAP following exposure to IAP only ($p = 0.07$) (table 5.2), as would be expected after exposure to a pulmonary vasodilator. This is in keeping with previous studies where the lack of systemic effect after exposure to NO [80,82,83,100,103,105,165] and IAP [47,151,152,167] has been described. The lack of effect of NO on MPAP in this study is not readily explicable. Thus apparent lack of effect of NO has also been observed in other studies, where a 30% non-response rate to NO has been described [118,172].

The lack of systemic effect of both NO and IAP is due to the short half-lives of the agents used - seconds in the case of NO and minutes in the case of IAP. IAP would be more likely to produce systemic hypotension due to its longer half-life if delivered in high doses. In addition, there is the

theoretical risk that if sufficiently high doses of IAP are administered, prostacyclin metabolites may reach the venous circulation and result in an increase in right to left shunting through the pulmonary circulation and a worsening of oxygenation (i.e. act as an intravenously-delivered vasodilator downstream). This effect was not observed in this series, nor in a patient to whom the candidate had previously administered doses of IAP as high as 200 ng/kg/min [170].

The reduction in $A\text{-}aDO_2$ and Q_s/Q_t and an increase in PaO_2/FiO_2 in most patients exposed to these two SPV's confirmed an improvement in oxygenation. There was worsening of these parameters in two patients in the NO group. A larger study would possibly determine the significance of this finding (i.e. chance finding, measurement error, due to underlying pathology or a consistent finding). Again, there is a well-recognised non-response rate of patients with ARDS to NO. of approximately 30% of exposed patients [118,172]. This non-response rate is not dose-dependent. NO has been shown to be effective in the parts per billion concentration range, so that the candidate is unable to explain the non-response of some patients in the NO group on the basis of inadequate dose. The NO delivery system used was reliable (i.e. adequate delivered concentrations were measured in the inhaled gas) and again is unlikely to the cause of non-response in some patients in the NO group. What is of interest was that even the non-responders in the NO group benefited from exposure to IAP. This gave further impetus to the candidate for continued investigation of IAP therapy.

This study was too small to determine a differential effect or response to treatment on the basis of the underlying pathology (eg primary versus secondary ARDS). The underlying pathology could also not be correlated with non-response, as some NO non-responders then went on to respond to IAP. It should also be noted that it is unlikely that this effect (i.e. some NO non-responders going on to respond to IAP) is unlikely to be due to perceived increase in minute ventilation due to nebuliser use, as the delivered V_m remained constant throughout the study period.

Short-term exposure to the two agents used in this study did not result in a marked increase in metHb levels in the four patients from whom measurements were obtained. Low levels of metHb during NO and IAP administration have subsequently been confirmed [173].

The effects reported in this study occurred at doses of NO and IAP which had previously been shown to be clinically useful, but which at the time this study was conducted had not been compared to each other in adult subjects with ARDS. A subsequent study by Zwissler and colleagues in 1996 [174] in adult subjects with ARDS confirmed their earlier favourable comparison of IAP versus NO in dogs [175]. Their comparison in human subjects was between IAP (up to 25 ng/kg/min) and NO (up to 8 ppm). A further comparative study published in the same year as the candidate's paper on these findings (1996) [176] was by Walmrath and co-workers[173]. They compared NO and IAP in 16 patients with ARDS using individually-titrated doses of each agent. A mean dose of NO of 17.8 ppm was compared with a mean IAP dose of 7.5 ng/kg/min. These authors again confirmed a comparable response to the two agents, with a more marked improvement in indices of oxygenation than in MPAP [173]. They further noted a 25% non-response rate to both agents, a finding previously reported for NO [177]. These studies [173,175] and the work of the candidate described in this Chapter and published in 1996 [176] all confirm the efficacy of IAP as a SPV, compared to NO in the adult human.

The advantage of IAP in terms of ease of administration, together with the promising results in patients with ARDS in this as well as previous series [151,152,167,174] encouraged the candidate to further investigate IAP as a SPV of some potential. Comparisons of IAP and NO have also been described for use in the assessment of animals and patients with pulmonary hypertension [142,178-180] and to assess the effect on right ventricular performance [168]. A study in paediatric patients (n = 3) with ARDS has compared IAP favourably with NO [171].

IAP in patients with ARDS appears to be at least as effective as NO in the doses selected and described in this study. Indeed the results of the candidate's pilot study show IAP to produce more consistent improvements in indices of oxygenation than NO. However, the optimal dose of IAP required to improve oxygenation is not known (and is the subject of study in Chapter 7). A single dose-response curve reported by the candidate in a patient with severe ARDS suggested a dose of 30 - 40 ng/kg/min was as effective as a dose of 50 ng/kg/min [170]. It is therefore possible that an equivalent effect to low dose NO (10 ppm or less) may be obtained at somewhat lower doses of IAP. Indeed, the comparative study by Walmrath [173] showed a mean IAP dose of 7.5 ng/kg/min to be effective. However, the delivery method used in that study was not identical to that used by the candidate. The actual mass of epoprostenol deposited in ventilated alveoli (i.e. the effective dose) as a fraction of the mass aerosolised into the ventilator circuit, would be less than the delivered doses stated above. The mass deposited would also depend on the aerosol delivery method. Previous authors estimate that as little as 10 per cent of nebulised drug may be deposited in the alveoli of ventilated patients [156,158].

The cost of IAP therapy is somewhat daunting (approximately R200 - 400/hour) in the adult patient. This will be a factor in the long-term place of this agent in the therapy of conditions, such as ARDS, where prolonged treatment with a SPV may be required. Despite this, IAP appears to be an effective SPV that is simple to administer and is worthy of further investigation.

5.5 Summary

NO 10 ppm and IAP 50 ng/kg/min were compared as SPV's in five patients with hypoxaemia secondary to ARDS. IAP improved oxygenation more consistently than NO, as measured by PaO_2/FiO_2, shunt fraction and $A\text{-}aDO_2$. Neither agent had a significant effect on MPAP. Neither SPV resulted in systemic haemodynamic changes, indicating true pulmonary selectivity.

Chapter 6

6.0 THE PULMONARY TOXICITY OF INHALED AEROSOLISED PROSTACYCLIN THERAPY.

6.1 Introduction

Prostacyclin (PGI_2) is an endogenous, potent vasodilator [135]. Its other major effect is platelet inhibition, mediated by an increase in cAMP[77,133,135,181]. Synthetic PGI_2 (epoprostenol) has also been used therapeutically as an intravenous vasodilator and anticoagulant [77,123]. More recently, PGI_2 has emerged as a selective pulmonary vasodilator (SPV) for conditions where hypoxaemia and pulmonary hypertension are part of the pathophysiological spectrum, such as in ARDS[151,169,182].

When delivered to patients as inhaled aerosolised prostacyclin (IAP), PGI_2 meets the criteria for pulmonary selectivity. The active drug is delivered to ventilated lung units, improving V/Q matching and thereby oxygenation [170]. Also, because of the short duration of action of the drug and the small doses delivered to the pulmonary vasculature, there is no systemic hypotension associated with the administration of IAP [183]. These features make IAP therapy a viable alternative to nitric oxide (NO) as a SPV, with the advantage that it is technically much simpler to administer than NO [176] (see Chapters 3, 5 and 7). Nevertheless, the potential pulmonary toxicity of IAP has not been fully investigated. Several studies have defined the pulmonary and systemic haemodynamic consequences of IAP administration[151,152,171,173-175,179,182,184-186]. However, no studies had addressed the possible pulmonary toxicity of IAP prior to commencement of the study conducted by the candidate and described in this Chapter. The main concern with IAP therapy is

the high alkalinity (pH +/-10.5) of the glycine buffer in which PGI_2 is dissolved prior to administration. The highly alkaline diluent, and consequently the prostacyclin solution, are potentially irritant to the lung.

The current study was designed to investigate the presence of pulmonary inflammatory changes (as defined by pulmonary neutrophil infiltration) in anaesthetised piglets exposed to high dose IAP administration for up to 8 hours.

6.2 Materials and Methods.

See Chapter 3 for further details of the Materials and Methods used for this study.

6.2.1 Animals

Animal Ethics Committee approval was obtained prior to commencement of the study. The study subjects were six large white/landrace cross piglets with a mass of up to 25 kg. The piglets were anaesthetised by inhalational induction with halothane 3 - 5% in oxygen. Intubation of the trachea was performed without recourse to muscle relaxants (with a 6 mm cuffed endotracheal tube). Anaesthesia was maintained with halothane (0.5 - 1%) in oxygen-enriched air (FiO_2 0.4 - 0.5), using the system shown in figure 3.3, and intravenous pentobarbitone infused, at 8 mg/kg/hr.

The piglets breathed spontaneously. Monitoring consisted of -

- continuous IABP via a femoral arterial line,
- continuous pulse oximetry (SpO_2) and
- hourly rectal temperature recordings.

Normal saline solution (0.9%) was infused to maintain hydration via a femoral venous catheter at 2 ml/kg/hr, using a volumetric infusion pump. The piglets were turned from side to side every two hours during the study period to minimise gravitational effects, such as pulmonary atelectasis.

6.2.2 Aerosol therapy

Aerosol therapy was delivered, as described in Chapter 3, using a Sidestream jet nebuliser. The continuous delivery of aerosol into the breathing circuit is depicted in figure 3.3.

The piglets were randomly assigned to one of three treatment groups –

Control (C) animals - Pig 0 (14 kg) and pig 1 (22 kg). Pig I received aerosolised normal saline solution (0.9%) at 15 ml/hr, the maximum rate at which the Sidestream nebuliser is able to produce aerosol (see Chapter 4). The aerosol therapy lasted for 8 hours. As there were no inflammatory changes seen in the lungs of Pig 1 due to aerosolised normal saline solution, Pig 0 received no aerosol therapy at all and was immediately euthanased.

Diluent (D) treated animals - Pig 2 (21 kg) and Pig 4 (13.5 kg) received aerosol therapy (Pig 2 for 7 hours and Pig 4 for 8 hours) with only the alkaline glycine diluent. The rate of aerosolisation was 15ml/hr.

Prostacyclin (P) group - Pig 5 (14 kg) and pig 6 (11 Kg) received IAP (10 μg/ml) at 15 ml/hr (pig 4 for 5 hours and pig 5 for 7 hours).

Note: Pig 3 died from Malignant Hyperthermia after 30 minutes and was not subjected to study – hence the numbering system i.e. pigs 0, 1, 2, 4, 5 and 6).

6.2.3 Pathology

At the end of the aerosol therapy all pigs were euthanased with a lethal intravenous dose of pentobarbitone. The trachea and both lungs were removed from all 6 pigs by median sternotomy and sent to the Pathology Laboratory *en-bloc*. Sputum samples were collected via tracheal

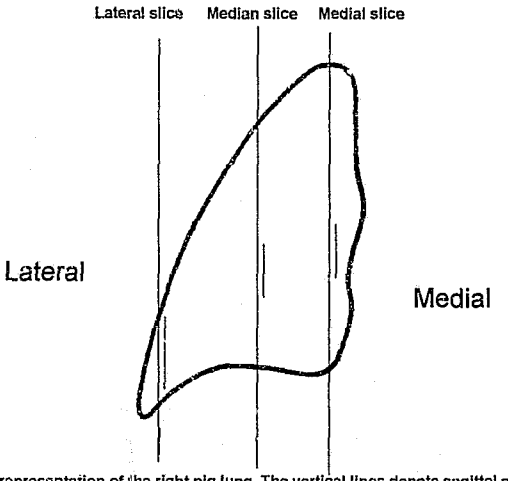
suction for bacteriological assessment of possible infection prior to removal of the lungs. The tissue samples from each pig were coded according to treatment and were sent for pathology without disclosure of treatments (i.e. pathologists were blinded as to treatment group).

Portions of tissue were taken for light microscopic and ultrastructural examination by using sharp-edged razor blades to maintain the integrity of the tissue as follows.

- One cross-sectional slice of trachea from each animal was taken one centimetre above the carina, to avoid areas of possible mechanical trauma caused by endotracheal intubation.
- One cross-section of each of the right and left main stem bronchus were also taken.
- Sections of **anterior** and **posterior** aspects of parenchyma from medial and median sagittal slices of the right and left lung, and one section from a lateral slice of each lung were taken as shown schematically in figure 6.1. A total of 5 areas of parenchyma were therefore biopsied from each of the right and left lung of each pig.

6.2.3.1 Light microscopy

Biopsies for light microscopy were immediately immersed in 10% buffered formal saline and fixed for at least 24 hours. The tissue was processed through increasing grades of ethanol and embedded in paraffin wax. Six micron sections were stained with Harris haematoxylin and 1% alcoholic eosin and examined by light microscopy.



Note: Diagramatic representation of the right pig lung. The vertical lines denote sagittal slices of lung from which sections of parenchyma (double lines) were removed for analysis. Anterior and posterior specimens were taken from medial and median slices. The procedure was repeated for the left lung.

Figure 6.1 **Site of biopsies taken from the pig lung (anterior view)**

Each section was graded according to the degree of acute inflammatory response (Histological Grade) reflected by the numbers of polymorphonuclear leucocytes (PMN's) recorded in each field of view.

Histological grade –

-	**Grade 0**	**(absent acute inflammation)**	**< 5 PMN's**
-	**Grade 1**	**(mild)**	**5 to 10 PMN's**
-	**Grade 2**	**(moderate)**	**11-25 PMN's**
-	**Grade 3**	**(severe acute inflammation)**	**> 25 PMN's**

Five individual, randomly-selected fields (400x magnification) from each **tracheal section** and each right and left **main stem bronchus** section, from each pig, were analysed. Grading of each field of view was performed according to the numbers of PMN's counted within the lining epithelium (excluding the lumen), the lamina propria, and the submucosa (of each trachea and main stem bronchus).

With respect to analysis of the **bronchioles** (the distal airways), PMN's within the epithelium, lamina propria and submucosa of 20 randomly selected bronchiolar cross sections were examined in each of the five sections of lung parenchyma (400x magnification). A total of 200 individual bronchioles were examined for each pig (5 fields, in each of 4 quadrants, in each of 5 sections from each lung).

Grading of the numbers of PMN's found within the **interstitium** (including alveoli and capillaries but excluding bronchi and bronchioles) of each pig lung was performed by bisecting two (400x magnification) fields, in 5 regions of each of the 5 lung specimens obtained, using a marker. The numbers of PMN's in contact with the upper edge of the opaque marker bar were counted. Thus a total of 100 interstitial measurements were taken for each pig (2 fields, in each of 5 regions, in each of 5 specimens, from each lung).

The histological grades (0 – 3 as defined above) for each subject by site, layer, field and quadrant are tabulated in table 6.1 (summary) and in Appendix B.

6.2.3.2 Electron microscopy

Tissue slices up to a maximum of 3 mm in thickness were immediately immersed in a freshly prepared fixative solution comprising 4% paraformaldehyde and 5% glutaraldehyde in 0.2M cacodylate buffer, pH 7.4 for 24 hours. The samples were post-fixed in 1% osmium tetroxide, dehydrated in increasing grades of ethanol and embedded in araldite. Semi-thin sections (0.5 - 1 μm) of each sample were stained with 0.2% toluidine blue in 5% borax and viewed by light microscopy. Representative ultrathin sections (50-90 nm) of the samples were then cut, mounted on 200 mesh copper grids, contrast enhanced with lead citrate and uranyl acetate solutions and viewed with a Phillips 410 (Phillips, Holland) transmission electron microscope.

Following the PMN count as described in 6.2.3.1 above, the light microscope slides and electron micrographs were reviewed by an independent pathologist for signs of PMN infiltration.

6.3 Statistical analysis

All analyses were done separately for each site, namely: trachea, right main stem bronchus, left main stem bronchus, bronchioles and alveoli. The statistical analysis of the raw data (tabulated in Appendix B) was carried out using the Epidemiological Graphics Estimation and Testing (EGRET) package, Analysis module (version 0.26.6) (SERC and CYTEL) statistical program. Two statistical models were used to determine differences between the three groups C, D and P (as described in 6.1.2 above) with regard to "treatment effect" (i.e. presence of PMN infiltration associated with the treatment received)

Table 6.1

Summary of histological grades by site (all layers at each site) for each treatment group.

Site	Treatment group	Histological grade
		mean value (range 0-3)
Trachea	Control	0.10
	Diluent	0.8
	Prostacyclin	1.25
Main stem bronchi	Control	0.02
	Diluent	0.19
	Prostacyclin	0.24
Bronchioles	Control	0.07
	Diluent	0.09
	Prostacyclin	0.04
Alveoli	Control	0.00
	Diluent	0.09
	Prostacyclin	0.01

The first statistical model used was **logistic regression.** For this analysis the PMN count (histological grade) was defined either as zero or greater than zero. Logistic regression was then used to find any factors associated with a histological grade greater than zero. This method does not take into account correlation that exists between measurements taken on the same pig (p-values may therefore be artificially low i.e. exaggerate the significance). However, using this model, if factors were found not to be significant, they were then dropped from further analysis by the generalised estimating equation (GEE) described below. The summary findings using this method are tabulated in table 6.2.

The second model used for analysis was the ***generalised estimating equation*** (GEE). *This is a* refinement of the logistic regression model, taking into account correlation between measurements from the same pig. The GEE model with a compound symmetry variance structure was fitted for each site, using only factors found to be significant in the logistic regression model. Summary statistics using this model are tabulated in table 6.3. Both statistical methods determine the correlation between *presence of PMN's* (more than 5/high power field) and treatment effect and do not further take into account the histological grade (other than to separate it into grade 0 *and anything other than grade 0*).

The overall histological appearance is discussed below – i.e. the pathologists impression upon examination of light and electron microscopy. Some idea of the degree of PMN infiltration is also conveyed by the mean histological grades discussed below and tabulated in table 6.1.

6.4 Results

The animals remained physiologically stable throughout the study period - MAP, HR, temperature and arterial oxygen saturation (SpO_2) remained within normal limits throughout the study period in all subjects (see Appendix C).

Table 6.2

Analysis of the pig data for association of treatment with evidence of PMN infiltration – using logistic regression.

Site	Treatment	Odds ratio	p-value
Trachea	Control	1	
	Diluent	8.5	**0.009**
	Prostacyclin	22.6	**< 0.001**
RMS bronchus	Control	1	
	Diluent	2.9	0.24
	Prostacyclin	3.6	0.14
LMS bronchus	Control	(excluded, as all values were zero)	
	Diluent	1	
	Prostacyclin	2.1	0.14
Bronchioles	Control	1	
	Diluent	4.5	**<0.001**
	Prostacyclin	0.6	**0.032**
Alveoli	Control	1	
	Diluent	1.2	0.7
	Prostacyclin	0.2	**0.012**

Key –

LMS - left main stem

RMS - right main stem

PMN - polymorphonuclear neutrophil

Table 6.3

Analysis of the pig data for association of treatment with evidence of PMN infiltration – using the general estimating equation analysis.

Site	Treatment	Odds Ratio	p-value
Trachea	Control	1	
	Diluent	8.1	0.054
	Prostacyclin	21.0	**0.006**
RMS bronchus	Control	1	
	Diluent	2.7	0.26
	Prostacyclin	3.3	0.19
LMS Bronchus	Control	(excluded, as all values were zero)	
	Diluent	1	
	Prostacyclin	2.0	0.12
Bronchioles	Control	1	
	Diluent	58.3	**<0.001**
	Prostacyclin	1.0	1
Alveoli	Control	1	
	Diluent	1.2	0.7
	Prostacyclin	0.2	**0.03**

Key –

LMS - left main stem

RMS - right main stem

PMN - polymorphonuclear neutrophil

Pathology

The histological grade of PMN's (0 – 3) seen in each field of each quadrant in each segment/specimen (by layer) are tabulated in Appendix B. The summary histological grades by site are tabulated in table 6.1. Pig 0 was found to have a chronic (and therefore pre-exisiting) tracheitis, with associated lymphocytic infiltration. As these changes were chronic, the tracheal findings for pig 0 were excluded from statistical analysis.

Independent review of the pathology slides, by pathologists blinded to the treatment group, confirmed mild acute suppurative tracheitis in all the animals in the D and P groups. The main bronchi and bronchioles were minimally involved in the same animals. Changes were confined to the superficial layers (epithelium and lamina propria) of the trachea. These findings were not associated with positive sputum cultures, strengthening the view that the changes were probably due to chemical/sterile inflammation. The control animals (C group) showed minimal evidence of PMN presence and no diagnoses of inflammation were made in these animals on histological review. As can be seen in table 6.1 the histological grade at each site is very low, even in the animals with a diagnosis of acute tracheitis.

Independent blinded review of the electron micrographs revealed –

- Pig 5 (group P) showed evidence of PMN infiltration of the epithelium and lamina propria of the trachea, as well as fluid exudation in these areas. There was some evidence of PMN's in the submucosa, with PMN's readily seen in the small vessels (figure 6.2).
- Pig 6 (group P) showed occasional PMN's in the tracheal epithelium (figure 6.3), with accumulations of PMN's in the lamina propria and the submucosa.

There was no other evidence on electron microscopy of acute inflammatory changes in any of the other subjects or sites. These findings correlated to some extent with the statistical findings below which showed a significant "treatment effect" confined mostly to the trachea for the group P and D pigs.

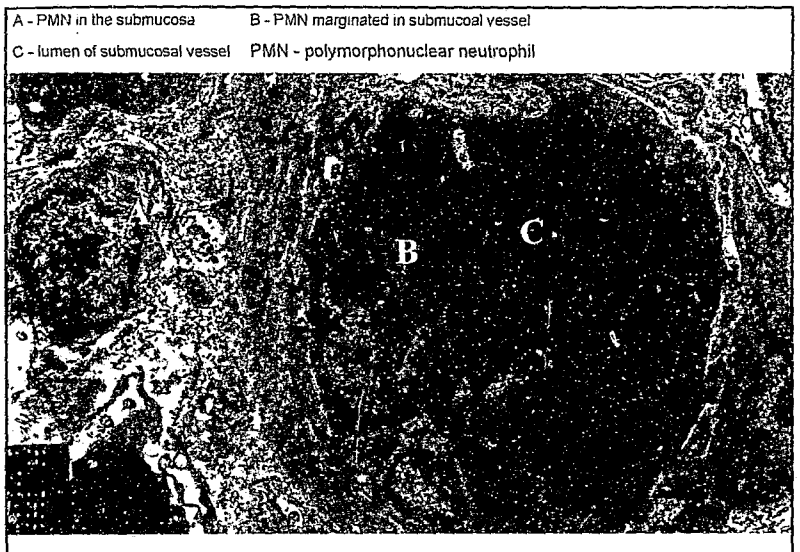


Figure 6.2 **Electron micrograph from trachea of Pig 5 (group P)**

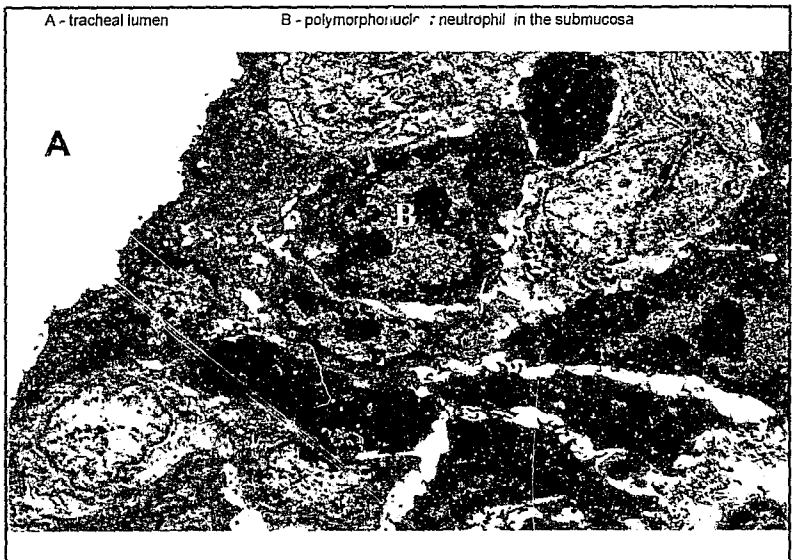


Figure 6.3 **Electron micrograph from trachea of Pig 6 (group P)**

Statistical analyses

The summary statistics for the logistic regression analysis are shown in table 6.2, which indicated a "treatment effect" (i.e. associated PMN infiltration as assessed by presence of PMN's of more than 5/high power field) for the trachea in both the P (odds ratio 22.6, p value < 0.001) and D (odds ratio 8.5, p value 0.009) groups. There was also a significant effect in the bronchioles of the D group (odds ratio 4.5, p value < 0.001).

Table 6.3 shows the results of the GEE analysis. This more stringent analysis again showed a "treatment effect" for the P group) in the trachea (odds ratio 21.0, p value 0.006). The values for the D group approached significance (odds ratio 8.1, p value 0.054). The "treatment effect" was again seen in the D group in the bronchioles (odds ratio 58.3, p value < 0.001).

6.5 Discussion

This study showed that the alkaline buffer used to dilute prostacyclin, and in which prostacyclin was delivered during IAP therapy, was associated with mild acute inflammation of the trachea as defined by PMN infiltration (≥5/HPF). This statement is based on the histological findings described above which showed acute suppurative tracheitis only in the animals that received either diluent aerosol or IAP (D and P groups), not in the control animals (C group). The tracheitis was sterile (no growth on sputum culture in any subject), supporting the diagnosis of chemical inflammation of the trachea. Also, the changes were predominantly in the epithelium and lamina propria layers, indicating a response to an irritant on the luminal surface of the trachea. The ventilation scan described in Chapter 4 (figure 4.4) confirmed a high concentration of tracer over the trachea when a similar aerosol delivery system was used. This effect is due to rain-out of large aerosol particles in the large airways (discussed in more detail in Chapter 4). This may explain the predominance of the inflammatory changes at this site.

The histological findings of acute tracheitis (in the D and P group animals) were supported by ultrastructural changes (figures 6.2 and 6.3). As well, there was statistical evidence for a strong association between the presence of PMN's (more than 5/high power field) and the D and P treatment groups with respect to the trachea (tables 6.2 and 6.3). The statistical evidence was less convincing for other sites. In the case of the bronchioles, the D group was strongly associated with the presence of PMN's while the P group (which was also delivered in glycine diluent) showed no effect (table 6.3).

These findings must to be interpreted in the light of the only two other studies that have examined the potential pulmonary toxicity of IAP therapy[187,188]. In these studies small numbers of lambs were exposed to IAP therapy for up to 8 hours, before the lungs were examined for evidence of inflammation. Coincidentally, the period of 8 hours of exposure to treatment was also chosen by the candidate, for several reasons –

- this is the maximum period during which the candidate was able to maintain anaesthetised piglets in a stable condition and
- this was deemed to be an adequate period to allow for the development of early acute inflammatory changes (following discussion with Pathologists).

In the clinical setting, however, patients may be exposed to IAP therapy for longer periods – up to days at a time. Hence, the candidate's request for caution in the use of IAP in the clinical setting, stated at the end of this chapter.

In the first comparable study [187] (which appeared following commencement of the study described in this Chapter), the animals' lungs were lavaged and examined for inflammatory cells and mediators following exposure to IAP therapy. No evidence of inflammation was found. In the second study (which appeared subsequent to completion of the study described in this Chapter), by the same investigators [188], the lungs were removed and examined by light and electron

microscopy for evidence of inflammation. Again, no inflammation was found. Both studies employed doses/volumes of IAP appropriate for the clinical treatment of humans (28 ng/kg/min[188]).

The current study demonstrated the presence of only mild superficial acute tracheitis in pigs exposed to short-term glycine diluent aerosol or IAP. The aerosols in this study were administered at approximately **nine times** the maximum volume or dose of diluent that would be given to an adult human (on a dose/mass basis) during IAP therapy (see Chapters 3, 5 and 7 for details of dosing of aerosol for adults receiving IAP therapy). An adult receiving a prostacyclin dose of 50 ng/kg/min (the maximum dose administered to any human subject during the conduct of studies for this thesis) during IAP therapy would require only approximately 5 ml/hr of prostacyclin solution (50 µg/ml concentration). Therefore, a subject would also receive only 5 ml/hr of alkaline diluent aerosol during IAP therapy. Doses or volumes of aerosol therapy used in the current study were intentionally high to maximise a treatment effect if it existed, but do not bear direct relationship to the clinical setting.

The statistical evidence in this study was somewhat conflicting. This was due to the large number of zero values in the analysis, as seen in the raw data (Appendix B). The large number of zeros (indicating Grade 0 inflammation as defined above) was testimony itself to the low level of inflammatory changes actually witnessed in these specimens. The sample size ($n = 6$) in this study was small, although in keeping with the other animal studies ($n = 14$) [188,189]. Larger samples may have yielded more definitive results. This was beyond the resources of the candidate to perform.

The significance and natural history of the PMN infiltration described in this study are not known. There may also be significant inter-species variation in the degree of inflammation mounted in

response to exposure to the alkaline diluent (see studies [187] and [188]). In all the reported literature to date on IAP therapy, there are no reports of inflammation of the airways in the human subject attributable to IAP therapy. Although the period of exposure to aerosol in the study described in this chapter is similar to the other available animal studies[186,189], it is conceivable that this period of exposure is too short. It may require a longer period of exposure to elicit a strong inflammatory response, if this response were indeed to develop. This time factor may then be responsible, in part, for the lack of inflammation (as opposed to minor PMN infiltration) observed in the groups exposed to diluent or IAP. This is reflected in the large number of zero values in the raw data (Appendix B). As the natural history of the PMN infiltration is not known at present, this must remain an area for further investigation.

IAP therapy is only used in ARDS patients as a rescue therapy, when oxygenation is not possible by conventional means, in order to reduce lung toxicity from exposure to high inspired concentrations of oxygen. Administration of IAP in these circumstances is then on the principle of "balance of risks" (risk of toxicity of IAP therapy versus risk of oxygen toxicity). Although the findings in this study suggest caution in the administration of high volumes of aerosolised glycine diluent during IAP therapy, they are somewhat inconclusive. There is contradictory evidence from the two other animal studies [187,188] and the well-documented, widespread safe use of IAP in humans[28,77,123,134,135,152,166,167,169,170,182,183,190-201]. IAP has also been given safely to healthy volunteers [77,123]. Therefore, particularly in light of the low histological grade of the PMN infiltration found in response to alkaline diluent (table 6.1), the candidate proceeded with the conduct of the study described in Chapter 7. Indeed, the presence of neutrophils does not in itself constitute or indicate tissue *damage* or *destruction*. It may be that the diluent is a chemotactic agent for neutrophils. Nonetheless, the smallest practical volumes of glycine diluent would be used for the solution and delivery of IAP in the study described in Chapter 7, where IAP would be administered to critically ill patients with ARDS as rescue therapy.

6.6 Conclusion

Very high doses of alkaline glycine diluent delivered as an aerosol to anaesthetised pigs was associated with mild acute tracheitis. The inflammation/PMN infiltration, was mild, was not associated with tissue damage, and was induced only by very high doses of alkaline glycine diluent. Despite the demonstrated safety of this therapy in other animal studies and in humans, the findings suggest caution in the administration of IAP therapy. The findings of this study however, do not preclude the judicious use of IAP therapy in human subjects for rescue therapy where the balance of therapeutic risks (high concentrations of inspired oxygen versus IAP therapy) favours IAP therapy.

6.7 Summary

Pigs (mass up to 25 kg) were exposed to aerosolised normal saline (or no therapy at all), the control group (C group), aerosolised alkaline glycine diluent (D group) or IAP (P group) for up to 8 hours. Pigs in the D and P groups developed mild acute sterile tracheitis, involving the superficial layers of the trachea shown histologically and ultrastructurally. The D group also showed inflammatory changes in the bronchioles. These findings are somewhat inconsistent with similar animal studies and with clinical experience of the use of IAP in humans. The findings do however, suggest caution in the use of high volumes of aerosolised alkaline glycine diluent during IAP therapy. In light of these findings, in the subsequent study described in Chapter 7, the candidate administered IAP only to critically ill patients with ARDS (IAP as rescue therapy) in the smallest practical volume of glycine diluent (see Chapters 3 and 7 for details).

Chapter 7

7.0 THE DOSE-RESPONSE RELATIONSHIP BETWEEN INHALED AEROSOLISED PROSTACYCLIN AND INDICES OF OXYGENATION.

7.1 Introduction

Hypoxaemia is by definition present in varying degrees in all patients with ARDS [3]. Occasionally the hypoxaemia is severe enough to pose an increased risk of morbidity or mortality, either *per se* or due to oxygen toxicity sustained as a result of therapy. When this is the case, the use of a SPV may offer some therapeutic advantages, by improving the V/Q ratio.

Theoretically, a SPV is delivered in the inspiratory gas only to areas of ventilated lung. The SPV, with a very short duration of action, then exerts it's vasodilator effect on pulmonary vessels within the ventilated lung unit. This in turn improves blood flow to the ventilated regions, of what is a heterogeneous/regional process in ARDS, and improves the V/Q ratio. The selectivity demonstrated by SPV's is therefore twofold, firstly with respect to effect only on the pulmonary and not the systemic circulation and secondly due to it's effect only on vessels within ventilated lung units. NO has been used extensively in this role since the 1980's [81,202]. More recently IAP delivered by jet or ultrasonic nebuliser has also been used as a SPV for hypoxaemia in ARDS[28,134,135,152,164,167,170,183,197,199]. Currently there is sufficient evidence to support the role of IAP as a SPV for hypoxaemia due to ARDS, in addition to it's use for pulmonary hypertension [142,178,185,203]. The findings of the pilot study described in Chapter 5 support the further investigation of IAP as a SPV. However, dose-response relationships for IAP versus indices of oxygenation and systemic and pulmonary pressures have not been clearly defined.

The aims of this study were to determine the presence or absence of dose-response relationships between IAP (over the dose range 0 – 50 ng/kg/min) versus indices of oxygenation (PaO_2 /FiO_2, A-aDO_2) and versus shunt fraction. In addition, the systemic levels of one of the major metabolites of prostacyclin, 6-ketoPGF1$_\alpha$ were measured, to determine the extent of systemic absorption of prostacyclin during IAP therapy. Due to the ephemeral nature of the parent compound it is not possible to readily measure prostacyclin levels. Platelet function studies were carried out to detect any anti-platelet effect of the circulating systemic 6-ketoPGF1$_\alpha$. Cardiovascular parameters (CI, MAP and MPAP) in response to increasing IAP dose would also be noted, to ensure the selective action of IAP within the pulmonary circulation (i.e. document lack of systemic effect).

At the time of commencement of the investigational work for this study there were no similar studies investigating the dose-effect of IAP. At the time of writing of this thesis there is only one other similar study[174]. This study is discussed in 7.5 below.

7.2 Materials and Methods

7.2.1 Patients

Institutional ethics committee approval was obtained prior to enrolling patients in this study. Nine adult patients, six male and three female with a mean age of 62 years (range 33 – 77) and a mean APACHE II score [153] of 16 (range 8 – 20) were enrolled in the study. Inclusion criteria were: witnessed informed consent obtained (from the legal patient surrogate and confirmed verbally with the patient as soon as the patient was able to communicate effectively), age ≥ 18 years, receiving mechanical ventilation in the ICU and ARDS due to any cause. All patients had a LIS of ≥ 2.5 (mean 2.7, range 2.5 – 3), indicating severe ARDS by this scoring system [27]. Exclusion criteria were: a bleeding diathesis (INR ≥ 1.5 or APTT ≥ 45 seconds) or any head injury. Patient diagnoses and demographic details are listed in table 7.1. The subjects were all in-patients in the Department of Intensive Care at the Sir Charles Gairdner Hospital. Patients received mechanical ventilation via endotracheal tube or tracheotomy tube

Table 7.1

Patient demograhic, diagnostic and ventilation details.

No.	Age	Sex	APACHE	LIS	Diagnosis	ICU day	V_m	PEEP	FiO_2
1	51	m	14	3	Legionella pneum.	2	12.4	10	0.8
2	75	m	19	3	AAA repair	13	21	12	0.75
3	56	m	20	2.5	Pancreatitis	3	12	10	0.5
4	77	m	8	2.5	Aspiration pneumonitis	2	22.4	10	0.5
5	76	m	11	2.75	Pancreatitis	13	11.2	2.5	0.6
6	52	f	20	2.75	Pneumococcal pneum.	3	18	5	0.5
7	68	f	20	2.75	Pulmonary contusion	8	21	10	0.5
8	70	m	20	2.5	G.neg. septicaemia	6	9	10	0.55
9	33	f	16	2.5	Adrenal surgery	2	10.3	5	1.0

Key –

No. - patient number

Age - age in years

APACHE - APACHE II score on the day of study enrolment

LIS - Murray Lung Injury Score

ICU day - day of ICU stay on which patient met study entry criteria

V_m - exhaled minute ventilation at time of study enrolment

PEEP - positive end expiratory pressure in centimetres of water

FiO2 - fractional inspired concentration of oxygen

AAA - abdominal aortic aneurysm

G.neg - gram negative

pneum - pneumonia

during the study period. Sedative agents and muscle relaxants as required were administered to enable patient compliance with tracheal intubation and mechanical ventilation (as described in Chapter 3).

FiO_2 (mean 0.6, range 0.5 – 1.0) (see table 7.1) was titrated to produce a PaO_2 of at least 50 mmHg. V_m (see table 7.1) was adjusted to maintain $PaCO_2$ within the physiological range (35 – 45 mmHg), where the pulmonary pathology permitted. Peak inflation pressure was maintained at or below 40 cmH_2O in all instances (allowing mean V_t of approximately 8 ml/kg). Ventilator parameters were not altered during the study period.

Patients were maintained in the supine position, with a maximum "head-up" elevation of 15 degrees throughout the study period (maximum time of 4 hours). During this time no nursing interventions (e.g. washing, dressing changes, airway suctioning), drug administration or intravenous fluid bolus administration were permitted. Constant infusions of vasopressors, inotropes and maintenance fluids, present prior to study enrolment, were continued unchanged during the study period.

7.2.2 Physiological monitoring

All subjects enrolled in this study had the following physiological monitoring (see Chapter 3 for details) –

Pressures –

- IABP (systolic, diastolic and mean pressures).
- CVP (mean pressure).
- PA pressures (systolic, diastolic and mean pressures).
- PCWP.

All pressures were zeroed with reference to the mid-axillary line.

Cardiac output –

Cardiac output (CO) was measured using the standard thermodilution technique.

Pulse oximetry –

Continuous pulse oximetry (SpO_2) was employed in all patients.

7.2.3 Blood gas measurements

Blood gas measurements were carried out every 30 minutes/at each dose interval (7 measurements per patient) using blood drawn from both the radial arterial line ("arterial") and the right atrial port of the pulmonary artery catheter ("mixed venous"). PAO_2 and $A\text{-}aDO_2$ were calculated using the standard equations as was the shunt fraction (Q_s/Q_t) (see table 3.2).

7.2.4 Drug delivery

Prostacyclin aerosol –

The synthetic analogue of prostacyclin (epoprostenol sodium, Flolan, GlaxoWellcome, Boronia, Victoria, Australia) was supplied in vials of 500 micrograms (μg). This was diluted in a 0.188% w/v solution of glycine in saline (0.147%w/v), prior to administration – the "diluent". The diluent was highly alkaline with a pH of 10.5 (+/- 0.3) and was more viscous than normal saline solution.

IAP was delivered continuously by jet nebuliser into the inspired gas being delivered to the subject as depicted in figure 3.5. The Medic-Aid Sidestream jet nebuliser (Medic-Aid Ltd., U.K.) was used

in this study. The MMD of diluted prostacyclin particles produced by this jet nebuliser was 3.38 μm, as measured by LPA (see Chapter 4 for details). The nebuliser driving gas was delivered at a pressure of 420 kPa, via a flowmeter at 6 l/min. However, the mechanical ventilator compensated for the additional gas flow into the ventilator circuit, so that PEEP, mean and peak airway pressures were unaltered. The exhaled V_m registered by the pneumotachograph of the ventilator, however, included the increased gas flow provided by the driving gas.

The actual mass of IAP deposited in the respiratory tract by this technique was not known, as some of the nebulised particles were lost by rain-out in the ventilator circuit and some were exhaled. That sufficient drug was deposited in the distal respiratory tree was inferred from the observed clinical effect and the performance of the jet nebuliser described in Chapter 4.

The concentration of prostacyclin solution (μg/ml) to be delivered at each dose increment was determined by adding appropriate volumes of the diluent to the vial of epoprostenol/prostacyclin. In this way 6 syringes were prepared of equal volume with 0, 10, 20, 30, 40 or 50 μg/ml concentrations of prostacyclin solution. The delivered dose of IAP could then accurately be increased every 30 minutes, in 10 ng/kg/min increments, over the dose range 0 – 50 ng/kg/min. This was done by using the syringe with the appropriate concentration of solution, delivered at the required rate (as described in 3.5.2). The nebuliser was primed with 1 ml of the new concentration at the start of each 30 min. period/increment change.

The aim of the 30 minute dosing interval was to reach a steady physiological state at each dose of IAP. This relatively short interval would however also limit confounding physiological changes as a result of the underlying disease process. Once the maximum dose of 50 ng/kg/min had been delivered for 30 minutes, the dose was again reduced to 0 ng/kg/min for a further 30 minutes to determine whether physiological parameters returned to baseline values.

7.2.5 Measurement of 6-keto prostaglandin F1 α levels

Prostacyclin is rapidly hydrolysed to its major metabolite 6-keto prostaglandin F1 $_{\alpha}$ (6-ketoPGF1$_{\alpha}$) *in vitro* and *in vivo* [137-139,154,155]. 6-ketoPGF1$_{\alpha}$ is more stable in the circulation than the parent compound and its level is measurable in plasma [154]. Plasma levels of 6-ketoPGF1$_{\alpha}$ were therefore measured as an estimate of the degree of absorption of the parent compound from the respiratory tract into the pulmonary and thence the systemic circulation.

Blood for the measurement of plasma 6-ketoPGF1$_{\alpha}$ levels was drawn from the arterial line as described in Chapter 3. These samples were placed in a centrifuge within 5 minutes of collection and spun at 4,000 rpm for 10 minutes. The plasma was then decanted by pipette into plastic storage tubes, which were stored at – 70°C until analysis. Analysis of plasma levels of 6-kPGF1$_{\alpha}$ was by radioimmuoassay (RIA) (see Chapter 3 for details). The recovery of ^{3}H 6-ketoPGF1$_{\alpha}$ through this procedure was approximately 45% and the within and between assay variations were 5 and 12% respectively.

7.2.6 Platelet aggregation studies

Blood samples were obtained from the radial arterial line into a vacuum tube blood collection system (Vacutainer ™) (see Chapter 3 for details). Platelet aggregation studies were carried out on these samples within 10 min. if possible, or batched and done within two hours while the samples were gently agitated on an agitation table. Platelet aggregation in response to ADP (1 μmol/l) was measured after the method of Burghuber and colleagues [77], as described in Chapter 3.

7.2.7 Statistical analysis

A generalised linear regression model (see model below) was used to determine the gradient of a regression line taken on each measured or calculated variable over the dose range of IAP employed in the study (0 – 50 ng/kg/min). A p value of ≤ 0.05 was used to define a significant increase or decrease in each variable in response to an increasing dose of IAP. This model excluded effects due to chance and factors related to repeated measures in the same subjects.

Generalized linear regression model –

$$y_{ij} = \mu + s_i + \alpha_j + e_{ij}$$

Where - $i = 1,2, ...9$ (patients)

$j = 1,2, ...7$ (doses)

y_{ij} = outcome for patient i under dose j

s_i = random subject effect

α_j = dose effect

e_{ij} = random error

A sample size calculation was carried out after 5 subjects had been studied using the technique described by Wong and Lachenbruch [204]. The equation for this calculation is described on page 350 of the paper by Wong and Lachenbruch [204] and is summarised below –

Sample size calculation –

$$N = 12(K-1)(Z_{1-\alpha}/2 + Z_{1-\beta})^2\sigma^2/[B^2(K+10]$$

Where - N = total sample size

N = nK, where n is the sample size per group and K being equally-spaced doses

α = level of significance

β = probability of type II error

Z = upper 100 x th percentile of the standard normal distribution

B = the amount of change in slope for a one unit increase in dose

σ = standard deviation

In order to show a dose-response relationship for the indices of oxygenation and 6-ketoPGF1α using the generalised linear regression model with an α (level of significance) = 0.05 and a 1-β (power) = 90%, the following sample sizes would be required–

- PaO_2/FiO_2 - n = 2 (for an observed gradient of 5)
- Shunt fraction - n = 5 (for an observed gradient of 0.5)
- A-aDO_2 - n = 3 (for an observed gradient of 3)
- 6-ketoPGF1α - n = 2 (for an observed gradient of 40)

This calculation was not done for the cardiovascular parameters, as they were measured and/or calculated primarily to ensure the pulmonary selectivity of IAP, by showing lack of effect on the systemic blood pressure. The final sample size of n = 9 would therefore have at least a 90% power to show a dose-effect on the indices of oxygenation, shunt and systemic levels of 6-ketoPGF1α.

In addition, the Wilcoxon ranked sums test for related measures was used to compare PaO_2/FiO_2, A-aDO_2 and Q_s/Q_t at IAP doses of 0, 10 and 50 ng/kg/min. The Wilcoxon test was also used to compare the parameters at baseline (dose 0 ng/kg/min) and at the end of the study (dose again 0 ng/kg/min).

7.3 Results

Raw data are tabulated in Appendix D. Summary statistics are tabulated in Appendix E. The results are depicted in figures 7.1- 7.8 and tables 7.2, 7.3 and 7.4. All values shown graphically are either mean values (n = 9) or individual values (as indicated on the figures). Error bars in the figures indicate the standard error of the mean (SEM).

7.3.1 Indices of oxygenation

PaO_2/FiO_2 showed a highly significant dose-dependent increase by generalised linear regression ($p = 0.003$). The A-aDO_2 gradient also decreased over the study period ($p = 0.01$). The largest incremental improvement (i.e. difference in mean values between successive dose intervals) in both values was between the baseline (IAP dose = 0 ng/kg/min) and 10 ng/kg/min. The graphs in figures 7.1 and 7.2 illustrate this and the subsequent reduction in rate of dose-related change in each parameter (flattening of the slope). The maximum effect (largest change from baseline, as determined by the difference between the mean values at baseline and subsequent dose) was produced by the maximum dose of IAP (50 ng/kg/min). The reduction in shunt fraction with increasing IAP dose tended towards statistical significance ($p = 0.07$). (Appendix E).

The indices of oxygenation, both PaO_2/FiO_2 and A-aDO_2, returned to baseline when the 50 ng/kg/min dose was discontinued.

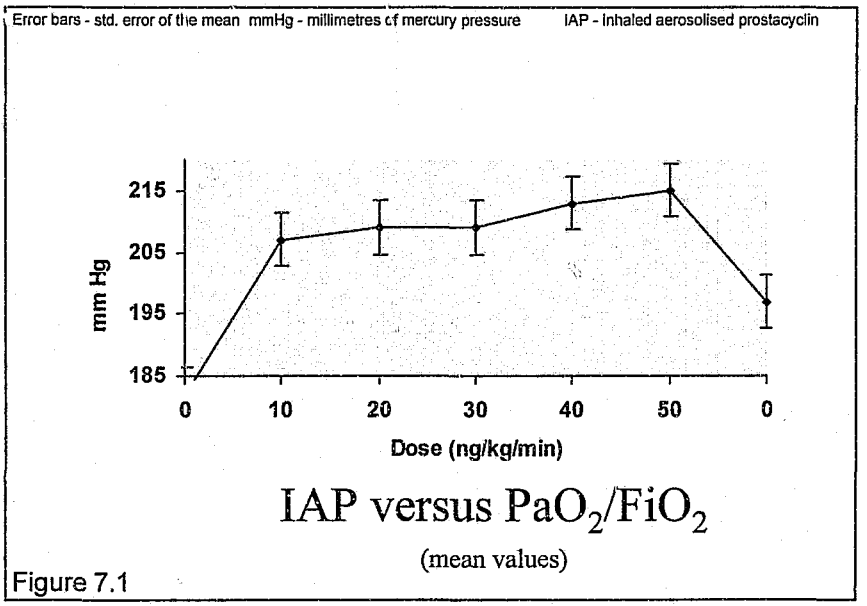


Figure 7.1

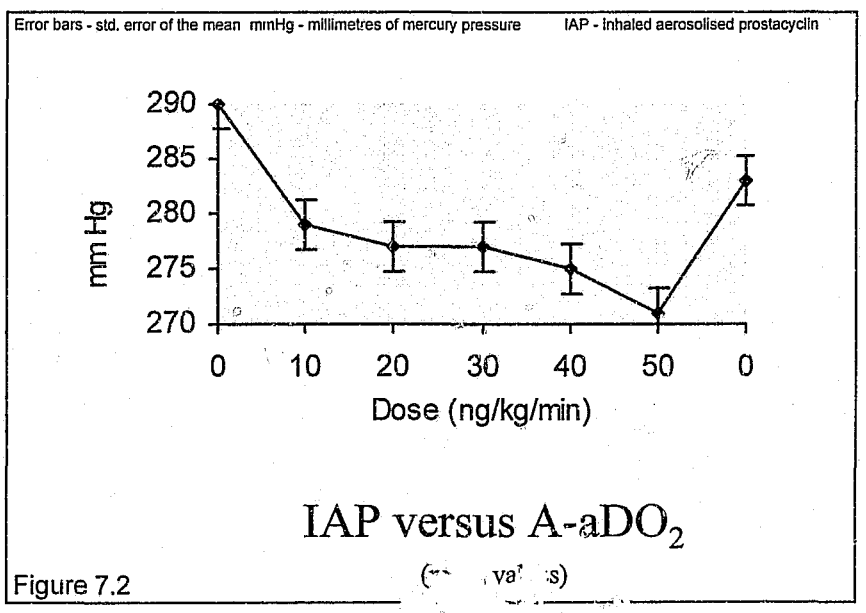


Figure 7.2

There was no significant statistical difference between PaO_2/FiO_2 ($p = 0.1$) and A-aDO_2 ($p = 0.4$) at the start of the study period (IAP dose = 0 ng/kg/min) versus the end of the study period (IAP dose = 0 ng/kg/min).

Comparison using the Wilcoxon ranked sums test for related measures at IAP doses of 0,10 and 50 ng/kg/min for PaO_2/FiO_2, A-aDO_2 and shunt fraction are shown in table 7.2. When the Bonferroni correction for multiple comparisons was applied, there were no statistical differences in the parameters between IAP doses of 10 or 50 ng/kg.min, nor between IAP doses 0 and 10 ng/kg/min, despite the largest incremental change occurring for this step from 0 to 10 ng/kg/min (see above). There was a tendency to statistical significance for PaO_2/FiO_2 between IAP doses 0 vs 50 ng/kg/min (table 7.2).

Table 7.3 shows the individual PaO_2/FiO_2 values for the 9 subjects at each dose interval.

Table 7.2 **Comparison of the median values of PaO_2/FiO_2, A-aDO_2 and shunt fraction at IAP doses 0, 10 and 50 ng/kg/min, using the Wilcoxon ranked sums test for related measures.**

IAP dose (ng/kg/min)	0 vs 10	0 vs 50	10 vs 50
Median values -			
PaO_2/FiO_2	187.2 vs 183.2	•187.2 vs 202.2	183.2 vs 202.2
A-aDO_2	238 vs 247	238 vs 237	247 vs 237
Shunt fraction (%)	28 vs 21.4	28 vs 18	21.4 vs 18

Key –

IAP dose -inhaled aerosolised prostacyclin dose in nanograms per kilogram per minute

PaO_2/FiO_2 -arterial partial pressure of oxygen/fractional inspired concentration of oxygen ratio in mmHg

A-aDO_2 -alveolar arterial partial pressure of oxygen gradient in mmHg

vs -versus

• -$p < 0.008$ – approaches significance, allowing for the Bonferroni correction for multiple comparisons

Table 7.3

Individual PaO_2/FiO_2 values for each patient.

IAP dose	0	10	20	30	40	50	0	ng/kg/min
Patient no.				PaO_2/FiO_2				
1	105.5	97.4	108.5	116.1	126.6	137.2	135.5	
2	97.1	182.4	110.9	98.9	102.8	100.8	86.8	
3	212	231.4	201	231.6	231	262.8	255.2	
4	155.2	150.8	155	165.4	160	171.6	166.4	
5	129.2	146.5	155.3	146.2	155.5	145	109.3	
6	358.4	420.4	416	410.2	398.6	367.2	367.6	
7	187.2	183.2	193.6	198.8	205.6	202.2	179.4	
8	189	212.7	280	251	291	284	225	
9	204	235	257	260	245	267	244	
mean	181.9	206.6	208.6	208.7	212.9	215.3	196.6	

Key –

IAP dose -dose of inhaled aerosolised prostacyclin in ng/kg/min

PaO_2/FiO_2 .arterial partial pressure of oxygen/fractional inspired oxygen concentration ratio (mmHg)

7.3.2 Cardiovascular parameters

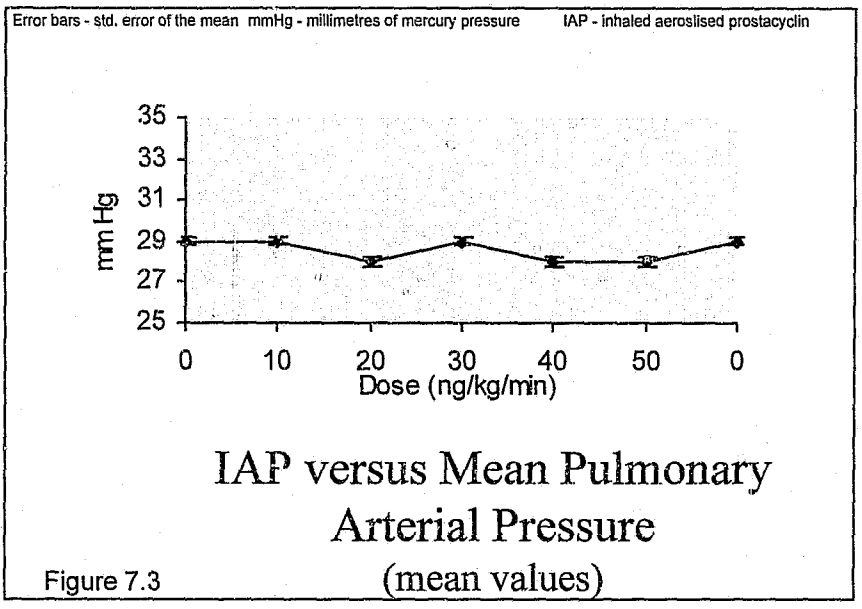
There was no statistically significant dose-related change in MPAP ($p = 0.38$) (figure 7.3), while there was a trend towards increase in MAP ($p = 0.07$) (figure 7.4) and CI ($p = 0.06$) (figure 7.5) over the study period. None of the patients entered into the study had significant pulmonary hypertension (mean MPAP at enrolment was 29 mmHg and the range was from 24-43 mmHg).

7.3.3 6-ketoPGF1$_\alpha$ plasma level

There was a linear and significant ($p = 0.001$) increase in plasma levels of this prostacyclin metabolite over the study period (figure 7.6). The maximum level of 6-ketoPGF1$_\alpha$ was seen at the maximum dose of IAP, as determined by the difference in the mean values of 6-ketoPGF1$_\alpha$ at dose 0 versus dose 50 ng/kg/min of IAP. The level of 6-ketoPGF1$_\alpha$ trended towards baseline when the 50 ng/kg/min dose was discontinued (figure 7.6).

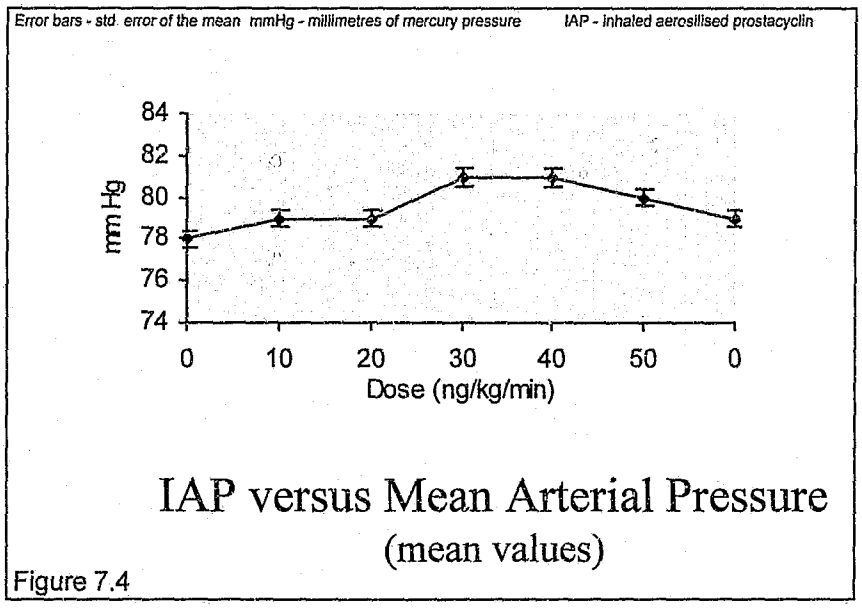
7.3.4 Platelet function studies

The results for the effect on platelet function studies of the dose range of IAP studied are shown in table 7.4. There was no demonstrable effect of dose of IAP versus platelet function as determined either by maximum aggregation (figure 7.7) or aggregation at 4 minutes (figure 7.8). In the case of both parameters there was a wide variation between subjects at baseline and at each subsequent dose interval. Mean values (and range) for the two measurements at baseline were, maximum aggregation, 41% (15 – 100) and aggregation at 4 minutes, 35% (5 – 100). Individual values are tabulated in Appendix D.



IAP versus Mean Pulmonary Arterial Pressure
(mean values)

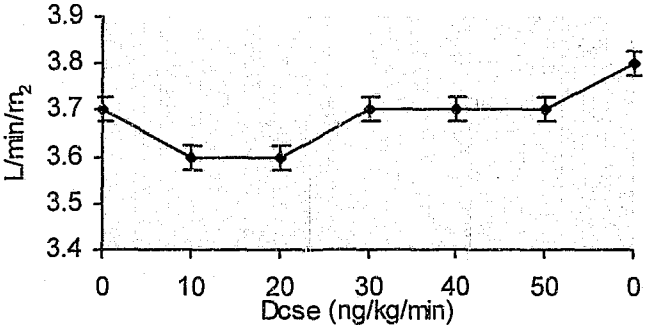
Figure 7.3



IAP versus Mean Arterial Pressure
(mean values)

Figure 7.4

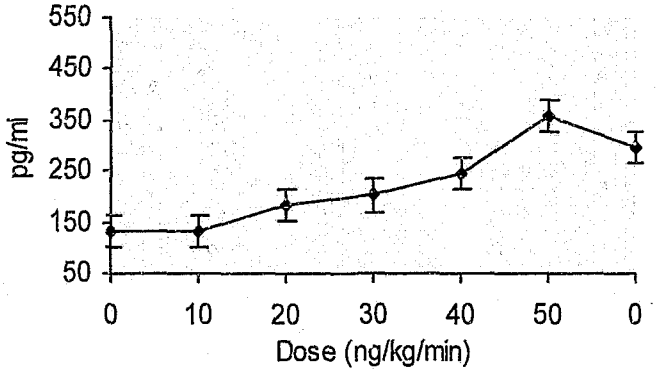
Error bars - std. error of the mean IAP - inhaled aerosolised prostacyclin L/min/m² - litres per minute per square metre



IAP versus Cardiac Index
(mean values)

Figure 7.5

Error bars - std. error of the mean pg/ml - picograms per millilitre IAP - inhaled aerosolised prostacyclin



6-Keto Prostaglandin $F_1\alpha$ Level
(mean values)

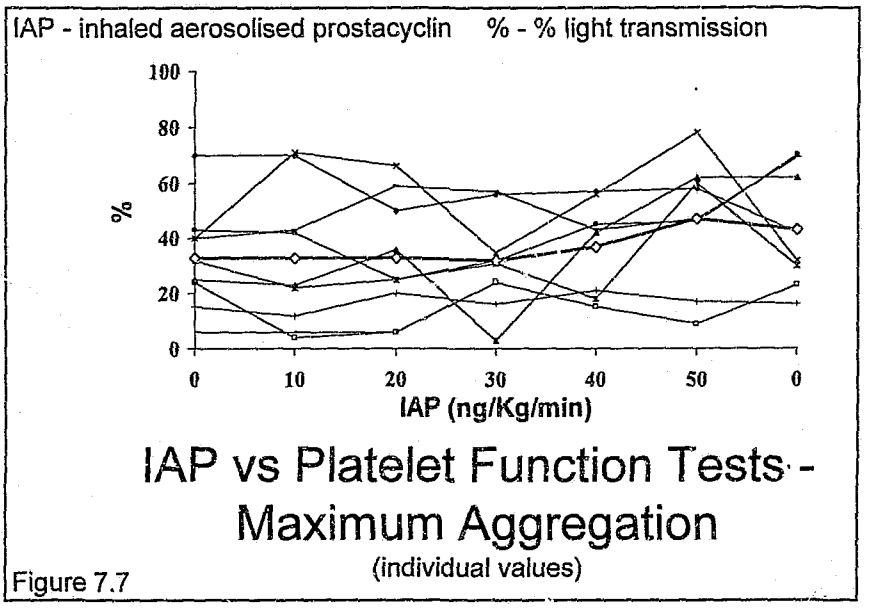
Figure 7.6

Table 7.4

Platelet function over the dose range 0 – 50 ng/kg/min.

Measurement	Parameter estimate	SEM	t	p
Maximum aggregation (%)	1.203	0.896	1.34	0.186
Aggregation at 4 minutes (%)	0.863	0.916	0.94	0.351

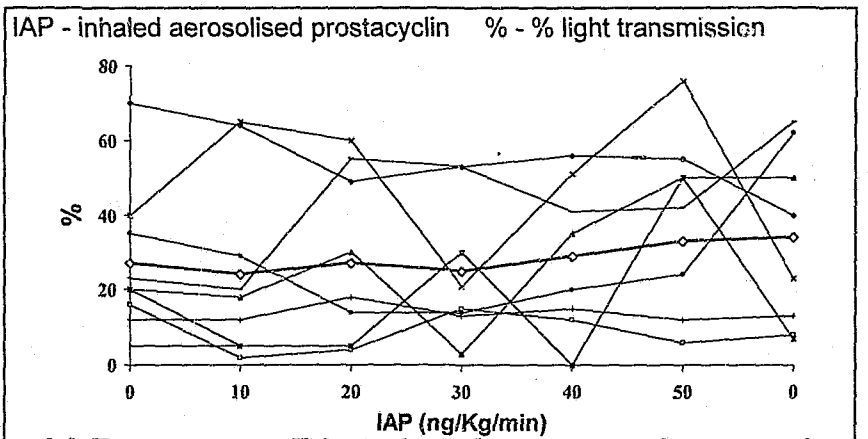
Key –
SEM -standard error of the mean
% -percentage light transmission



IAP vs Platelet Function Tests - Maximum Aggregation

(individual values)

Figure 7.7



IAP versus Platelet Aggregation at 4 minutes

(individual values)

Figure 7.8

7.4 Discussion

This study demonstrated the basic properties of IAP as a SPV. This potent vasodilator, when inhaled, resulted in an improvement in oxygenation, without a systemic hypotensive effect, in this group of patients with severe ARDS (i.e. no dose-dependent decrease in systemic blood pressure as IAP dose increased). This study also showed that this effect on oxygenation is dose-responsive, at least over the dose range used in the study. The mechanism by which IAP is able to achieve this improvement in oxygenation has previously been shown to be via an improvement in V/Q matching as demonstrated by the multiple inert gas elimination technique [167]. Also, the shunt fraction is reduced by IAP therapy as shown by the results of the pilot study reported in Chapter 5 (figure 5.2) and the results of this study. Reduced shunt fraction is an indication of improved V/Q matching as there is no evidence for the closure of anatomical right to left shunts by the application of IAP.

The blunting of the initial rapid improvement in oxygenation (from 0 to 10 ng/kg/min incremental change) may have been due to recirculation of the 6-ketoPGF1$_\alpha$, which then acted as a non-selective pulmonary vasodilator as it re-entered the pulmonary circulation. This assumption however was not supported by a marked accompanying reduction in MPAP as 6-ketoPGF1$_\alpha$ levels increased and was contradicted by the fact that the maximum improvement in oxygenation was seen at the highest dose of IAP (i.e. ongoing improvement in oxygenation as IAP dose increased).

Indeed, there was no marked effect on MPAP, and this was probably because of the relatively normal baseline PA pressures in the study group. The fact that the CI did not significantly change also served to demonstrate the efficacy of IAP as a SPV. The total blood flow through the lung was unchanged, but because oxygenation improved, it is assumed that the flow was redirected within the lung from non-ventilated to ventilated areas, thus improving V/Q matching. This was borne out by a reduction in shunt fraction with increasing dose of IAP. That IAP achieved its

effect in the manner described above was supported by tests of the efficiency of the drug delivery system (discussed in Chapter 4). The nebuliser used was able to generate respirable particles of prostacyclin in glycine buffer solution. These respirable particles were delivered to ventilated areas of the lung.

This study was also able to demonstrate a significant dose-related increase in systemic serum levels of prostacyclin metabolite. A systemic hypotensive effect due to 6-ketoPGF1$_\alpha$, a less potent vasodilator than the parent compound, was not seen in this group of patients. Platelet dysfunction due to systemic absorption of prostacyclin and its metabolites was also not demonstrated by this study. This is in conflict with a previous *in vivo* [77] study and the results of the study described in Chapter 8, where extremely low levels (picograms per ml) of prostacyclin and its metabolites were shown to produce potent anti-platelet effects.

The inability of this study to demonstrate dose-responsive antiplatelet effects may relate to the wide variation in baseline values of platelet function in this group of very ill patients, indicating disordered platelet function on entry to the study. The effects of systemically-absorbed prostacyclin/6-ketoPGF1$_\alpha$ may therefore not be apparent, in this population, due to the high level of background noise. Noise, which would not be present in controlled *in vitro* studies (such as described in Chapter 8 [205]) or *in vivo* studies in volunteers [77]. In any event, an antiplatelet effect within the pulmonary circulation, where prostacyclin concentrations would be highest during IAP therapy, has theoretical advantages in ARDS where platelet and white cell aggregation are part of the pathological process [30,32,206,207]. Finally, the sample size in this study was probably not large enough, given the wide baseline variation in platelet function, to demonstrate an antiplatelet effect. Using the equation for sample size described in 7.2.7 above, a sample size of at least 62 subjects would be required to demonstrate a dose-related effect on platelet function. Consequently a more controlled population (with smaller baseline variation in platelet function) would require study to be

able to determine an antiplatelet effect of absorbed prostacyclin metabolite. This is the subject of study in Chapter 8.

In ARDS, SPV therapy is primarily directed at the severe hypoxaemia associated with the syndrome. In this role, a SPV may be able to improve oxygenation, allowing a reduction in FiO_2 to less toxic levels during mechanical ventilation. Oxygen toxicity remains a real concern when treating ARDS [208,209]. Secondly, SPV therapy may also be utilised for the pulmonary hypertension seen occasionally in patients with ARDS, or other causes of pulmonary hypertension.

The only similar study, of which the candidate is aware, that addresses the dose-response relationship between IAP and oxygenation, is by Zwissler and co-authors [174]. That study was published after commencement of the investigational work for the study described here. The study by Zwissler also differs from the current study in a number of aspects. That study investigated the dose-response relationships to 0, 1, 10 and 25 ng/kg/min of IAP versus oxygenation and haemodynamic indices, where the dose was increased every 15 minutes. The Zwissler study was able to demonstrate an effect, though modest, on MPAP, which the candidate was not. That study also did not investigate 6-ketoPGF1$_\alpha$ levels, nor the possible effect this might have on platelet function. The two studies concur on a number of points – there is a significant dose-related effect of IAP on oxygenation in patients with ARDS (using similar statistical methods), there is no significant difference between various doses of IAP on indices of oxygenation when compared (table 7.2), the best improvement in oxygenation occurred between 0 and 10 ng/kg/min and there was no significant effect on shunt fraction in either study. Both studies also demonstrated the individual variations in response to the same dose range of IAP (table 7.4) with respect to extent of response and timing of response. IAP, like nitric oxide therapy, and indeed as for other vasoactive therapies, requires titration to individual response. In this way the lowest (but most effective dose) is chosen for maximum effect, with minimum adverse

effects. The current study would support 10 ng/kg/min as a reasonable starting dose of IAP for hypoxaemic patients with ARDS.

The reasons for the wide patient variability in response to SPV's, as demonstrated again by this study (Table 7.3), are not readily apparent, but would include –

- variation in individual response to vasoactive agents,
- heterogeneity of disease processes included under the banner of ARDS (see Table 7.1 for various primary diagnoses in this group of patients),
- variation in the time at which patients were entered in the study and hence variation in stage of ARDS at which patient was studied (see Table 7.1 for day of enrolment), although patients were enrolled as soon as they met inclusion criteria and
- some variation in the actual dose of drug deposited in alveoli depending on variations between ventilators.

All of these factors would support the candidate's advice to titrate treatment to effect and not rely on "cookbook" doses. This is true for many treatments in critically ill patients

IAP and NO have both now been well-demonstrated to fulfill the role of an efficacious SPV. IAP has the advantage of simplicity of delivery, being a viable SPV in the operating room and in the transport environment. Currently a number of other agents are undergoing investigation either as SPV's, including other eicanosoids [210] and nitric oxide donors [198,211-214] or as enhancers of SPV function eg zaprinast [215]. The major roles of these agents remain the treatment of hypoxaemia and pulmonary hypertension. The additional administration of an enhancer of hypoxic pulmonary vasoconstriction such as almitrine bimesylate or phenylephrine [216] may improve the V/Q matching effect of the SPV, another area requiring further study.

7.5 Conclusions

This study confirmed the efficacy of IAP as a SPV for the treatment of hypoxaemia due to ARDS. The study also demonstrated (with a power greater than 90%) the dose-related actions of IAP on indices of oxygenation, when delivered by this simple delivery system. Systemic levels of 6-ketoPGF1$_\alpha$ increased during IAP therapy, indicating systemic absorption, but no consequent anti-platelet effect of IAP therapy could be demonstrated.

7.6 Summary

This study was carried out to determine the efficacy of and dose response relationships to IAP, when used as a SPV in patients with severe hypoxaemia due to the ARDS. This was an unblinded interventional prospective clinical study in a university tertiary referral centre, general intensive care unit. Nine adult patients with severe ARDS (LIS ≥ 2.5) were enrolled. All patients received IAP over the dose range 0 to 50 ng/kg/min. The IAP was delivered via a jet nebuliser placed in the ventilator circuit. Dose increments were 10 ng/kg/min every 30 min. Cardiovascular parameters, indices of oxygenation and shunt fraction were measured or calculated at each dose interval, as were platelet aggregation and systemic levels of prostacyclin metabolite. Generalised linear regression was used to determine a dose effect of IAP on the measured parameters. Wilcoxon ranked sums test for related measures was used to compare the effects of various doses of IAP.

IAP acted as a SPV, with a statistically significant dose-related improvement in PaO_2/FiO_2 ($p = 0.003$) and A-aDO_2 ($p = 0.01$). Systemic prostacyclin metabolite levels increased significantly with delivered IAP dose ($p = 0.001$). There was no significant dose effect on systemic or pulmonary arterial pressures, nor on platelet function, as determined by platelet aggregation in response to challenge with adenosine diphosphate. IAP is an efficacious SPV, with marked dose-related improvement in oxygenation and with no demonstrable effect on systemic arterial pressures over the dose range 0 – 50 ng/kg/min. Despite significant systemic levels of prostacyclin metabolite, there was no demonstrable platelet function defect.

Chapter 8

8.0 *IN VITRO* ASSESSMENT OF THE EFFECT OF EXTREMELY LOW CONCENTRATIONS OF PROSTACYCLIN AND PROSTACYCLIN METABOLITES ON PLATELET FUNCTION.

8.1 Introduction

Inhaled aerosolised prostacyclin (IAP) is used as a SPV in the treatment of severe hypoxaemia and pulmonary hypertension, as may occur in ARDS [134,151,152,191]. Since the earliest reports of IAP therapy, there has been an awareness of the potential for prostacyclin and its metabolites to reach the systemic circulation during inhalation therapy [77,123].

Prostacyclin rapidly hydrolyses to 6-ketoPGF1α (half-life 18-29 minutes [154]), its major metabolite, as well as a number of lesser metabolites [138,139,141,217]. Both prostacyclin and its major metabolite, 6-ketoPGF1α, are potent inhibitors of platelet aggregation, acting via cAMP [77,181,218-220]. A recent report indicated that systemic plasma levels of 6-ketoPGF1α reached during IAP therapy exceeded 600 pg/ml [221]. The results from the study described in Chapter 7 confirmed similar levels of 6-ketoPGF1α in the systemic circulation of subjects receiving IAP therapy. The candidate was, however, unable to confirm antiplatelet effects due to the presence of prostacyclin metabolite in the systemic circulation *in vivo* (Chapter 7), despite a strong theoretical basis for expecting such an antiplatelet effect. Reasons for the discrepancy between expected and actual findings were discussed in Chapter 7.

The aim of the current study was to determine whether extremely low concentrations of prostacyclin or its metabolites, as may be encountered in the systemic circulation during IAP therapy, affected platelet function in a controlled environment *in vitro*. Platelet aggregation

responses (to adenosine diphosphate (ADP) and collagen), and thrombelastography (TEG) would both be used to measure platelet function.

8.2 Method

Institutional ethics committee approval and written informed consent was obtained prior to recruiting eight healthy adult subjects (4 male and 4 female, aged 28 to 46 years) to participate in the study. None of the subjects were taking medication known to affect platelet function, such as the oral contraceptive pill or aspirin. None were smokers, as smoking may also affect platelet function[222].

8.2.1 Blood samples

Blood samples for platelet aggregation studies were obtained from an antecubital vein using a 21G winged infusion set (Surflo, Terumo Medical Corp., Elkton, MD., USA) drawn into a vacuum tube blood collection system (Vacutainer ™), as described in Chapter 3. Each sample was collected into a siliconised glass tube containing 1 part 3.8% sodium citrate per 9 parts blood.

Blood for the TEG was collected in a plastic syringe prior to testing in the TEG.

8.2.2 Platelet aggregation studies

Various dilutions of prostacyclin in a 100 µl volume were added immediately to each 5 ml blood sample, collected as above, using a micro-pipette, to produce final prostacyclin concentrations of 10 pg/ml, 100 pg/ml or 500 pg/ml. A control sample, to which 100 µl of normal saline was added, was included in each test series for each subject.

Platelet aggregation studies were carried out on these samples within 10 min., as described in Chapter 3. Platelet aggregation was performed using a Daiichi Monitor IV aggregation recorder (Helena Laboratories, Beaumont, Texas, USA). Platelet aggregation was induced with adenosine

diphosphate (ADP) sodium salt in buffer in final concentrations of 1 μmol/L and 8 μmol/L. Reagent Horm collagen in a final concentration of 10 μmol/L was also used to induce aggregation. The maximal rate of aggregation (slope) and the extent of aggregation (maximal and after 4 minutes) were determined as a percentage of light transmission (see figure 3.1 for an example of a platelet aggregation trace obtained).

8.2.3 Thrombelastography (TEG)

Two computerised TEG machines were used, providing four channels simultaneously. Venous blood (0.35 ml) was added to disposable plastic cups in each channel. Various dilutions of prostacyclin were then added in a 10 μl volume, using a micro-pipette, to produce final prostacyclin concentrations in each of the cups of 10 pg/ml, 100 pg/ml or 500 pg/ml. A control sample (10 μl of normal saline) for each series was used for each subject. The order of the TEG channels used for each prostacyclin concentration was randomised for each subject. The R, K and the MA of each TEG run were recorded (see Chapter 3 for definitions).

8.2.4 Statistical analysis

Statistical analysis included comparison of the baseline and treatment groups, for maximal rate of aggregation and extent of aggregation (max. and after 4 min.), by means of Wilcoxon Signed Rank tests. The Wilcoxon Signed Rank test was also used to compare the MA of the TEG at the various concentrations of prostacyclin to control values. P values < 0.05 were considered statistically significant.

8.3 Results

Subject demographic data are shown in table 8.1. All raw data from this study are tabulated in Appendix F.

8.3.1 Platelet aggregation studies

The results for ADP (8 μmol/L) and collagen are shown in figure 8.1. The results for ADP 1 μmol/L are not shown graphically, for the sake of clarity, but are tabulated in table 8.2. These results show that there was a significant reduction in **maximum platelet aggregation** in response to both 1 μmol/L and 8 μmol/L ADP at prostacyclin concentrations of 10 pg/ml, 100 pg/ml and 500 pg/ml. The maximum platelet aggregation response to collagen was significantly impaired only at the 100 pg/ml and 500 pg/ml concentrations of prostacyclin.

Platelet aggregation at 4 minutes in response to ADP (1 μmol/L and 8 μmol/L) and collagen (10 μmol/L) was significantly reduced at the 100 pg/ml and 500 pg/ml prostacyclin concentrations, but not at the 10 pg/ml prostacyclin concentration. The findings for platelet aggregation at 4 minutes are shown in figure 8.1 and table 8.2.

The rates of aggregation (slope) in response to challenge with ADP (1 μmol/L and 8 μmol/L) and collagen (10 μmol/L) are shown in table 8.3. These results show the **rate of aggregation** (slope) to ADP 1 μmol/L was significantly decreased at all the prostacyclin concentrations tested. The rate of aggregation in response to ADP (8 μmol/L) or collagen (10 μmol/L) was significantly reduced at the 100 pg/ml and 500 pg/ml prostacyclin concentrations, but not at the 10 pg/ml level where the p values were 0.08 (ADP 8 μmol/l) and 0.1 (collagen) respectively.

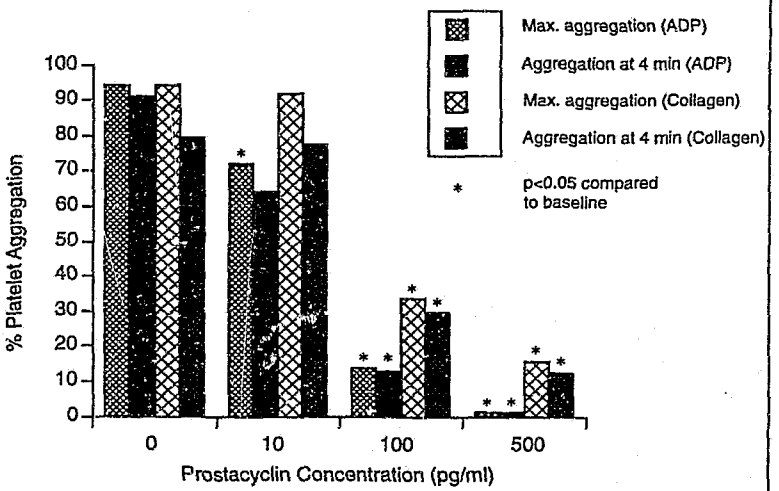
8.3.2 TEG

The maximum amplitudes (MA) obtained by thrombelastography (TEG) at baseline and at each of the three concentrations of prostacyclin are shown in table 8.4. There were no significant differences between the MA's at the various concentrations compared to baseline. The mean R and K times for each concentration of prostacyclin remained within normal limits (Appendix F).

Table 8.1

Subject demographics.

Subject no.	Age (years)	Gender
I	44	female
II	28	female
III	31	male
IV	41	female
V	39	male
VI	46	male
VII	28	female
VIII	36	male



Platelet aggregation in response to ADP and collagen

Figure 8.1 **(maximum and at 4 minutes)**

Table 8.2

Mean and standard deviations for platelet aggregation (% light transmission) in response to ADP and Collagen.

	Prostacyclin concentration (pg/ml)			
	0	**10**	**100**	**500**
Agonist				
ADP (1 μmol/L)				
- % light transmission				
- (mean (SD)				
- maximum	62.3(24.6)	*26.7(29.8)	*0(0)	*0(0)
- at 4 minutes	39.8(36.1)	16.2(28.0)	*0(0)	*0(0)
ADP (8 μmol/L)				
- % light transmission				
- (mean (SD)				
- maximum	94.1(7.6)	*72.0(25.5)	*13.6(32.8)	*0(0)
- at 4 minutes	90.8(8.2)	64.3(26.5)	*13.0(32.9)	*0(0)
Collagen (10 μmol/L)				
- % light transmission				
- (mean (SD)				
- maximum	94.5(5.8)	92.0(8.3)	33.7(32.3)	*15.6(3.9)
- at 4 minutes	79.6(7.9)	77.6(28.9)	*29.6(31.9)	*12.5(3.6)

Key –
ADP - adenosine diphosphate
SD - standard deviation
* - $p < 0.05$ compared to baseline
pg/ml - picograms per millilitre

Table 8.3

Rate of aggregation (slope) in response to challenge with ADP or collagen.

	Prostacyclin concentration (pg/ml)			
	0	10	100	500
Agonist -				
ADP (1 μmol/L) **slope**				
-mean (SD)	58(6.4)	17.8(19)*	0(0)*	0(0)*
ADP (8 μmol/L) **slope**				
-mean (SD)	52.5(9.02)	34.6(18.1)	0.6(1.2)*	0(0)*
Collagen (10 μmol/L) **slope**				
-mean (SD)	58.1(2.4)	49.6(13.3)	6.8(8.3)*	2.2(0.8)*

Key -

ADP - adenosine diphosphate

SD - standard deviation

* - $p < 0.05$ compared to baseline

pg/ml - picograms per millilitre

Table 8.4

Maximum amplitude of the thrombelastogram in response to various concentrations of prostacyclin.

MA	Prostacyclin Concentration (pg/ml)			
	0	10	100	500
Mean	50.7	54	49.4	46.7
SD	6.3	9.2	11.3	13.5

Key –

MA - maximum amplitude

pg/ml - picograms per millilitre

SD - standard deviation

8.4 Discussion

This study demonstrated a significant platelet aggregation defect *in vitro* at prostacyclin concentrations as low as 10 pg/ml. These findings indicated that extremely low systemic plasma levels of prostacyclin and 6-ketoPGF1α may be associated with a platelet aggregation defect. The clinical significance of a potential platelet aggregation defect, which may develop during IAP, therapy has to be interpreted in the light of the essentially normal TEG over the entire prostacyclin concentration range tested.

Platelet aggregation responses to agonists such as ADP and collagen are a sensitive method of assessing platelet inhibition by antiplatelet drugs [223]. The concentration of agonists required to induce aggregation is a measure of the potency of the inhibition. The finding of dose-related inhibition of platelet aggregation to high concentrations of ADP and collagen indicate that prostacyclin is a potent inhibitor of platelet aggregation, even at the low levels that occurred during IAP therapy (see Chapter 7).

However, platelet aggregation responses do not assess the contribution of other coagulation elements that occur *in vivo*. Thrombelastography provides a dynamic assessment of clot formation in whole blood [221]. The MA of the TEG is a measure of the maximum strength of clot, which is dependent on the interaction between fibrinogen and platelets [221]. Moreover, because the TEG uses whole blood, thrombin is generated which is a potent activator of platelet aggregation. This may explain the relatively normal MA in this study, despite the inhibition of platelet aggregation in response to ADP and collagen.

The normal TEG findings, therefore, suggest that platelet aggregation in response to potent agonists *in vivo* (e.g. thrombin) may be preserved. An alternative explanation may be that the TEG is not a sensitive measure of platelet dysfunction, due to platelet activation by other pathways during whole blood coagulation. In any event, a normal TEG does not indicate normal

platelet function in this setting (i.e. TEG has a very low sensitivity). The results also indicate that platelet aggregation studies rather than TEG are appropriate for monitoring platelet function during IAP therapy or other situations where low levels of prostacyclin are encountered.

IAP has been shown to be a potent selective pulmonary vasodilator (SPV)[134,151,152,191]. Its use in reducing pulmonary arterial pressure and improving oxygenation has been demonstrated in patients with ALI by the candidate (Chapters 5 and 7) and by other authors [134,152]. However, the toxicity of IAP until now has not been well elucidated. One area of concern with regard to toxicity of this therapy is the known potent anti-platelet effects of both prostacyclin and its metabolite 6-ketoPGF1α.

A recent report [221], as well as the findings described in Chapter 7 indicate levels of 6-ketoPGF1α of over 600 pg/ml in the plasma of patients with ARDS being treated with IAP in doses of up to 50 ng/kg/min. This study was conducted to determine whether these levels of 6-ketoPGF1α, encountered in the systemic circulation during IAP therapy *in vivo*, were responsible for a significant platelet aggregation defect *in vitro*. The *in vivo* tests of platelet function carried out during the conduct of the study described in Chapter 7 were inconclusive. This study clearly demonstrated reduced platelet aggregation in response to agonists of platelet aggregation *in vitro*. The failure of the *in vivo* testing described in Chapter 7 to show a similar effect may reflect the known difference in performance between platelet aggregation studies when carried out immediately (or soon) on *in vitro* samples as opposed to those obtained and stored for some time[219]. As discussed in Chapter 7, the subjects in that study were all critically ill and had disordered platelet function at baseline. The subjects in the current study were all normal, healthy adults.

An assumption drawn in the conduct of this study is that although plasma levels of 6-ketoPGF1α were reported during IAP therapy, due to the ephemeral nature of prostacyclin, the platelet

aggregation defect seen *in vivo* would be a result of the action on platelets, via cAMP, of both prostacyclin and its metabolites, principally 6-ketoPGF1α. So, although prostacyclin was added to the test tube in this study, rapid hydrolysis of the prostacyclin to 6-ketoPGF1α *in vitro* would result, and the anti-platelet effect would be due to both the parent compound and the metabolite, much as would occur *in vivo*. It is not possible however to equate with absolute certainty the levels of 6-ketoPGF1α in patients receiving IAP therapy [224] (also see Chapter 7) and the amount of prostacyclin added *in vitro* in this study. A platelet aggregation defect in response to ADP and collagen, associated with raised plasma levels of 6-ketoPGF1α, was not confirmed in the series of patients described in Chapter 7[224].

8.5 Conclusion

Extremely low concentrations of prostacyclin, as may be seen in the systemic circulation during IAP therapy, had potent antiplatelet effects as determined by platelet aggregation studies *in vitro*. The TEG, however, was unaffected, suggesting either the preservation of normal haemostasis or poor sensitivity of the TEG for the study of platelet inhibition. This study confirmed that platelet aggregation studies and not TEG should be used for monitoring of platelet function when IAP is administered. The findings also support the recommendations that IAP therapy not be used in patients with a known bleeding diathesis or where potential bleeding is a severe risk (such as in closed head injury).

8.6 Summary

Platelet aggregation in response to ADP and collagen, as well as measurement of the MA by TEG, was undertaken *in vitro* using venous blood from eight healthy subjects exposed to extremely low concentrations of prostacyclin (0, 10, 100 and 500 pg/ml). This was done to determine the possible direct effects of low concentrations of prostacyclin that might spill over into the systemic circulation during the administration of inhaled aerosolised prostacyclin (IAP).

There were statistically significant reductions in parameters of platelet aggregation in response to the agonists.ADP (1 and 8 μmol/L) and collagen (10μmol/L) following exposure to as little as 10 pg/ml of prostacyclin *in vitro*. The MA of the TEG was unchanged over the entire range of prostacyclin concentrations studied.

The results indicate that low concentrations of prostacyclin or prostacyclin metabolite which are observed during IAP therapy may be associated with a marked platelet aggregation defect *in vitro*. The TEG did not detect this antiplatelet effect.

Chapter 9

9.0 CONCLUSIONS

Effective therapeutic manipulation of the pulmonary circulation has been elusive. The application of therapies primarily designed for control of the systemic circulation, to the pulmonary circulation, have been hampered by a lack of selectivity. Intravenous (IV) administration of vasodilators (such as glyceryl trinitrate (GTN) or sodium nitroprusside (SNP), for action within the pulmonary circulation, are very effective at reducing mean pulmonary arterial pressure (MPAP). However, they usually also affect the systemic circulation and cause systemic hypotension. Systemic hypotension is an extremely undesirable effect in the setting of right ventricular hypertrophy due to chronic pulmonary hypertension. Any reduction in mean arterial pressure (MAP) may also reduce right ventricular (RV) perfusion and reduce the effectiveness of RV function. The resultant RV dysfunction may be precisely the reason why a pulmonary vasodilator was required in the first instance (i.e to reduce RV afterload and improve performance). The effect on the systemic circulation can be reduced by using very short-acting vasodilators such as PGI_2 or prostaglandin E_1. Given by the IV route, there is minimal effect on the systemic circulation due to the short duration of action of these agents.

Regardless of the selective action of short-acting IV agents on the pulmonary circulation, another problem peculiar to the pulmonary circulation exists. That is the effect that IV administration of vasodilators has on the ventilation-perfusion (V/Q) ratio in the lung. IV pulmonary vasodilators act on all pulmonary vessels and blunt the effect of the hypoxic pulmonary vasodilator (HPV) response. As a consequence there is reduced matching of ventilation and perfusion, with non-ventilated areas of the lung receiving an inappropriately large blood flow (i.e. low V/Q ratio). The effect is an increase in venous admixture (reduced V/Q ratio) and hypoxaemia. Because the

resultant hypoxaemia is due to venous admixture, it is not readily treatable with supplemental oxygen therapy. The hypoxaemia may not be a critical issue where pulmonary vasodilators are used specifically for pulmonary hypertensive conditions. However, where oxygenation is marginal or where the treatment of hypoxaemia is the primary aim (such as in ARDS), then this side-effect of IV pulmonary vasodilators is unacceptable.

Nitric oxide (NO) provided the first therapeutic hope for selective vasodilatation of the pulmonary vascular bed. In addition to selectivity for the pulmonary circulation, NO (because it is an inhaled gas) acts only within ventilated areas of the lung, improving V/Q matching and oxygenation. This dual selectivity makes it a useful agent for treatment of pulmonary conditions where pulmonary hypertension and/or hypoxaemia are present. ARDS is such a condition, making it a useful pathological model for the study of SPV's. The mainstay of treatment of ARDS is supportive only at present. Until the advent of NO and ventilatory modes associated with lower morbidity, it was not uncommon for the supportive care to result in significant patient morbidity in the form of oxygen toxicity and baro- or volutrauma (see Chapter 1). NO therapy would then seem ideal for use as a SPV in ARDS – to improve oxygenation and limit morbidity attributable to high concentrations of supplemental oxygen. Outcome studies emerging currently ho[illegible] indicate only a short-lived improvement in oxygenation and less impressive long-term benefit of NO therapy in patients with ARDS [109,225,226].

NO therapy also has a significant number of difficulties associated with its delivery. In addition, its optimal use (time of initiation, duration of therapy etc.) in the patient with lung disease has not been well-defined to date. At the time of writing, therefore, there is no evidence to suggest improved outcome attributable to NO therapy [109,225,226]. Further investigation and refinement of NO therapy are therefore indicated. Also, the search for and investigation of other agents for use as SPV's are indicated. For these reasons the candidate decided to undertake research into the use of IAP as a SPV i.e. SPV's provide for a degree of control of the pulmonary circulation not

previously attainable, and NO (although extensively used) may not be the ideal SPV. The clinical model used for investigation were patients with severe ARDS. This is the one group of patients (the other being patients with varying causes of pulmonary hypertension) who might benefit from refinement of the use of SPV's – to improve the supportive care they receive and limit the morbidity they might suffer as a result of this supportive care. The studies carried out and described in this thesis were undertaken to determine whether IAP is a safe and efficacious SPV for patients with ARDS.

The method of delivery of aerosol during IAP therapy was validated prior to commencement of any further investigational work. The candidate was able to show adequate function of the nebuliser systems used in terms of production of particles in the respirable range and widespread and distal deposition of particles in an animal model. The actual mass of active drug deposited in the distal airways was not determined. This is probably not an important issue, as IAP therapy is titrated to effect. What the candidate showed though is that the drug is indeed acting where it was theoretically postulated it would (i.e. the distal airways), by showing an improvement in oxygenation and a reduction in shunt fraction.

IAP was then compared to NO as a SPV in a pilot study, to decide whether IAP is in fact a viable option to NO as a SPV. That is, there would be no point studying IAP as a SPV if it did not have at least similar actions to NO. This proved to be the case, with the therapeutic effect of IAP being comparable and in some cases superior to NO. This work has subsequently been validated in the literature.

Having identified a viable delivery system and the fact that IAP is indeed a SPV worthy of further study, the candidate undertook to investigate the safety of IAP therapy.

previously attainable, and NO (although extensively used) may not be the ideal SPV. The clinical model used for investigation were patients with severe ARDS. This is the one group of patients (the other being patients with varying causes of pulmonary hypertension) who might benefit from refinement of the use of SPV's – to improve the supportive care they receive and limit the morbidity they might suffer as a result of this supportive care. The studies carried out and described in this thesis were undertaken to determine whether IAP is a safe and efficacious SPV for patients with ARDS.

The method of delivery of aerosol during IAP therapy was validated prior to commencement of any further investigational work. The candidate was able to show adequate function of the nebuliser systems used in terms of production of particles in the respirable range and widespread and distal deposition of particles in an animal model. The actual mass of active drug deposited in the distal airways was not determined. This is probably not an important issue, as IAP therapy is titrated to effect. What the candidate showed though is that the drug is indeed acting where it was theoretically postulated it would (i.e. the distal airways), by showing an improvement in oxygenation and a reduction in shunt fraction.

IAP was then compared to NO as a SPV in a pilot study, to decide whether IAP is in fact a viable option to NO as a SPV. That is, there would be no point studying IAP as a SPV if it did not have at least similar actions to NO. This proved to be the case, with the therapeutic effect of IAP being comparable and in some cases superior to NO. This work has subsequently been validated in the literature.

Having identified a viable delivery system and the fact that IAP is indeed a SPV worthy of further study, the candidate undertook to investigate the safety of IAP therapy.

The main concerns regarding toxicity of IAP therapy were –

- would the highly alkaline buffer in which prostacyclin is dissolved prior to administration as an aerosol cause pulmonary inflammation and
- as a potent anti-platelet agent, would IAP therapy cause a disturbance of platelet function and a bleeding diathesis?

The results of the animal study described in this thesis suggest caution in the volume of alkaline glycine buffer administered during IAP therapy. Pigs receiving nine times the volume of alkaline glycine buffer aerosol developed mild acute tracheitis. The human literature and subsequent animal studies, where clinical doses of IAP were delivered, have not substantiated a risk of acute airway inflammation.

Although significant levels of prostacyclin metabolite were demonstrated in the systemic circulation of human subjects receiving IAP, there was no demonstrable effect on platelet function *in vivo*. A subsequent *in vitro* study, however, did demonstrate the potent anti-platelet action of extremely low concentrations of prostacyclin and/or its metabolites. Reasons for the discrepancy between *in vivo* and *in vitro* findings were discussed in detail. In essence, alternative pathways of platelet activation (e.g. via thrombin) may preserve platelet aggregation *in vivo*. The thrombelastograph (TEG), which measures whole blood clotting, does not show an anti-platelet effect in response to extremely low concentrations of prostacyclin and/or its metabolites. This probably reflects what occurs *in vivo*, rather than the more "artificial", but more sensitive platelet aggregation studies. Also, the findings regarding platelet dysfunction in human subjects with ARDS receiving IAP were not conclusive. However, since prostacyclin has a well-defined site of action in the platelet and since these effects are borne out in the *in vitro* study, the candidate does advise caution in the use of IAP in patients where possible bleeding as a result of IAP therapy may be hazardous (e.g. closed head injury).

Having determined the relative safety of this method of delivery of prostacyclin (i.e. IAP), the candidate then undertook to examine the dose-response relationships of IAP versus indices of oxygenation. IAP therapy improved indices of oxygenation in patients with severe ARDS in a dose-responsive manner over the range 0 – 50 ng/kg/min. There was wide inter-individual response to IAP and in the clinical setting the dose of IAP should be titrated to effect. Associated with the administration of IAP, there was an increase in systemic levels of prostacyclin metabolite. No systemic hypotension resulted from this, nor could an anti-platelet effect be attributed to the systemic levels of prostacyclin metabolite *in vivo*. A worsening of oxygenation due to a reduction of V/Q ratio caused by the downstream effect (i.e. a non-selective pulmonary vasodilator effect) of prostacyclin metabolite could not be shown.

On completion of his research into IAP as a SPV the candidate concluded that –

- IAP administered to patients with hypoxaemia due to ARDS is efficacious (improves oxygenation as determined by PaO_2, $A\text{-}aDO_2$ and shunt fraction in some patients).
- IAP is safe with the proviso that volumes of IAP solution aerosolised during IAP therapy be kept to a minimum to avoid the irritant effect on airways of the alkaline glycine buffer diluent used to dissolve prostacyclin . A potential antiplatelet effect may result from the administration of IAP and this therapy should be avoided where bleeding is a risk.
- No marked effect of IAP could be demonstrated on cardiovascular parameters (either pulmonary or systemic), due to the relatively normal baseline pulmonary arterial (PA) pressures in the study groups and the pulmonary selectivity of IAP. Cardiac output (CO) was unchanged by IAP therapy despite a reduction in shunt fraction (total blood flow through the lung was unchanged, but the distribution was improved).

Clearly no outcome studies were carried out as part of the investigational work and the candidate is unable to comment on the effect of IAP on outcome.

Further study is required to correctly place IAP as a SPV in the therapeutic spectrum. Currently it is a useful agent for patients with severe ARDS who are severely hypoxaemic. IAP allows a reduction in inspired oxygen concentrations in the treatment of these patients. The concerns regarding the potential pulmonary toxicity due to the alkaline diluent used during IAP therapy also requires further investigation. The more stable analogue of prostacyclin, iloprost, has been used as an aerosol for selective pulmonary vasodilation [192], as has prostaglandin E_1 [227]. IAP has therefore opened the path to the investigation of other agents as SPV's. Once an efficacious, non-toxic SPV is identified, there still remains the task of determining optimal doses. However, the candiate suspects that any SPV will have to be titrated to effect due to the wide inter-individual variation to the currently-available SPV's.

Any research into the use of SPV's to place them in the therapeutic spectrum will ideally include large multi-centre trials to determine a positive effect on outcome (in terms of reduced morbidity and mortality) of long-term use of the agent. Initial outcome studies for NO, when used to treat pulmonary hypertension and hypoxaemia in patients with acute lung injury, have recently been reported [119,120]. The results have so far been disappointing with NO therapy conferring no outcome benefit in terms of reduced morbidity or mortality. These results are not entirely unexpected in view of the heterogenous patient population in which ALI/ARDS develops. Further analysis may yet identify subgroups of patients with ALI who will benefit from NO or other SPV therapy. Negative outcome studies, such as those which have recently appeared[119,120] will influence further research into alternate SPV's. These results may cause either a renewed effort to find a substitute for NO or an abandonment of research into SPV's as a non-viable therapeutic option.

The candidate has concentrated on the use of SPV's (and IAP in particular) for the treatment of hypoxaemia associated with ARDS.

There may be many other potential applications for SPV therapy such as –

- quantifying the response to pulmonary vasodilator therapy in severe pulmonary hypertension[182,200,210],
- short-term treatment of severe pulmonary hypertension [169,191]
- treatment of pulmonary hypertension or cardiac (RV) failure post coronary artery bypass grafting [195]
- pulmonary hypertension due to chronic hypoxia associated with chronic obstructive pulmonary disease, in the same way "home" oxygen is now used to reduce HPV.

All of these applications warrant further investigation. The candidate's interest in the use of IAP as a SPV was sparked by a case series of three patients [152]. This has resulted in the research reported in this thesis and also published in the peer-reviewed literature (as listed on pages vi and vii in the Preface and Acknowledgement section). Other assumptions regarding SPV's, such as the notion that simple NO donor molecules, such as sodium nitroprusside, administered as an aerosol would be just as effective as NO as a SPV have proven unfounded. Although pulmonary vasodilation results [228], administration of NO donor molecules as an aerosol causes venous admixture [213]. However, an interesting new area of SPV research has been into the use of NO donors in the form of NONOates. This group of agents, the NONOates, consist of nucleophiles derived from methionine and ornithine (e.g. putreanine, spermine, spermidine), combined with NO. The NONOate is stable as a solid and soluble in water. The solution is aerosolised and once in the lung releases NO over about a two hour period in concentrations of approximately 5 ppm [211-214]. The tertiary amine dimethylaminoethylputreanine-NO or DMAEP/NO is theoretically very attractive in that the polar nucleophile itself does not readily cross the alveolar-capillary barrier, while releasing NO into the alveolus. The NONOates appear to have the efficacy of NO, with all the delivery advantages of IAP.

In conclusion, IAP is a safe and efficacious agent for use as a SPV for the treatment of hypoxaemia in patients with severe ARDS. As such, it is a viable alternative to NO therapy. The entire group of SPV's requires further research to determine their place in the therapeutic spectrum.

Appendices

Appendix A

Raw data for pilot study comparing NO and IAP (Chapter 5).

RAW DATA FOR PILOT STUDY

Appendix A

Variable	Pre NO	Post NO	Pre IAP	Post IAP
PaO_2/FiO_2	90.3	189.1	135.1	191.1
(n=5)	265.4	264.6	181.6	208
	265.8	271.8	197.8	198
	54.2	50.7	46.6	126.3
Shunt	29.2	20.8	23.7	18
(n=5)	22.8	25.6	34	27.4
	18.8	13.3	18.7	12.5
	68	75	75	52
	23	16	20	19
$A\text{-}aDO_2$	394.2	324.6	363.6	332.3
(n=5)	184.5	186.6	226.3	213.4
	181.6	182.8	195.7	161.5
	613	619	616	550
	330	334	360	315
$PaCO_2$	40.4	40.6	39.1	31.4
(n=5)	37.7	35.8	37.5	37.2
	34.9	31.2	31.4	42.1
	45.7	43.1	50.1	36.6
	35.2	30.2	33.2	35.5
MPAP	30	33	39	33
(n=5)	20	19	18	18
	24	23	24	23
	36	39	50	34
	23	22	23	20
metHb	0.5	0.9	0.9	0.9
(n=4)	0.8	0.5	0.7	0.6
	0.5	0.8	0.8	0.6
	1	1.1	1.2	1.1
HR	101	98	103	97
(n=5)	95	95	96	95
	89	87	88	89
	113	114	117	109
	85	83	98	94
MAP	69	66	62	58
(n=5)	77	68	65	70
	80	80	63	63
	69	71	70	60
	67	79	61	65

Variable	Pre NO	Post NO	Pre IAP	Post IAP
CO	8.3	7.9	8.6	7.7
(n=5)	9.5	9.4	8.8	8.8
	9.4	11.8	11.7	9.2
	6.5	5.8	6.5	5.9
PCWP	15	18	19	19
(n=5)	16	12	11	13
	15	16	15	17
	21	19	19	17
	10	9	8	12
CVP	12	13	14	15
(n=5)	19	19	18	19
	15	15	15	15
	19	20	19	17
	9	11	9	8

Appendix B

Raw data for animal study into pulmonary toxicity of IAP– histological grade by subject, site and layer (Chapter 6).

PROSTACYCLIN PROJECT : PIG 0
COUNT OF PMN CELLS IN PROXIMAL AIRWAYS

LARGE CALIBER AIRWAYS :	FIELD 1	FIELD 2	FIELD 3	FIELD 4	FIELD 5	MEAN	MEDIAN
TRACHEA (AT CARINA)							
epithelium	0	3	1	1	1	1.2	1
lamina propria	0	3	1	3	2	1.8	2
submucosa : fibrous	0	3	0	3	0	1.2	0
submucosa : glands	0	3	2	3	1	1.8	2
RIGHT MAIN STEM BRONCHUS							
epithelium	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	0	0	0	0
LEFT MAIN STEM BRONCHUS							
epithelium	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	0	0	0	0

PROSTACYCLIN PROJECT : PIG 1
COUNT OF PMN CELLS IN PROXIMAL AIRWAYS

LARGE CALIBER AIRWAYS :	FIELD 1	FIELD 2	FIELD 3	FIELD 4	FIELD 5	MEAN	MEDIAN
TRACHEA (AT CARINA)							
epithelium	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0
submucosa : fibrous	0	0	0	0	1	0.2	0
submucosa : glands	0	0	0	0	1	0.2	0
RIGHT MAIN STEM BRONCHUS							
epithelium	0	1	0	0	0	0.2	0
lamina propria	0	0	0	0	0	0	0
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	1	0	0	0.2	0
LEFT MAIN STEM BRONCHUS							
epithelium	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	0	0	0	0

PROSTACYCLIN PROJECT : PIG 2
COUNT OF PMN CELLS IN PROXIMAL AIRWAYS

LARGE CALIBER AIRWAYS :	FIELD 1	FIELD 2	FIELD 3	FIELD 4	FIELD 5	MEAN	MEDIAN
TRACHEA (AT CARINA)							
epithelium	1	1	0	0	0	0.4	0
lamina propria	0	0	0	1	2	0.6	0
submucosa : fibrous	0	0	0	0	2	0.4	0
submucosa : glands	0	0	0	0	0	0	0
RIGHT MAIN STEM BRONCHUS							
epithelium	0	0	1	0	1	0.4	0
lamina propria	0	0	1	0	2	0.6	0
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	0	0	0	0
LEFT MAIN STEM BRONCHUS							
epithelium	0	1	1	0	1	0.6	1
lamina propria	0	0	0	0	0	0	0
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands		0	0	0	1	0.25	0

PROSTACYCLIN PROJECT : PIG 4
COUNT OF PMN CELLS IN PROXIMAL AIRWAYS

LARGE CALIBER AIRWAYS :	FIELD 1	FIELD 2	FIELD 3	FIELD 4	FIELD 5	MEAN	MEDIAN
TRACHEA (AT CARINA)							
epithelium	3	1	2	3	1	2	2
lamina propria	0	0	1	2	1	0.8	1
submucosa : fibrous	1	1	0	0	0	0.4	0
submucosa : glands	2	2	3	0	2	1.8	2
RIGHT MAIN STEM BRONCHUS							
epithelium	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	1	0	0.2	0
LEFT MAIN STEM BRONCHUS							
epithelium	2	2	2	0	0	1.2	2
lamina propria	0	1	0	0	1	0.4	0
submucosa : fibrous	0	1	0	0	0	0.2	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	0	0	0	0

PROSTACYCLIN PROJECT : PIG 5
COUNT OF PMN CELLS IN PROXIMAL AIRWAYS

LARGE CALIBER AIRWAYS :	FIELD 1	FIELD 2	FIELD 3	FIELD 4	FIELD 5	MEAN	MEDIAN
TRACHEA (AT CARINA)							
epithelium	0	0	0	0	0	0	0
lamina propria	3	3	3	3	3	3	3
submucosa : fibrous	3	3	3	3	3	3	3
submucosa : glands	3	3	3	3	3	3	3
RIGHT MAIN STEM BRONCHUS							
epithelium	0	0	0	0	0	0	0
lamina propria	1	0	1	1	1	0.8	1
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	2	0	0.4	0
LEFT MAIN STEM BRONCHUS							
epithelium	1	0	1	0	0	0.4	0
lamina propria	1	0	1	1	1	0.8	1
submucosa : fibrous	0	0	1	0	0	0.2	0
submucosa : muscle	0	0	1	0	0	0.2	0
submucosa : glands	0	1	0	1	1	0.6	1

PROSTACYCLIN PROJECT : PIG 6
COUNT OF PMN CELLS IN PROXIMAL AIRWAYS

LARGE CALIBER AIRWAYS :	FIELD 1	FIELD 2	FIELD 3	FIELD 4	FIELD 5	MEAN	MEDIAN
TRACHEA (AT CARINA)							
epithelium	2	0	1	1	1	1	1
lamina propria	2	1	1	3	1	1.6	1
submucosa : fibrous	0	0	0	3	3	1.2	0
submucosa : glands	0	0	0	3	3	1.2	0
RIGHT MAIN STEM BRONCHUS							
epithelium	0	0	0	0	0	0	0
lamina propria	0	0	0	0	1	0.2	0
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	0	0	0	0
LEFT MAIN STEM BRONCHUS							
epithelium	0	1	0	0	0	0.2	0
lamina propria	0	1	1	2	0	0.8	1
submucosa : fibrous	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0
submucosa : glands	0	0	0	1	0	0.2	0

PROSTACYCLIN PROJECT : PIG 0
COUNT OF PMN CELLS IN DISTAL AIRWAYS

BRONCHIOLES :	QUADRANT 1					QUADRANT 2					QUADRANT 3					QUADRANT 4					MEAN	MEDIAN
RIGHT LOWER LOBE - 4																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 5																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 6																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 7																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.05	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 8																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 9																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 10																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 11																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 12																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0.15	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 13																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0.05	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
																					0	
																					0.055	
																					0	

PROSTACYCLIN PROJECT : PIG 1
COUNT OF PMN CELLS IN DISTAL AIRWAYS

BRONCHIOLES :	QUADRANT 1					QUADRANT 2					QUADRANT 3					QUADRANT 4					MEAN	MEDIAN
RIGHT LOWER LOBE - 4																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	1	0	0	0	0	2	2	0	0	0	0	0	0	0.25	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 5																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	2	2	2	1	1	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0.55	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 6																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	1	1	0	0	0	0	0	1	1	0	2	2	2	0	0	0	0.5	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 7																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.15	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 8																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 9																						
epithelium	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0
lamina propria	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0.2	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 10																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0.2	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 11																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 12																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	1	0	0	0	0	1	1	0	0	0	0	1	2	2	0	0	0	0	0	0.4	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 13																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
																					0.01	
																					0.225	
																					0	

PROSTACYCLIN PROJECT : PIG 2
COUNT OF PMN CELLS IN DISTAL AIRWAYS

BRONCHIOLES :	QUADRANT 1					QUADRANT 2					QUADRANT 3					QUADRANT 4					MEAN	MEDIAN
RIGHT LOWER LOBE - 4																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	2	0	0.3	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 5																						
epithelium	0	3	0	1	0	3	3	0	0	0	0	0	0	0	0	0	3	3	0	0	0.8	0
lamina propria	0	2	0	1	0	2	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0.45	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 6																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	1	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0.2	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 7																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0.2	0
lamina propria	3	1	1	1	1	0	1	2	0	0	2	0	2	2	2	1	0	1	2	0	1.1	1
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 8																						
epithelium	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0.15	0
lamina propria	0	0	0	0	0	0	0	0	2	1	2	1	1	0	1	1	0	1	1	0	0.55	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 9																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	2	0	1	1	1	0	1	0	0	1	1	1	2	1	0	0	0	1	0	0	0.65	1
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 10																						
epithelium	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0
lamina propria	0	0	2	2	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0.3	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 11																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.1	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 12																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	1	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0.2	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 13																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0	0	0.2	0
lamina propria	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
																					0.14	
																					0.395	
																					0	

PROSTACYCLIN PROJECT : PIG 4
COUNT OF PMN CELLS IN DISTAL AIRWAYS

BRONCHIOLES :	QUADRANT 1					QUADRANT 2					QUADRANT 3					QUADRANT 4					MEAN	MEDIAN
RIGHT LOWER LOBE - 4																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	0	0	1	0.3	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	2	0	0.25	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 5																						
epithelium	1	2	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0.3	0
lamina propria	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0.25	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 6																						
epithelium	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0.2	0
lamina propria	0	1	0	1	2	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0.3	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 7																						
epithelium	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	3	0	0	0	0.3	0
lamina propria	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0.15	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 8																						
epithelium	0	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	1	1	0.3	0
lamina propria	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.1	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 9																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	1	0	1	0	0	0	1	1	1	0	0	0	0	2	0	0	0.35	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 10																						
epithelium	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0.25	0
lamina propria	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 11																						
epithelium	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.15	0
lamina propria	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	1	1	0	1	0	0.3	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 12																						
epithelium	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0.2	0
lamina propria	2	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0.3	0
submucosa : muscle	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0
LEFT LOWER LOBE - 13																						
epithelium	0	0	0	0	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0.2	0
lamina propria	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0.2	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
																					0.22	
																					0.225	
																					0.005	

PROSTACYCLIN PROJECT : PIG 5
COUNT OF PMN CELLS IN DISTAL AIRWAYS

BRONCHIOLES :	QUADRANT 1					QUADRANT 2					QUADRANT 3					QUADRANT 4					MEAN	MEDIAN
RIGHT LOWER LOBE - 4																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.05	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 5																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	2	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0.25	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 6																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 7																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 8																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 9																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 10																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 11																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.05	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 12																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.05	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 13																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
																					0	
																					0.04	
																					0	

PROSTACYCLIN PROJECT : PIG 6
COUNT OF PMN CELLS IN DISTAL AIRWAYS

BRONCHIOLES :	QUADRANT 1					QUADRANT 2					QUADRANT 3					QUADRANT 4					MEAN	MEDIAN
RIGHT LOWER LOBE - 4																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0.05	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 5																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 6																						
epithelium	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0
lamina propria	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	1	1	0	0.25	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 7																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0.2	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 8																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.15	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 9																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 10																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0.1	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 11																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 12																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.1	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 13																						
epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0.15	0
submucosa : muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
																					0.005	
																					0.1	
																					0	

COUNT OF PMN CELLS IN DEISTAL AIRWAYS
SUMMARY TABLE

	pigs 0	1	2	4	5	6	mean
epithelium	0	0	0	0	0	0	0
lamina propria	0	0	0	0	0	0	0
(muscularis)	0	0	0	0	0	0	0
average	0	0	0	0	0	0	0

PROSTACYCLIN PROJECT : PIG 0
COUNT OF PMN CELLS IN THE ALVEOLAR REGION

ALVEOLI :	QUADRANT 1	QUADRANT 2	QUADRANT 3	QUADRANT 4	QUADRANT 5	MEAN
RIGHT LOWER LOBE - 4	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 5	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 6	0 0	1 0	1 0	0 0	1 0	0.3
RIGHT LOWER LOBE - 7	0 0	0 0	0 0	1 0	0 0	0.1
RIGHT LOWER LOBE - 8	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 9	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 10	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 11	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 12	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 13	0 0	0 0	0 0	0 0	0 0	0

MEAN = 0.04

PROSTACYCLIN PROJECT : PIG 1
COUNT OF PMN CELLS IN THE ALVEOLAR REGION

ALVEOLI :	QUADRANT 1	QUADRANT 2	QUADRANT 3	QUADRANT 4	QUADRANT 5	MEAN
RIGHT LOWER LOBE - 4	0 0	0 0	1 0	0 0	0 0	0.1
RIGHT LOWER LOBE - 5	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 6	0 0	0 0	0 0	0 0	0 1	0.1
RIGHT LOWER LOBE - 7	0 0	0 0	0 1	1 0	0 1	0.3
RIGHT LOWER LOBE - 8	0 0	0 0	0 1	0 0	0 0	0.1
LEFT LOWER LOBE - 9	0 0	1 0	0 0	0 0	0 0	0.1
LEFT LOWER LOBE - 10	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 11	1 0	0 0	0 0	0 0	0 0	0.1
LEFT LOWER LOBE - 12	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 13	0 0	0 0	0 0	0 0	0 0	0

MEAN = 0.06

PROSTACYCLIN PROJECT : PIG 2
COUNT OF PMN CELLS IN THE ALVEOLAR REGION

ALVEOLI :	QUADRANT 1		QUADRANT 2		QUADRANT 3		QUADRANT 4		QUADRANT 5		MEAN
RIGHT LOWER LOBE - 4	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 5	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 6	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 7	1	1	2	0	0	0	0	0	2	0	0.6
RIGHT LOWER LOBE - 8	1	0	0	0	0	0	1	0	0	0	0.2
LEFT LOWER LOBE - 9	2	1	0	0	0	0	0	0	0	0	0.3
LEFT LOWER LOBE - 10	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 11	1	0	0	0	0	0	0	0	0	0	0.1
LEFT LOWER LOBE - 12	0	0	0	0	0	0	0	1	0	1	0.2
LEFT LOWER LOBE - 13	1	0	0	0	0	0	0	0	0	0	0.1

MEAN = 0.15

PROSTACYCLIN PROJECT : PIG 4
COUNT OF PMN CELLS IN THE ALVEOLAR REGION

ALVEOLI :	QUADRANT 1		QUADRANT 2		QUADRANT 3		QUADRANT 4		QUADRANT 5		MEAN
RIGHT LOWER LOBE - 4	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 5	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 6	0	0	0	0	0	1	0	0	0	0	0.1
RIGHT LOWER LOBE - 7	0	0	0	0	0	0	0	0	0	0	0
RIGHT LOWER LOBE - 8	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 9	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 10	0	0	1	0	1	0	0	0	0	0	0.2
LEFT LOWER LOBE - 11	0	0	0	0	1	0	0	0	0	0	0.1
LEFT LOWER LOBE - 12	0	0	0	0	0	0	0	0	0	0	0
LEFT LOWER LOBE - 13	0	0	0	0	0	0	0	0	0	0	0

MEAN = 0.04

PROSTACYCLIN PROJECT : PIG 5
COUNT OF PMN CELLS IN THE ALVEOLAR REGION

ALVEOLI :	QUADRANT 1	QUADRANT 2	QUADRANT 3	QUADRANT 4	QUADRANT 5	MEAN
RIGHT LOWER LOBE - 4	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 5	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 6	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 7	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 8	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 9	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 10	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 11	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 12	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 13	0 0	0 0	0 0	0 0	0 0	0

MEAN = 0

PROSTACYCLIN PROJECT : PIG 6
COUNT OF PMN CELLS IN THE ALVEOLAR REGION

ALVEOLI :	QUADRANT 1	QUADRANT 2	QUADRANT 3	QUADRANT 4	QUADRANT 5	MEAN
RIGHT LOWER LOBE - 4	0 0	0 0	0 0	1 0	0 0	0.1
RIGHT LOWER LOBE - 5	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 6	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 7	0 0	0 0	0 0	0 0	0 0	0
RIGHT LOWER LOBE - 8	1 0	0 0	0 0	0 0	0 0	0.1
LEFT LOWER LOBE - 9	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 10	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 11	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 12	0 0	0 0	0 0	0 0	0 0	0
LEFT LOWER LOBE - 13	0 0	0 0	0 0	0 0	0 0	0

MEAN = 0.02

COUNT OF PMN CELLS IN TRACHEA								
SUMMARY TABLE								
pigs :	0	1	2	4	5	6		mean
epithelium	1.20	0.00	0.40	2.00	0.00	1.00		0.77
lamina propria	1.80	0.00	0.60	0.80	3.00	1.60		1.30
submucosa : fibrous	1.20	0.20	0.40	0.40	3.00	1.20		1.07
submucosa : glands	1.8	0.2	0	1.8	3	1.2		1.33333
mean	1.50	0.10	0.35	1.25	2.25	1.25		1.12
COUNT OF PMN CELLS IN MAIN BRONCHIAL SEGMENTS								
SUMMARY TABLE								
pigs :	0	1	2	4	5	6		mean
epithelium	0.00	0.10	0.50	0.60	0.20	0.10		0.25
lamina propria	0.00	0.00	0.30	0.20	0.80	0.50		0.30
submucosa : fibrous	0.00	0.00	0.00	0.10	0.10	0.00		0.03
submucosa : muscle	0.00	0.00	0.00	0.00	0.10	0.00		0.02
submucosa : glands	0.00	0.10	0.13	0.10	0.50	0.10		0.15
mean	0	0.04	0.185	0.2	0.34	0.14		0.15083
COUNT OF PMN CELLS IN DISTAL AIRWAYS								
SUMMARY TABLE								
pigs :	0	1	2	4	5	6		mean
epithelium	0.00	0.01	0.14	0.22	0.00	0.01		0.06
lamina propria	0.06	0.23	0.40	0.23	0.04	0.10		0.17
(muscularis)	0.00	0.00	0.00	0.01	0.00	0.00		0.00
mean	0.0275	0.1175	0.2675	0.2225	0.02	0.0525		0.11792
COUNT OF PMN CELLS IN ALVEOLAR SPETA								
SUMMARY TABLE								
pigs :	0	1	2	4	5	6		mean
septa	0.04	0.08	0.15	0.04	0.00	0.02		0.06

COUNT OF PMN CELLS IN TRACHEA
SUMMARY TABLE

pigs :	0	1	2	4	5	6	mean
epithelium	1.20	0.00	0.40	2.00	0.00	1.00	0.77
lamina propria	1.80	0.00	0.60	0.80	3.00	1.60	1.30
submucosa : fibrous	1.20	0.20	0.40	0.40	3.00	1.20	1.07
submucosa : glands	1.80	0.20	0.00	1.80	3.00	1.20	1.33
mean	1.50	0.10	0.35	1.25	2.25	1.25	1.12

COUNT OF PMN CELLS IN MAIN BRONCHIAL SEGMENTS
SUMMARY TABLE

pigs :	0	1	2	4	5	6	mean
epithelium	0.00	0.10	0.50	0.60	0.20	0.10	0.25
lamina propria	0.00	0.00	0.30	0.20	0.80	0.50	0.30
submucosa : fibrous	0.00	0.00	0.00	0.10	0.10	0.00	0.03
submucosa : muscle	0.00	0.00	0.00	0.00	0.10	0.00	0.02
submucosa : glands	0.00	0.10	0.13	0.10	0.50	0.10	0.15
mean	0.00	0.04	0.19	0.20	0.34	0.14	0.15

COUNT OF PMN CELLS IN DISTAL AIRWAYS
SUMMARY TABLE

pigs :	0	1	2	4	5	6	mean
epithelium	0.00	0.01	0.14	0.22	0.00	0.01	0.06
lamina propria	0.06	0.23	0.40	0.23	0.04	0.10	0.17
(muscularis)	0.00	0.00	0.00	0.01	0.00	0.00	0.00
mean	0.03	0.12	0.27	0.22	0.02	0.05	0.12

COUNT OF PMN CELLS IN ALVEOLAR SPETA
SUMMARY TABLE

pigs :	0	1	2	4	5	6	mean
septa	0.04	0.08	0.15	0.04	0.00	0.02	0.06

COUNT OF PMN CELLS IN TRACHEA
SUMMARY TABLE

pigs :	0	1	2	4	5	6	mean
epithelium	1.20	0.00	0.40	2.00	0.00	1.00	0.77
lamina propria	1.80	0.00	0.60	0.80	3.00	1.60	1.30
submucosa : fibrous	1.20	0.20	0.40	0.40	3.00	1.20	1.07
submucosa : glands	1.80	0.20	0.00	1.80	3.00	1.20	1.33
mean	1.50	0.10	0.35	1.25	2.25	1.25	1.12

COUNT OF PMN CELLS IN MAIN BRONCHIAL SEGMENTS
SUMMARY TABLE

pigs :	0	1	2	4	5	6	mean
epithelium	0.00	0.10	0.50	0.60	0.20	0.10	0.25
lamina propria	0.00	0.00	0.30	0.20	0.80	0.50	0.30
submucosa : fibrous	0.00	0.00	0.00	0.10	0.10	0.00	0.03
submucosa : muscle	0.00	0.00	0.00	0.00	0 10	0.00	0.02
submucosa : glands	0.00	0.10	0.13	0.10	0.50	0.10	0.15
mean	0.00	0.04	0.19	0.20	0.34	0.14	0.15

COUNT OF PMN CELLS IN DISTAL AIRWAYS
SUMMARY TABLE

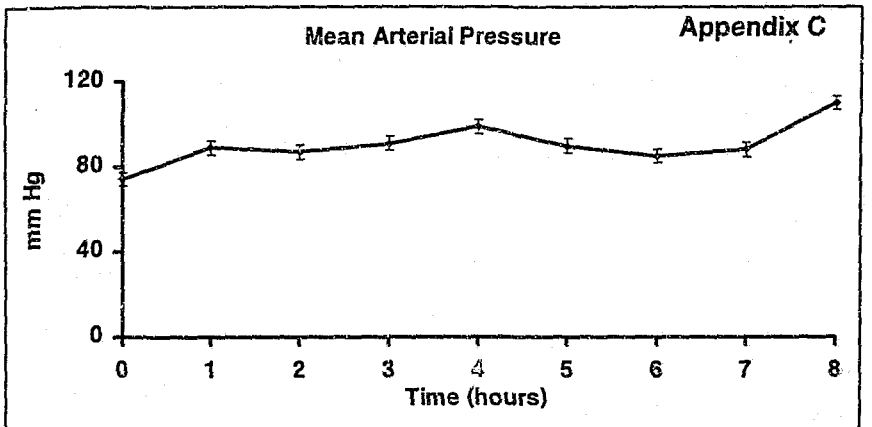
pigs :	0	1	2	4	5	6	mean
epithelium	0.00	0.01	0.14	0.22	0.00	0.01	0.06
lamina propria	0.06	0.23	0.40	0.23	0.04	0.10	0.17
(muscularis)	0.00	0.00	0.00	0.01	0.00	0.00	0.00
mean	0.03	0.12	0.27	0.22	0.02	0.05	0.12

COUNT OF PMN CELLS IN ALVEOLAR SPETA
SUMMARY TABLE

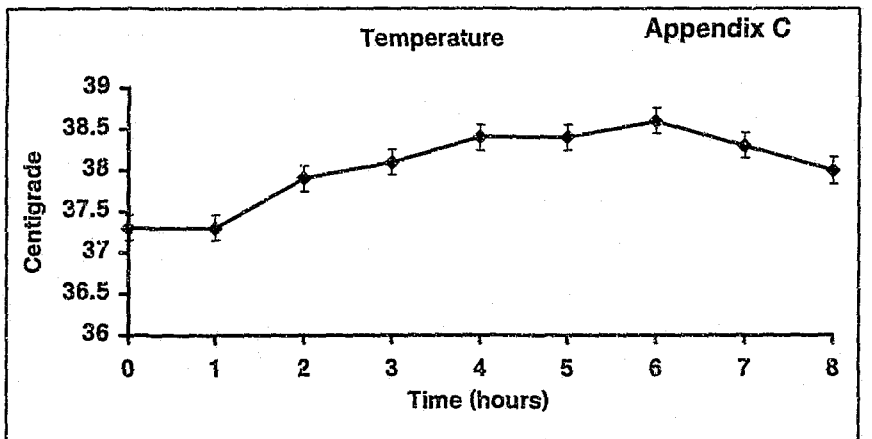
pigs :	0	1	2	4	5	6	mean
septa	0.04	0.08	0.15	0.04	0.00	0.02	0.06

Appendix C

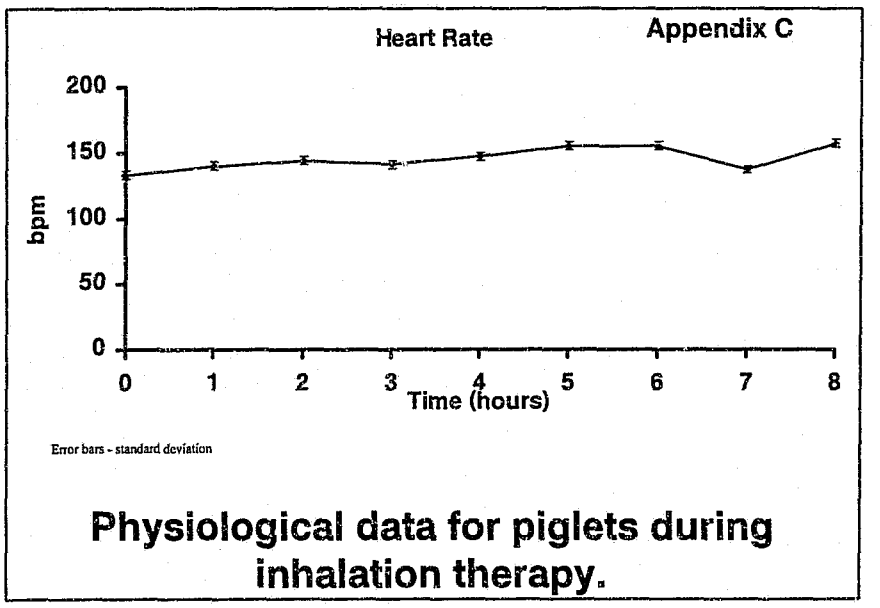
Summary physiological data for animal study into pulmonary toxicity of IAP (Chapter 6).



Physiological data for piglets during inhalation therapy.



Physiological data for piglets during inhalation therapy.



Physiological data for piglets during inhalation therapy.

Appendix D

Raw data for dose-response study (Chapter 7).

- Raw data for platelet function studies
- Individual subject data

RAW DATA FOR PLATELET FUNCTION STUDIES (CHAPTER 7)

Patient No	iAP Dose ng/kg/min	0	10	20	30	40	50	0
1.	max. aggreg.	70	70	50	56	57	58	42
2.		24	4	6	24	15	9	23
3.		25	23	36	3	42	62	
4.		40	71	66	35	56	78	32
5.		15	12	20	16	21	17	16
6.		32	22	25	31	18	60	30
7.		43	42	25	32	45	46	70
8.		18	19	17	14	10	19	13
9.		100	100	100	100	100	100	100
1.	agg@4min	70	64	49	53	56	55	40
2.		16	2	4	15	12	6	8
3.		20	18	30	3	35	50	
4.		40	65	60	21	51	76	23
5.		12	12	18	13	15	12	13
6.		20	5	5	30	0	50	7
7.		35	29	14	14	20	24	62
8.		5	7	10	7	5	11	3
9.		100	100	100	100	100	100	95

max aggreg. -Maximum aggregation in response to adenosine diphosphate.

agg @ 4min -Aggregation at 4 minutes in response to adenosine diphosphate.

ng/kg/min -nanograms per kilogram per minute

Note: All results tabulated are expressed as % light transmission

Patient		No :	1
Age :	51	Ventilation	Vm: 12.4
Gender :	m		rate:18/18
APACHE II :	14		Vt: 686
LIS :	3		PEEP: 10
PCWP :	14		PS: 20
Weight :	75kg		FiO_2: 0.8

Diagnosis : Pneumonia; CAL; asthma

Infusions : Mor/mid 8 N.sal 120 Salbut 20 Erythrom

Time Period	0	1	2	3	4	5	6
IAP dose	0	10	20	30	40	50	0

FiO2	0.8	0.8	0.8	0.8	0.8	0.8	0.8
SaO2	94.9	93.5	94.6	95.2	96.2	96.5	96.4
PaO2	84.4	77.9	86.8	92.9	101.3	109.8	108.4
SVO2	75.2	78.9	75.6	77.2	75.6	76.8	77.3
PVO2	44.5	48.1	44.3	44.5	43.6	45.3	45.5
A - aDO2	441	446.3	439.1	434.7	426.8	419.8	420.7
% Shunt	42%	49.90%	41.30%	41.40%	38.10%	36.60%	36.90%
DO2	1156	1148	1270	1184	1053	1091	1200
VO2	246	183	261	232	234	232	248
PaCO2	44.2	45.1	43.2	41.3	40.7	39.2	39.6
PaO_2/FiO_2	105.5	97.4	108.5	116.1	126.6	137.2	135.5
PCWP	14	15	14	14	15	13	14
CVP	16	16	16	16	16	16	15
C.O.	9.1	8	8.8	8.4	7.4	7.8	8.3
C.I.	4.8	4.2	4.7	4.4	3.9	4.1	4.4
H.R.	125	124	122	120	118	118	117
MAP	61	59	63	60	59	66	65
MPAP	38	36	36	36	34	33	34
SVR	396	430	427	419	465	523	463
SVRI	748	813	807	792	879	988	875
PVR	211	210	200	210	205	205	183
PVRI	399	397	378	397	387	387	346
Hb	11.2	=	=	=	=	=	=
Temp.	35.9			35.6	35.6	35.5	35.5

Patient No : 2

Age :	75	**Ventilation**	Vm: 21
Gender :	m		rate: 37
APACHE II :	19		Vt: 605
LIS :	3		PEEP: 12
PCWP :	12		PS: 20
Weight :	82.7		FiO_2: 0.75

Diagnosis : Post emergency AAA repair

Infusions : ng.feed 60

Time Period	0	1	2	3	4	5	6
IAP dose	0	10	20	30	40	50	0

FiO2	0.75	0.75	0.75	0.75	0.75	0.75	0.75
SaO2	93.3	98.6	96	94.2	94.7	94.5	91.8
PaO2	72.8	136.8	83.2	74.2	77.1	75.6	65.1
SVO2	60.9	66.2	65.7	64.3	63.7	63.6	62.9
PVO2	36.5	39.9	37	35.9	35.3	34.5	34
A - aDO2	415.8	353.3	398.1	406	403.3	404.8	409.1
% Shunt	33.30%	22.30%	30%	33.40%	31.60%	32.20%	37.30%
DO2	853	1002	943	931	895	927	892
VO2	298	340	302	298	296	307	283
PaCO2	53.2	52.6	55.6	56.4	56.1	55.3	57.6
PaO_2/FiO_2	97.1	182.4	110.9	98.9	102.8	100.8	86.8
PCWP	12	18	18	20	16	19	22
CVP	12	10	9	8	9	9	11
C.O.	7.3	8.1	7.9	7.9	7.5	7.9	7.6
C.I.	3.6	4	3.9	3.9	3.7	3.9	3.8
H.R.	116	121	112	112	111	121	125
MAP	78	80	75	78	83	78	85
MPAP	43	39	37	35	37	40	39
SVR	723	691	668	709	789	699	779
SVRI	1460	1396	1349	1432	1594	1412	1574
PVR	340	207	192	152	203	213	179
PVRI	687	418	388	307	410	430	362
Hb	8.9						
Temp.	38.3		37.6	37.6	37.6	37.6	37.6

Patient No : 3

Age :	56	**Ventilation:**	Vm: 12
Gender :	M		rate: 15
APACHE II :	20		Vt: 848
LIS :	2.5		PEEP: 10
PCWP :	18		CMV
Weight :	83.5		FiO_2: 0.5

Diagnosis : Pancreatitis; MOF

Infusions : Adrenaline @ 3ml / hr

Time Period	0	1	2	3	4	5	6
IAP dose	0	10	20	30	40	50	0

FiO2	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SaO2	98.2	98.7	97.6	98.5	98.3	98.4	98.2
PaO2	106	115.7	100.5	115.8	115.5	131.4	127.6
SVO2	75	73.1	68.9	71.8	71.8	71.9	73.9
PVO2	41.5	38.5	40.4	39.8	40.8	42.2	45.1
A - aDO2	186.9	185.4	201.9	187	187.6	171.3	176.5
% Shunt	18.70%	16.80%	17.90%	17.60%	17.90%	15.50%	16.60%
DO2	1432	1203	1295	1247	1189	1399	1549
VO2	350	325	388	352	332	392	398
PaCO2	53.4	47.5	47.8	48.8	49.5	51.6	51.4
PaO_2/FiO_2	212	231.4	201	231.6	231	262.8	255.2
PCWP	14	12	13	13	17	17	10
CVP	14	12	11	12	10	14	11
C.O.	10.6	9.6	9.4	10.8	9.9	10.4	11
C.I.	5.1	4.6	4.5	5.2	4.7	5	5.3
H.R.	113	112	111	115	116	117	120
MAP	69	69	63	67	69	71	65
MPAP	26	25	26	30	27	25	27
SVR	415	475	443	407	477	438	393
SVRI	867	993	926	851	997	915	821
PVR	91	108	111	126	81	62	124
PVRI	190	226	232	263	169	130	259
Hb	10.2	10.2	10.2	10.2	10.2	10.2	10.2
Temp.	37	37.2	37.3	37.6	37.7	37.9	38

Patient No : 4

Age :	77	**ventilation:**	Vm: 22.4
Gender :	M		rate:32/16
APACHE II :	8		Vt:700
LIS :	2.5		PEEP:10
PCWP :	16		PS:20
Weight :	80kg		

Diagnosis : gastric outlet obstruction - aspiration pneumonitis

Infusions : no inotropes

Time Period	0	1	2	3	4	5	6
IAP dose	0	10	20	30	40	50	0

FiO2	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SaO2	95.5	95.7	96.3	96.3	96.1	96.8	96.1
PaO2	77.6	75.4	77.5	82.7	80	85.8	83.2
SVO2	60.3	58.1	56.7	57	57.2	59.9	58.2
PVO2	33.1	31.3	30.4	31.5	31.4	32.2	33.5
A - aDO2	237.8	247.4	246.1	242.6	245.2	236.8	239.6
% Shunt	21.1	20.3	18.2	18	18.1	17.9	18.6
DO2	711	704	658	666	700	687	699
VO2	265	279	273	274	285	265	279
PaCO2	44.7	38.5	37.7	37	37.3	38.8	40.3
PaO_2/FiO_2	155.2	150.8	155	165.4	160	171.6	166.4
PCWP	12	12	17	14	14	15	13
CVP	11	9	11	10	10	8	9
C.O.	5.6	5.6	5.1	5.1	5.1	5.3	5.3
C.I.	2.9	2.9	2.6	2.6	2.6	2.7	2.7
H.R.	80	75	72	81	77	77	77
MAP	65	66	71	75	73	70	69
MPAP	24	24	25	26	26	24	24
SVR	786	800	941	1020	973	875	986
SVRI	1541	1568	1844	1999	1907	1715	1776
PVR	171	171	125	188	188	121	166
PVRI	335	335	245	368	368	237	325
Hb	10	10	10	10	10	10	10
Temp.	38.4	38.6	38.6	387	38.8	38.6	38.9

Patient Study No : 5

Age :	76	**ventilation**	Vm:11.2
Gender :	M		PEEP:2.5
APACHE II :	11		PC:yes
LIS :	2.75		Vt:700
PCWP :	13		rate:16
Weight :	85 kg		FiO_2:0.6

Diagnosis : Haemorrhagic pancreatitis; aspiration pneumonitis

Infusions : No inotropes

Time Period	0	1	2	3	4	5	6
IAP dose	0	10	20	30	40	50	0

FiO2	0.6	0.6	0.6	0.6	0.6	0.6	0.6
SaO2	96.5	97.8	97.8	97.4	97.5	96.9	93.8
PaO2	77.5	87.9	93.2	87.7	93.3	87	65.6
SVO2	75.6	77.1	79.7	79.4	81.1	83.3	77.3
PVO2	39.3	36.8	40.4	41.2	44.4	46.3	41.1
A - aDO2	259.9	279.5	273.1	275.5	266.6	270.9	295.7
% Shunt	29.2	25.5	27.4	28.9	30.1	35.8	40.4
DO2	1251	1334	1312	1457	1573	1574	1468
VO2	277	291	252	277	274	229	263
PaCO2	48.1	44.3	46.5	49.8	52.7	55.2	53
PaO_2/FiO_2	129.2	146.5	155.3	146.2	155.5	145	109.3
PCWP	10	13	11	8	12	9	6
CVP	4	5	6	7	7	8	8
C.O.	8.2	8.7	8.5	9.5	10.2	10.1	9.9
C.I.	4.1	4.3	4.2	4.7	5.1	5	4.9
H.R.	105	103	105	109	113	120	117
MAP	103	111	109	101	99	98	90
MPAP	25	29	27	29	27	31	25
SVR	966	975	969	792	714	713	671
SVRI	1942	1960	1948	1592	1435	1433	1349
PVR	146	147	104	177	118	174	162
PVRI	293	295	304	356	237	350	326
Hb	10.4	10.4	10.4	10.4	10.4	10.4	10.4
Temp.	35.7	35.7	35.9	36	36	36	36.1

Patient No : 6

Age :	52	**ventilation**	Vm: 18
Gender :	F		PEEP: 5
APACHE II :	17		PS: 20
LIS :	2.75		Vt: 600
PCWP :	10		rate:30/12
Weight :	40 kg		FiO_2: 0.5

Diagnosis : Scleroderma / Pneumococcal pneumonia

Infusions : No inotropes

Time Period	0	1	2	3	4	5	6
IAP dose :	0	10	20	30	40	50	0

FiO2	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SaO2	99.7	99.8	100	100.1	100.2	99.6	100.2
PaO2	179.2	210.2	208	205.1	199.3	183.6	183.8
SVO2	57.8	57.5	53.8	55.5	54.8	56.3	51.2
PVO2	29.6	28.3	27.9	27.4	27.1	27.6	27
A - aDO2	138.9	112	116.1	119.3	125.9	141.2	141.2
% Shunt	7.8	5.5	5.7	6.6	6.6	7.7	9.3
DO2	262	267	243	221	242	283	213
VO2	115	119	117	104	115	128	109
PaCO2	34.3	29.8	26.5	27.5	26.7	26.9	27.4
PaO_2/FiO_2	358.4	420.4	416	410.2	398.6	367.2	367.6
PCWP	17	18	18	18	19	19	17
CVP	16	16	14	15	13	15	16
C.O.	2.3	2.2	2.2	2.2	2.3	2.4	2.3
C.I.	1.7	1.6	1.6	1.6	1.7	1.8	1.7
H.R.	71	71	71	71	71	71	73
MAP	75	78	81	83	82	81	81
MPAP	27	30	29	31	30	29	30
SVR	2052	2255	2400	2473	2365	2200	2261
SVRI	2770	3044	3240	3339	3193	2970	3052
PVR	348	436	400	436	383	333	452
PVRI	470	589	540	589	517	450	610
Hb	11.9	11.9	11.9	11.9	11.9	11.9	11.9
Temp.	36.7	36.6	36.7	36.6	36.6	36.6	36.7

Patient Study No : 7

Age :	68	**ventilation**	Vm: 21
Gender :	F		PEEP: 10
APACHE II :	20		SIMV/PS
LIS :	2.75		Vt: 750
PCWP :	≤ 18		Rate:30/12
Weight :	100 kg		FiO_2: 0.5

Diagnosis : MVA: 1) Pulmonary contusion; 2) Biliary leak/abdomen.

Infusions : Nil

Time Period :	0	1	2	3	4	5	6
IAP dose :	0	10	20	30	40	50	0

FiO2	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SaO2	97.2	97	97.9	97.6	97.9	97.7	97
PaO2	93.6	91.6	96.8	99.4	102.8	101.1	89.7
SVO2	65	63.1	63	62.2	59.6	65.8	66.9
PVO2	35.7	34.3	33.2	34.1	33.1	37.3	38.7
A - aDO2	226	229	219.9	218.7	219	217	228
% Shunt	19	19	16	16.6	15	18	20
DO2	717.10	654	591	673.7	661.9	591	654
VO2	242.3	232.5	214.7	248.2	264	197	206.9
PaCO2	32.9	32.2	31.8	30.7	30.5	34.3	34.1
PaO_2/FiO_2	187.2	183.2	193.6	198.8	205.6	202.2	179.4
PCWP	15	13	14	14	13	14	14
CVP	11	12	11	12	14	12	13
C.O.	4.9	4.7	4.2	4.8	4.7	4.2	4.7
C.I.	2.5	24	2.1	2.4	2.4	2.1	2.4
H.R.	95	94	90	91	90	96	94
MAP	74	72	70	78	77	78	76
MPAP	24	23	23	24	25	25	27
SVR	1029	1021	1124	1100	1072	1257	1072
SVRI	2027	2011	2214	2167	2112	2476	2112
PVR	147	170	171	167	204	210	221
PVRI	290	335	337	329	402	414	435
Hb	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Temp.	37.6	37.7	37.8	37.9	38.1	38.1	38.2

Patient Study No : 8

Age :	70	**ventilation**	9
Gender :	Male		10
APACHE II :	16		vol. contr.
LIS :	2.5		850
PCWP :	17		10
Weight :	130		0.55

Diagnosis : Gram negative septicaemia

Infusions : Nor-adrenaline: 14, Adrenaline: 16, Dopamine: 3.

Time Period:	0	1	2	3	4	5	6
IAP dose:	0	10	20	30	40	50	0

FiO2	0.55	0.55	0.55	0.55	0.55	0.55	0.55
SaO2	98	98	98.6	98.2	98.7	98.7	98.1
PaO2	104	117	153.6	138.3	159.9	156.2	124.3
SVO2	76	71.8	82	70.5	84.2	68.5	68.8
PVO2	40.3	42.1	50.2	41.7	53.4	39.7	40.7
A - aDO2	238.2	221.9	185.8	199.6	177.1	180	213.1
% Shunt	28	21.4	23.2	17.9	12.5	14.3	18.1
DO2	927	1116	1230	1150	1304	1230	1221
VO2	215	308	230	340	217	395	378
PaCO2	43.7	46.2	45.7	47	47.6	48.2	47
PaO_2/FiO_2	189	212.7	280	251	291	284	225
PCWP	17	13	11	15	12	13	11
CVP	16	13	12	13	12	11	11
C.O.	9.6	9.3	9.9	9.3	10.4	9.8	10
C.I.	4.1	4	4.2	4	4.4	4.2	4.3
H.R.	105	104	100	101	100	100	102
MAP	80	80	77	81	79	78	80
MPAP	29	29	28	27	26	25	29
SVR	533	576	525	585	515	547	552
SVRI	1253	1354	1234	1375	1210	1285	1297
PVR	100	138	137	103	108	98	144
PVRI	235	324	322	242	254	230	338
Hb	90						
Temp.	38.2	38.2	38.3	38.3	38.3	38.4	38.4

Patient Study No : 9

Age :	33	**ventilation**	Vm 10.3
Gender :	F		PEEP 5
APACHE II :	15		PC 25
LIS :	2.5		Vt 593
PCWP :	14		rate 18
Weight :	81		FiO_2 1.0

Diagnosis : ARDS post resection of adrenal Ca

Infusions : Morphine/Midazolam

Time Period	0	1	2	3	4	5	6
IAP dose	0	10	20	30	40	50	0

FiO2	1	1	1	1	1	1	1
SaO2	98.8	98.9	99	99	99.2	98.8	98.7
PaO2	204	235.1	257.1	260	245	267 5	244
SVO2	74.7	76.2	77.7	77.6	78.3	78.2	78.7
PVO2	40.2	41.6	43.8	44.1	45	45	45.4
A - aDO2	465.6	434.9	411.6	407.8	422.4	400.8	423.6
% Shunt	39.1	31.1	33	31.5	36.1	34	36
DO2	708	1001	883	987	758	823	856
VO2	203	266	232	256	197	215	212
PaCO2	36.1	35.9	37.2	38.3	38.6	37.6	38
PaO_2/FiO_2	204	235	257	260	245	267	244
PCWP	12	13	11	11	10	9	10
CVP	11	10	9	8	8	10	8
C.O.	9	8.7	9	9.1	8.6	8.7	8.8
C.I.	4.7	4.6	4.7	4.8	4.5	4.6	4.6
H.R.	86	84	84	83	84	83	83
MAP	96	100	103	103	94	102	102
MPAP	25	25	25	25	23	23	23
SVR	684	828	836	835	800	846	855
SVRI	1300	1573	1588	1587	1520	1607	1625
PVR	116	120	124	105	121	138	127
PVRI	220	228	236	200	230	262	241
Hb	81	81	81	81	81	81	81
Temp.	37.5	37.5	37.5	37.5	37.5	37.4	37.5

Appendix E

Summary statistics for dose-response study (Chapter 7).

Summary Statistics

Variable	Parameter estimate	se	t	p
PaO_2/FiO_2	5.30	1.72	3.09	0.004
A-aDO_2	-3.00	1.14	-2.63	0.01
% shunt	-0.49	0.26	-1.87	0.07
MPAP	-0.12	0.13	-0.88	0.38
MAP	0.72	0.39	1.84	0.07
6-ketoPGF1α	41.60	11.67	3.56	0.001
Max. aggregation	1.20	0.89	1.34	0.18
Aggreg. – 4 minutes	0.86	0.91	0.94	0.35

max. aggregation - maximum platelet aggregation in response to adenosine diphosphate
aggre. – 4 min. - platelet aggregation at 4 minutes in response to adenosine diphosphate
se - standard error
t - t value
p - p value

Appendix F

Raw data for *in vitro* platelet aggregation study (Chapter 8).

Subject number :	1
Age :	44
Gender :	F

Date :	21/09/96
Medication :	NIL

Prostacyclin dose (pg / ml)	0	10	100	500
R	23	9.5	10.5	1.5
K	8	3	16	4
MA	50	65.5	38	59.5
Other				
Angle	43.5	67.5	34	65.5
Channel	1	2	3	4
Specimen code	0	1	5	10

Platelet aggregation response	0	10	100	500
ADP (max) 1 umol	100	0	0	0
Slope	45	0	0	0
ADP 4 min.	80	0	0	0
ADP (max) 8 umol	100	84	94	0
Slope	60	38	3.5	0
ADP 4 min.	100	67	94	0
10 ug Collagen (max)	100	100	94	24
Slope	59	60	22	3.5
4 min.	100	10	94	20

Subject number :	II
Age :	28
Gender :	F

Date :	21/09/96
Medication :	Nil

Prostacyclin dose (pg / ml)	0	10	100	500
R	19	26.5	31.5	22.5
K	7	8	10	37
MA	60.5	66.5	70	23
Other				
Angle	49.5	51	40.5	22.5
Channel	2	3	4	1
Specimen code	0	1	5	10

Platelet aggregation response	0	10	100	500
ADP (max) 1 umol	94	0	0	0
Slope	40	0	0	0
ADP 4 min.	94	0	0	0
ADP (max) 8 umol	100	63	15	0
Slope	60	12.5	1	0
ADP 4 min.	100	42	10	0
10 ug Collagen (max)	97	96	77	17
Slope	60	53	18	2
4 min.	97	96	65	14

Subject number :	III
Age :	31
Gender :	M

Date :	21/09/9[illegible]
Medication :	Nil

Prostacyclin dose (pg / ml)	0	10	100	500
R	33	31.5	36.5	28.5
K	14.5	16.5	12	16
MA	51	44	44	62
Other				
Angle	28.5	24.5	37.5	27
Channel	3	4	1	2
Specimen code	0	1	5	10

Platelet aggregation response	0	10	100	500
ADP (max) 1 umol	51	0	0	0
Slope	45	0	0	0
ADP 4 min.	20	0	0	0
ADP (max) 8 umol	92	33	0	0
Slope	60	7.5	0	0
ADP 4 min.	92	26	0	0
10 ug Collagen (max)	93	87	20	17
Slope	60	22	4	3.5
4 min.	93	80	15	15

Subject number :	IV
Age :	41
Gender :	F

Date :	21/09/96
Medication :	
Minipill	

Prostacyclin dose (pg / ml)	0	10	100	500
R	37.5	23	30	41.5
K	14	9	10	28
MA	56.5	56.5	59.5	53
Other				
Angle	32	44	37.5	18
Channel	4	1	2	3
Specimen code	0	1	5	10

Platelet aggregation response	0	10	100	500
ADP (max) 1 umol	39	9	0	0
Slope	34	1	0	0
ADP 4 min.	5	6	0	0
ADP (max) 8 umol	88	36	0	0
Slope	67	26	0	0
ADP 4 min.	85	35	0	0
10 ug Collagen (max)	93	87	15	15
Slope	56	56	2	2
4 min.	85	85	10	10

Subject number :	V
Age :	39
Gender :	M

Date :	21/09/96
Medication :	Nil

Prostacyclin dose (pg / ml)	0	10	100	500
R	37.5	24	40.5	33
K	12.5	11.5	16	16.5
MA	49.5	51	47.5	44
Other				
Angle	34.5	33	30	26.5
Channel	1	2	3	4
Specimen code	0	1	5	10

Platelet aggregation response	0	10	100	500
ADP (max) 1 umol	45	50	0	0
Slope	32	31	0	0
ADP 4 min.	30	35	0	0
ADP (max) 8 umol	100	95	0	0
Slope	35	47	0	0
ADP 4 min.	90	90	0	0
10 ug Collagen (max)	100	100	14	12
Slope	60	60	1.5	1.5
4 min.	100	100	10	10

Subject number :	VI
Age :	46
Gender :	M

Date :	21/09/96
Medication :	Nil

Prostacyclin dose (pg / ml)	0	10	100	500
R	35	19	33	35.5
K	16	15	17.5	18
MA	46	50	44	48
Other				
Angle	25	29	23.5	23.5
Channel	2	3	4	1
Specimen code	0	1	5	10

Platelet aggregation response	0	10	100	500
ADP (max) 1 umol	55	38	0	0
Slope	40	31	0	0
ADP 4 min.	20	10	0	0
ADP (max) 8 umol	94	84	0	0
Slope	51	38	0	0
ADP 4 min.	90	80	0	0
10 ug Collagen (max)	89	89	20	15
Slope	55	40	3	2
4 min.	85	85	18	11

Subject number :	VII
Age :	28
Gender :	F

Date :	21/09/96
Medication :	Minipill

Prostacyclin dose (pg / ml)	0	10	100	500
R	35	29	31.5	31.5
K	12.5	16	11.5	10
MA	52.5	48.5	42.5	55
Other				
Angle	32.5	27.5	33.5	37.5
Channel	3	4	1	2
Specimen code	0	1	5	10

Platelet aggregation response	0	10	100	500
ADP (max) 1 umol	77	82	0	0
Slope	27	40	0	0
ADP 4 min.	70	79	0	0
ADP (max) 8 umol	79	81	0	0
Slope	59	60	0	0
ADP 4 min.	75	80	0	0
10 ug Collagen (max)	84	77	12	12
Slope	55	40	1.5	1.5
4 min.	81	70	10	10

Subject number :	VIII
Age :	36
Gender :	M

Date :	21/09/96
Medication :	Nil

Prostacyclin dose (pg / ml)	0	10	100	500
R	32.5	19.5	24	47
K	18.5	15	15.5	21
MA	41.5	44.5	42.5	37.5
Other				
Angle	23.5	28.5	38	20
Channel	4	1	2	3
Specimen code	0	1	5	10

Platelet aggregation response	0	10	100	500
ADP (max) 1 umol	38	35	0	0
Slope	41	39	0	0
ADP 4 min.	0	0	0	0
ADP (max) 8 umol	100	100	0	0
Slope	48	48	0	0
ADP 4 min.	95	95	0	0
10 ug Collagen (max)	100	100	18	13
Slope	60	60	2	1.5
4 min.	100	95	15	10

References

1. Fox GA, McCormack DG. A new look at the pulmonary circulation in acute lung injury. Thorax 1992;47:743-7.

2. Zapol WM, Snider MT. Pulmonary hypertension in severe acute respiratory failure. N Engl J Med 1977;296:476-80.

3. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke KJ, Hudson LD, et al. Report of the American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination. Intensive Care Med 1994;20:225-32.

4. Fishman AP. Pulmonary hypertension. In: Schlant RC, Alexander W, editors. The heart, arteries and veins. New York: McGraw Hill, 1994:1857-74.

5. Prichard JS. Pulmonary hypertension. In: Weatherall DJW, Ledingham JGG, Warrell DA, editors. Oxford Textbook of Medicine. Oxford: Blackwell, 1996:2505-14.

6. Warrell DA, Evans JW, Clarke RO, Kingaby GP, West JB. Pattern of filling in the pulmonary capillary bed. J Appl Physiol 1972;32:346-56.

7. Nunn JF. The pulmonary circulation. In: Applied Respiratory Physiology. 4th ed. Oxford: Butterworth and Heinaman, 1993:135-55.

8. Benumof JL. Mechanism of decreased blood flow in atelectatic lung. J Appl Physiol 1988;64:68-77.

9. West JB, Dollery CT. Distribution of blood flow and the pressure-flow relations of the whole lung. J Appl Physiol 1965;20:175-82.

10. Grossman W, Braunwald E. Pulmonary Hypertension. In: Braunwald E, editor. Heart Disease: a textbook of cardiovascular medicine. Philadelphia: WB Saunders, 1992:790-816.

11. Rich S, Dantzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, et al. Primary pulmonary hypertension: A national prospective study. Ann Intern Med 1987;107:216-23.

12. Palevsky HI, Schloo BL, Pietra GG, Weber KT, Janicki JS, Rubin E, et al. Primary pulmonary hypertension. Vascular structure, morphometry, and responsiveness to vasodilator agents. Circulation 1989;80:1207-21.

13. Pietra GG, Edwards WD, Kay JM, Rich S, Kernis J, Schloo BL, et al. Histopathology of primary pulmonary hypertension: A qualitive and quantative study of pulmonary blood vessels from 58 persons in the National Heart, Lung and Blood Institute Primary Pulmonary Hypertension Registry. Circulation 1989;80:1198-1206.

14. Paraskos JA, Adelstein SJ, Smith RE, Richman FD, Grossman W, Dexter L, et al. Late prognosis of acute pulmonary embolus. N Engl J Med 1973;289:55-8.

15. Harvey RM, Ferrer MI, Richards DW, Courand A. Influence of chronic pulmonary disease on the heart and circulation. Am J Med 1951;10:719-25.

16. Enson Y, Giuntini C, Lewis ML, Morris TQ, Ferrer MI, Harvey RM. The influence of hydrogen ion concentration and hypoxia on the pulmonary circulation. J Clin Invest 1964;43:1146-62.

17. Abraham AS, Cole RB, Green ID, Hedworth-Whitty RB, Clarke SW, Bishop JM. Factors contributing to the reversible pulmonary hypertension in patients with acute respiratory failure studied by serial observation during recovery. Circ Res 1969;24:51-60.

18. Fishman AP. Hypoxia on the pulmonary circulation - how and where it acts. Circ Res 1976;38:221-31.

19. Bryan E, Marshall MD. Regulation of the pulmonary circulation. In: Stanley TH, Sperry RJ, editors. Anaesthesia and the Lung. Dordrecht: Kluwer, 1992:3-16.

20. Marshall BE, Hanson CW, Frasch F, Marshall C. Role of hypoxic pulmonary vasoconstriction in pulmonary gas exchange and blood flow distribution. Intensive Care Med 1994;20:379-89.

21. Warren JB, Maltby NH, MacCormack D, Barnes PJ. Pulmonary endothelium-derived relaxing factor is impaired in hypoxia. Clin Sci 1989;77:671-6.

22. Brashers VL, Peach MJ, Rose CE. Augmentation of hypoxia-induced pulmonary vasoconstriction in the isolated rat lung by in vitro antagonists of endothelium-dependent relaxation. J Clin Invest 1988;82:1495-1502.

23. Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM. Inhaled nitric oxide; a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. Circulation 1991;83:2038-47.

24. Reid L. Structural and functional reappraisal of the pulmonary arterial system. The Scientific Basis of Medicine Annual Reviews. London: Athlone Press, 1968:289-307.

25. Zapol WM, Rie MA, Frikker M, Snider MT, Quinn DA. Pulmonary circulation during adult respiratory distress syndrome. In: Zapol WM, Falke KJ, editors. Acute respiratory failure. New York: Marcel Dekker, 1985:241-74.

26. Rinaldo JE, Rogers RM. Adult respiratory distress syndrome. Changing concepts of lung injury and repair. N Engl J Med 1982;306:900-9.

27. Murray JF, Matthay MA, Luce JM, Flick MR. An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 1988;138:720-3.

28. Scheeren T, Radermacher P. Prostacyclin (PGI_2): new aspects of an old substance in the treatment of critically ill patients. Intensive Care Med 1997;23:146-58.

29. Shapiro BA, Peruzzi WT. Respiratory care. In: Miller RD, editor. Anaesthesia. 4th ed. New York: Churchill Livingstone, 1994:2397-2439.

30. Meduri GU, Kohler G, Headley S, Tolley E, Stentz F, Postlethwaite A. Inflammatory cytokines in the BAL of patients with ARDS. Persistent elevation over time predicts poor outcome. Chest 1995;108:1303-14.

31. Meduri GU. Pulmonary fibroproliferation and death in patients with late ARDS. Chest 1995;107:5-6.

32. Kollef MH, Schuster DP. The acute respiratory distress syndrome. N Engl J Med 1995;332:27-37.

33. Bone RC, Balk R, Slotman G, Maunder R, Silverman H, Myers TM, et al. Adult respiratory distress syndrome; sequence and importance of development of multiple organ failure. Chest 1992;101:320-6.

34. Hall J, Wood LDH. Oxygen therapy in the critically ill patient. In: Hall J, Schmidt GA, Wood LDH, editors. Principles of Critical Care. New York: McGraw Hill, 1992:165-74.

35. Bernard GR, Reines HD, Haiushka PV, Higgins SB, Metz C, Swindell BB, et al. Prostacyclin and thromboxane A_2 formation is increased in human sepsis syndrome. Effects of cyclooxygenase inhibition. Am Rev Respir Dis 1991;144:1095-1101.

36. Bone RC. The pathogenesis of sepsis. Ann Intern Med 1991;115:457-69.

37. Chapman MJ. Adult respiratory distress syndrome: an update. Anaesth Intensive Care 1994;22:255-66.

38. Brett SJ, Simon J, Gibbs R, Pepper JR, Evans TW. Impairment of endothelium-dependent pulmonary vasodilation in patients with primary pulmonary hypertension. Thorax 1996;51:89-91.

39. Kharitonov SA, Cailes J, Black CM, du Bois RM, Barnes PJ. Decreased nitric oxide in the exhaled air of patients with systemic sclerosis with pulmonary hypertension. Thorax 1997;52:1051-5.

40. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. N Engl J Med 1995;333:214-21.

41. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. N Engl J Med 1993;328:1732-9.

42. Miyata M, Ueno Y, Sekine H, Ito O, Sakuma F, Koike H, et al. Protective effect of beroprost sodium, a stable prostacyclin analogue, in development of monocrotaline-induced pulmonary hypertension. J Cardiovasc Pharmacol 1996;27:20-6.

43. Welsh DJ, Scott P, Plevin R, Wadsworth R, Peacock AJ. Hypoxia enhances cellular proliferation and inositol 1,4,5-triphosphate generation in fibroblasts from bovine pulmonary artery but not from mesenteric artery. Am J Respir Crit Care Med 1998;158:1757-62.

44. Christman BW, McPherson CD, Newman JH, King GA, Bernard GR, Groves BM, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med 1992;327:70-5.

45. Egermayer P, Town GI, Peacock AJ. Role of serotonin in the pathogenesis of acute and chronic pulmonary hypertension. Thorax 1999;54:161-8.

46. Archer SL, Djaballah K, Humbert M, Weir KE, Fartoukh M, Dall'ava-Santucci J, et al. Nitric oxide deficiency in fenfluramine- and dexfenfluramine-induced pulmonary hypertension. Am J Respir Crit Care Med 1998;158:1061-7.

47. Radermacher P, Santak B, Wust HJ, Tarnow J, Falke KJ. Prostacyclin for the treatment of pulmonary hypertension in adult respiratory distress syndrome: Effects on pulmonary capillary pressure and ventilation-perfusion distributions. Anesthesiology 1990;72:238-44.

48. Radermacher P, Santak B, Wust HJ, Tarnow J, Falke KJ. Prostacyclin and right ventricular function in patients with pulmonary hypertension associated with ARDS. Intensive Care Med 1990;16:227-32.

49. Mathur PN, Powles P, Pugsley SO, McEwan MP, Campbell EJM. Effect of digoxin on right ventricular function in severe chronic airflow obstruction: A controlled clinical trial. Ann Intern Med 1981;95:283-8.

50. Fuster V, Steele PM, Edwards WD, Gersh BJ, McGoon MD, Frye RL. Primary pulmonary hypertension: natural history and the importance of thrombosis. Circulation 1984;70:580-7.

51. Dantzker DR, Brook CJ, Dehart P, Lynch JP, Weg JG. Ventilation-perfusion distributions in the adult respiratory distress syndrome. Am Rev Respir Dis 1979;120:1039-52.

52. Presberg K, Yanos J, Monk R, Corbridge T, Wood LDH. The role of mixed venous hypoxaemia in the pulmonary hypertension and shunt of canine low-pressure pulmonary oedema. Am Rev Respir Dis 1989;139:A416.

53. Medoff BD, Harris RS, Kesselman H, Venegas J, Amato MBP, Hess D. Use of recruitment maneuvers and high-positive end-expiratory pressure in a patient with acute respiratory distress syndrome. Crit Care Med 2000;28:1210-16.

54. Amato MBP, Barbas CSV, Medeiros DM, Magaldi RB, Schettino GP, Lorenzi-Filho G, et al. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. N Engl J Med 1998;338:347-54.

55. Amato MBP, Barbas CSV, Medeiros DM, Schettino GDP, Lorenzi-Filho G, Kairalla RA, et al. Beneficial effects of the "open lung approach" with low distending pressures in acute respiratory distress syndrome. A prospective randomized study on mechanical ventilation. Am J Respir Crit Care Med 1995;152:1835-46.

56. Brazzi L, Pelosi P, Gattioni L. Prone position in mechanically-ventilated patients. Monaldi Arch Chest Dis 1998;58:410-14.

57. Richards G, White H, Hopley M. Rapid reduction of oxygenation index by employment of a recruitment technique in patients with ARDS. Combined Congress Critical Care Society of Southern Africa & South African Pulmonary Society, July 2000. (abstract).

58. Lapinsky SE, Aubin M, Mehta S, Boiteau P, Slutsky AS. Safety and efficacy of a sustained inflation for alveolar recruitment in adult respiratory failure. Intensive Care Med 1999;25:1297-1301.

59. Goldring RM, Turino GM, Cohen G, Jameson AG, Bass BG, Fishman AP. The catecholamines in the pulmonary arterial pressor response to hypoxia. J Clin Invest 1962;41:1211-20.

60. Lupi-Herrera E, Bialostozky D, Sobrino A. The role of isoproterenol in pulmonary artery hypertension of unknown aetiology. Chest 1981;79:292-6.

61. Klinke WP, Gilbert JAL. Diazoxide in primary pulmonary hypertension. N Engl J Med 1980;302:91-2.

62. Groves BM, Rubin LJ, Frosolono MF, Cato AE, Reeves JT. A comparison of the acute haemodynamic effects of prostacyclin and hydralazine in primary pulmonary hypertension. Am Heart J 1985;110:1200-4.

63. Niarchos AP, Whitman HH, Goldstein JE, Laragh JH. Haemodynamic effects of captopril in pulmonary hypertension of collagen vascular disease. Am Heart J 1982;104:834-8.

64. Woodmansey PA, O'Toole L, Channer KS, Morice AH. Acute pulmonary vasodilator properties of amlodipine in humans with pulmonary hypertension. Heart 1996;75:171-3.

65. Malcic I, Richter D. Verapamil in primary pulmonary hypertension. Br Heart J 1985;53:345-7.

66. Cchen ML, Kronzon I. Adverse effects of phentolamine in primary pulmonary hypertension. Ann Intern Med 1981;95:591-2.

67. Fullerton DA, Kirson LE, Jones SD, McIntyre RC. Adenosine is a selective pulmonary vasodilator in cardiac surgical patients. Chest 1996;109:41-6.

68. Nootens M, Schrader B, Kaufman E, Vestal R, Long W, Rich S. Comparative acute effects of adenosine and prostacyclin in primary pulmonary hypertension. Chest 1995;107:54-7.

69. Weir EK, Rubin LJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, et al. The acute administration of vasodilators in primary pulmonary hypertension. Experience from the NIH registry on primary pulmonary hypertension. Am Rev Respir Dis 1989;140:1623-30.

70. Rich S, Brundage BH. High-dose calcium channel blocking therapy for primary pulmonary hypertension: Evidence for long-term reduction in pulmonary arterial pressure and regression of right ventricular hypertrophy. Circulation 1987;76:135-41.

71. Rich S, Kaufman E, Levy PS. The effect of high doses of calcium channel blockers on survival in primary pulmonary hypertension. N Engl J Med 1992;327:76-81.

72. Radermacher P, Huet Y, Pluskwa F. Comparison of ketanserin and sodium nitroprusside in patients with severe ARDS. Anesthesiology 1988;68:152-7.

73. Radermacher P, Santak B, Becker H, Falke KJ. Prostaglandin E1 and nitroglycerin reduce pulmonary capillary pressure but worsen V/Q distributions in patients with ARDS. Anesthesiology 1989;70:601-6.

74. Sibbald WJ, Driedger AA, McCallum D, Cunningham D, Cheung H. Nitroprosside infusion does not improve biventricular performance in patients with hypoxaemic respiratory failure. J Crit Care 1986;1:197-203.

75. Kerins DM, Murray R, FitzGerald GA. Prostacyclin and Prostaglandin E_1; Molecular mechanisms and therapeutic utility. Prog Hemost Thromb 1991;10:307-37.

76. Hanneman L, Reinhart K, Meier-Hellerman A, Bredle DL. Prostacyclin in septic shock. Chest 1994;105:1504-10.

77. Burghuber OC, Silberbauer K, Haber P, Sinzinger H, Elliott M, Leithner C. Pulmonary and antiaggregatory effects of prostacyclin after inhalation and intravenous infusion. Respiration 1984; 45:450-4.

78. Devitt HH, Burka JF, Jones R, Amy RW, King EG. Hemodynamic and pathologic effects of prostacyclin on oleic acid-induced pulmonary injury. Surgery 1988;103:213-20.

79. Griffith TM, Edwards DH, Davies RL, Harrison TJ, Evans KT. EDRF coordinates the behaviour of vascular resistance vessels. Nature 1987;329:442-5.

80. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43:109-42.

81. *Vallance P, Collier J. Biology and clinical relevance of nitric oxide. BMJ 1994;309:453-7.*

82. Kam PCA, Govender G. Nitric oxide: basic science and clinical applications. Anaesthesia 1994;49:515-21.

83. Anggard E. Nitric oxide: mediator, murderer and medicine. Lancet 1994;343:1199-1206.

84. Zapol WM, Rimar S, Gillis N, Marletta M, Bosken H. Nitric oxide and the lung. Am J Respir Crit Care Med 1994; 49:1375-80.

85. Fink MP, Payen D. The role of nitric oxide in sepsis and ARDS: synopsis of a roundtable conference held in Brussels on 18-20 March 1995. Intensive Care Med 1996;22:158-65.

86. Hurford WE. Inhaled nitric oxide therapy. Current Opinion in Anaesthesia 1996;9:117-26.

87. Stamler JS. Redox signalling: nitrosylation and related target interactions of nitric oxide. Cell 1994;78:931-6.

88. Fratacci MD, Frostell C, Chen TY, Wain JC, Robinson DR, Zapol WM. Inhaled nitric oxide. A selective pulmonary vasodilator of heparin-protamine vasoconstriction in sheep. Anesthesiology 1991;75:990-9.

89. Rossaint R, Falke KJ, Lopez F, Slama K, Pison U, Zapol WM. Inhaled nitric oxide for the adult respiratory distress syndrom. N Engl J Med 1993;328:399-405.

90. Sanna A, Kurtansky A, Veriter C, Stanescu D. Bronchodilator effect of inhaled nitric oxide in healthy men. Am J Respir Crit Care Med 1994;150:1702-4.

91. Lowenstein CJ, Dinerman JL, Snyder SH. Nitric oxide: A physiologic messenger. Ann Intern Med 1994;120:227-37.

92. Kubes P, Suzuki M, Grainger DN. Nitric oxide: An endogenous modulator of leucocyte adhesion. Proc Natl Acad Sci 1991;88:4651-4.

93. Roberts JD, Roberts CT, Jones R, Zapol WM, Bolch KD. Continuous nitric oxide inhalation reduces pulmonary arterial structural changes, right ventricular hypertrophy and growth retardation in the hypoxic newborn rat. Circ Res 1995;76:215-22.

94. Higenbottam TW, Pepke-Zaba J, Scott J, Woolman P, Coutts C, Wallwork J. Inhaled endothelium-derived relaxing factor in primary pulmonary hypertension. Am Rev Respir Dis 1988;137:A107

95. Klemm P, Thiemermann C, Winklmaier G, Martorana PA, Henning R. Effects of nitric oxide synthase inhibition combined with nitric oxide inhalation in a porcine model of endotoxin shock. Bri J Pharmacol 1995;224:363-8.

96. Dahm P, Blomquist S, Martenson L, Thorne J, Zoukas E. Circulatory and ventilatory effects of intermittant nitric oxide inhalation during porcine endotoxaemia. J Trauma 1994;37:769-77.

97. Romand JA, Pinsky MR, Firestone L, Zar HA, Lancaster JR. Effect of inhaled nitric oxide on pulmonary haemodynamics after acute lung injury in dogs. J Appl Physiol 1994;76:1356-62.

98. Payen D, Gatecel C, Guinard N. Inhalation of low dose of nitric oxide and IV L-arg in ARDS patients. Am Rev Respir Dis 1993;147:A720

99. Puybasset L, Stewart T, Rouby JJ, Cluzel P, Mourgeon E, Belin M, et al. Inhaled nitric oxide reverses the increase in pulmonary vascular resistance introduced by permissive hypercapnia in patients with acute respiratory distress syndrome. Anesthesiology 1994;80:1254-67.

100. Young JD, Brampton WJ, Knighton JD, Finfer SR. Inhaled nitric oxide in acute respiratory failure in adults. Br J Anaesth 1994;73:499-502.

101. Puybasset L, Rouby JJ, Mourgeon E, Stewart T, Cluzel P, Arthaud M, et al. Inhaled nitric oxide in acute respiratory failure: dose-response curves. Intensive Care Med 1994;20:319-27.

102. Samama CM, Diaby M, Fellahi JL, et al. Inhibition of platelet aggregation by inhaled nitric oxide in patients with ARDS. Anesthesiology 1995;83:56-65.

103. Gerlach H, Pappert D, Lewandowski K, Rossaint R, Falke KJ. Long-term inhalation with evaluated low doses of nitric oxide for selective improvement of oxygenation in patients with adult respiratory distress syndrome. Intensive Care Med 1993;19:443-9.

104. Bigatello LM, Hurford WE, Kacmarek RM, Roberts JD, Zapol WM. Prolonged inhalation of nitric oxide in patients with severe adult respiratory distress syndrome. Anesthesiology 1994;80:761-70.

105. Zapol WM. Minidose inhaled nitric oxide: less is better. Intensive Care Med 1993;19:433-4.

106. Miller OI, Tang SF, Keech A, Celermajer JS. Rebound pulmonary hypertension on withdrawal from inhaled nitric oxide. Lancet 1995;346:51-2.

107. Nishimura M, Hess D, Kacmarek RM, Ritz R, Hurford WE. Nitrogen dioxide production during mechanical ventilation with nitric oxide in adults. Anesthesiology 1995;82:1246-54.

108. Clutten-Brock J. Two cases of poisoning by contamination of nitrous oxide with the higher oxides of nitrogen during anaesthesia. BrJ Anaesth 1967;39:388-92.

109. Westphal K, Strouhal U, Byhahn C, Hommel K, Behne M. Inhalation of nitric oxide in severe lung failure. Anaesthsiol Reanim 1998;23:144-8.

110. Abman SH, Griebel JL, Parker DK, Schmidt JM, Swanton D, Kinsella JP. Acute effects of inhaled nitric oxides in children with severe hypoxaemic respiratory failure. J Pediatr 1994;124:881-8.

111. Channick RN, Newhart JW, Johnson F, Williams P, Auger WR, Fedullo PF, et al. Pulsed delivery of inhaled nitric oxide in patients with primary pulmonary hypertension. An ambulatory delivery system and initial clinical tests. Chest 1996;109:1545-9.

112. Shiel FOM. Morbid anatomical changes in the lungs of dogs after inahalation of higher oxides of nitrogen during anaesthesia. Br J Anaesth 1967;39:413-24.

113. Greenbaum R, Bay J, Hargreaves MD, et al. Effects of higher oxides of nitrogen on the anaesthetised dog. Br J Anaesth 1967;39:393-403.

114. Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airway. Am J Resir Crit Care Med 1994;149:538-51.

115. Centres for Disease Control. Recommendations for occupational safety and health standards. MMWR 1988;37(supplement):21

116. Nader ND, Knight PR, Bobela I, Davidson BA, Johnson KJ, Morin F. High-dose nitric oxide inhalation increases lung injury after gastric aspiration. Anesthesiology 1999;91:741-9.

117. Vaughan D, Yanay O, Zimmerman JJ. Deciphering the oxyradical inflammation Rosetta stone: O2-NO, OONO-, polymorphonuclear neutrophils, poly (ADP-ribose) synthetase, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome. Crit Care Med 1999;27:1666-9.

118. Hart CM. Nitric oxide in adult lung disease. Chest 1999;115:1407-17.

119. Dellinger RP. Inhaled nitric oxide in acute lung injury and acute respiratory distress syndrome. Inability to translate physiological benefit to clinical outcome benefit in adult clinical trials. Intensive Care Med 1999;25:881-3.

120. Lundin S, Mang H, Smithies M, Stenquist O, Frostell C, European Study Group of Inhaled Nitric Oxide. Inhalation of nitric oxide in acute lung injury: results of a European multicentre study. Intensive Care Med 1999;25:911-19.

121. Vane JR, Botting RM. Pharmacodynamic profile of prostacyclin. Am J Cardiol 1995;75:3A-10A.

122. Vane JR, Anggard E, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990;323:27-36.

123. Szczeklik A, Gryglewski RJ, Nizankowska E, Nizankowska R, Musial J. Pulmonary and anti-platelet effects of intravenous and inhaled prostacyclin in man. Prostaglandins 1978;16:651-60.

124. Szczeklik A, Gryglewski RJ, Nizankowski R, Musial J, Pieton R, Mruk J. Circulatory and anti-platelet effects of intravenous prostacyclin in healthy men. Pharmacol Res Comm 1978;10:545-56.

125. Tremoli E, Colli S, Paoletti R. Mode of action of PGI_2 and its stable derivative iloprost on platelets and leukocytes. Thromb Res Suppl 1990;11:33-42.

126. Long W, Rubin LJ. Prostacyclin and PGE_1: Treatment of pulmonary hypertension. Am Rev Respir Dis 1987;136:773-6.

127. Bihari DJ, Tinker J. The therapeutic value of vasodilator prostaglandins in multiple organ failure associated with sepsis. Intensive Care Med 1988;15:2-7.

128. Hyman AL, Spannhake EW, Kadowitz PJ. Prostaglandins and the lung. Am Rev Respir Dis 1978;117:111-36.

129. Benumof JL. Respiratory physiology and function during anaesthesia. In: Miller RD, editor. Anaesthesia. New York: Churchill Livingstone, 1994:577-620.

130. Hyslop S, deNucci G. The mechanisms and significance of the coupled release of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI_2) from endothelial cells. Wien Klin Wochenschr 1991103:422-34.

131. Hermiller JB, Bambach D, Thompson JM. Vasodilators and prostaglandin inhibitors in primary pulmonary hypertension. Ann Intern Med 1982 97:480-9.

132. Meyrick B, Niedermeyer ME, et al. Pulmonary hypertension and increased vasoreactivity caused by repeated indomethacin treatment in sheep. J Appl Physiol 1985;59:443-52.

133. Moncada S, Vane JR. Prostacyclin and blood coagulation. Drugs 1981;21:430-7.

134. Wetzel RC. Aerosolized prostacyclin. In search of the ideal pulmonary vasodilator. *Anesthesiology* 1995;82:1315-17.

135. Silvester W. Prostacyclin in review: its physiology and role in intensive care. Intensive Care World 1997;15:22-39.

136. Campbell WB, Halushka PV. Lipid-derived autacoids. In: Hardman JG, Limbird LE, editors. Goodman & Gilman's: The pharmacological basis of therapeutics. New York: McGraw Hill, 1996:601-16.

137. Belch JJ, Greer I, McLaren M, Walker J, Forbes CD. Measurement of prostacyclin metabolites. Lancet 1983 Dec 24-31;2:1504

138. Machleidt C, Forstermann U, Anhut H, Hertting G. Formation and elimination of prostacyclin metabolites in the cat in vivo as determined by radioimmunoassay of unextracted plasma. Eur J Pharmacol 1981;74:19-26.

139. Rosenkranz B, Fischer C, Weimer KE, Frolich JC. Metabolism of prostacyclin and 6-keto-prostaglandin F1 alpha in man. J Biol Chem1980;255:194-8.

140. Rosenkranz B, Fischer C, Reiman I, Weimer KE, Beck G, Frolich JC. Identification of the major metabolite of prostacyclin and 6- keto-prostaglandin F1 alpha in man. Biochem Biophys Acta 1980;619:207-13.

141. Rosenkranz B, Fischer C, Reiman I, Weimer KE, Beck G, Frolich JC. Metabolism of prostacyclin and 6-keto-prostaglandin F1 alpha in man. Artery 1980;8:18-22.

142. Zobel G, Dacar D, Rodl S, Friehs I. Inhaled nitric oxide versus inhaled prostacyclin and intravenous versus inhaled prostacyclin in acute respiratory failure with pulmonary hypertension in piglets. Pediatr Res 1995;38:198-204.

143. McKay CR, Brundage BH, Ports TA, Parmley WW, Chatterjee K. Long-term clinical results after vasodilator evaluation in patients with primary and secondary pulmonary hypertension: acute haemodynamic comparisons and long-term survival. Int J Cardiol 1989;22:311-19.

144. Rubin LJ, Mendoza J, Hood M, McGoon MD, Barst RJ, Williams WB, et al. Treatment of primary pulmonary hypertension with continuous intravenous prostacyclin. Ann Intern Med 1990; 112:485-91.

145. Haywood GA, Adams KF, Gheorghiade M, McKenna WJ. Is there a role for epoprostenol in the management of heart failure? Am J Cardiol 1995;75:44A-50A.

146. Virgolini I, Fitscha P, Linet OI, O'Grady J, Sinzinger H. A double-blind, placebo-controlled trial of intravenous prostacyclin in 108 patients with ishaemic peripheral vascular disease. Prostaglandins 1990;39:657-64.

147. Bihari DJ, Smithies M, Gimson A, Tinker J. The effects of vasodilation with prostacyclin on oxygen delivery and uptake in critically ill patients. N Engl J Med 1987;317:397-403.

148. Pascual JM, Fiorelli AI, Bellotti GM, Stolf NA, Jalene AD. Prostacyclin in the management of pulmonary hypertension after heart transplant. J Heart Transplant 1990;9:644-651.

149. Long W, Rubin LJ, et al. Randomized trial of conventional therapy alone vs. conventional therapy and continuous infusions of prostacyclin in primary pulmonary hypertension. Am Rev Respir Dis 1993;147:A538

150. Goldman AP, Delius RE, Deanfield JE, Macrae DJ. Nitric oxide is superior to prostacyclin for pulmonary hypertension after cardiac operations. Ann Thorac Surg 1995;60:300-6.

151. Welte M, Zwissler B, Habazettl H, Messmer K. PGI_2 aerosol versus nitric oxide for selective pulmonary vasodilation in hypoxic pulmonary vasoconstriction. Eur Surg Res 1993;25:329-40.

152. Walmrath D, Schneider T, Pilch J, Grimminger F, Seeger W. Aerosolised prostacyclin in adult respiratory distress syndrome. Lancet 1993;342:961-2.

153. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: A severity of disease classification system. Crit Care Med 1985;13:818-29.

154. Machin SJ, Chamone DA, Defreyn G, Vermylen J. The effect of clinical prostacyclin infusions in advanced arterial disease on platelet function and plasma 6-keto PGF1 alpha levels. Br J Haematol 1981;47:413-22.

155. Berry CN, Hoult JR. 6-keto-prostaglandin E1: its formation by platelets from prostacyclin and resistance to pulmonary degradation. Pharmacology 1983;26:324-30.

156. O'Riordan TG, Greco MJ, Perry RJ, Smaldone GC. Nebulizer function during mechanical ventilation. Am Rev Respir Dis 1992;145:1117-22.

157. Tandon R, McPeck M, Smaldone GC. Measuring nebulizer output. Aerosol production vs gravimetric analysis. Chest 1997;111:1361-5.

158. Durbin CD. Aerosol therapy in mechanically ventilated patients. Chest 1994;106:336-7.

159. Watterberg KL, Clark AR, Kelly HW, Murphy S. Delivery of aerosolized medication to intubated babies. Pediatr Pulmonol 1991;10:136-41.

160. Alvine GF, Rodgers P, Fitzsimmons KM, Ahrens RC. Disposable jet nebulizers. How reliable are they? Chest 1992;101:316-19.

161. Coleman DM, Kelly HW, McWilliams BC. Determinants of aerosolized albuterol delivery to mechanical ventilated infants. Chest 1996;109:1607-13.

162. Hess D, Fisher D, Williams P, Pooler S, Kacmarek RM. Medication nebulizer performance. Effects of diluent volume, nebulizer flow, and nebulizer brand. Chest 1996;110:498-505.

163. Brett SJ, Quinlan GJ, Mitchell J, Pepper JR, Evans TW. Production of nitric oxide during surgery involving cardiopulmonary bypass. Crit Care Med 1998;26:272-8.

164. van Heerden PV, Power BM, Leonard RC. Re: Delivery of inhaled aerosolised prostacyclin (IAP). Anaesth Intensive Care 1996;24:624-5.

165. Gerlach H, Rossaint R, Pappert D, Falke KJ. Time-course and dose-response of nitric oxide inhalation for systemic oxygenation and pulmonary hypertension in patients with adult respiratory distress syndrome. Eur J Clin Invest 1993;23:499-502.

166. Bein T, Pfeifer M, Riegger GAJ, Taeger K. Continuous intraarterial measurement of oxygenation during aerosolized prostacyclin administration in severe respiratory failure. N Engl J Med 1994;331:335-6.

167. Walmrath D, Schneider T, Pilch J, Schermuly R, Grimminger F, Seeger W. Effects of aerosolized prostacyclin in severe pneumonia. Impact of fibrosis. Am J Respir Crit Care Med 1995;151:724-30.

168. Zwissler B, Welte M, Messmer K. Effects of inhaled prostacyclin as compared with inhaled nitric oxide on right ventricular performance in hypoxic pulmonary vasoconstriction. J Cardiothorac Vasc Anesth 1995;9:283-9.

169. Haas NA, Schulze-Neick I, Lange PE. Intratracheal prostacyclin for resuscitation in primary pulmonary hypertension. Lancet 1995;346:643

170. van Heerden PV, Webb SAR, Hee G, Corkeron M, Thompson WR. Inhaled aerosolized prostacyclin as a selective pulmonary vasodilator for the treatment of severe hypoxaemia. Anaesth Intensive Care 1996;24:87-90.

171. Pappert D, Busch T, Gerlach H, Lewandowski K, Radermacher P, Rossaint R. Aerosolized prostacyclin versus inhaled nitric oxide in children with severe acute respiratory distress syndrome. Anesthesiology 1995;82:1507-11.

172. Holzmann A, Bloch KD, Sanchez LS, Filippov G, Zapol WM. Hyporesponsiveness to inhaled nitric oxide in isolated, perfused lungs from endotoxin-challenged rats. Am J Physiol 1996; 271:L981-L986.

173. Walmrath D, Schneider T, Schermuly R, Olschewski H, Grimminger F, Seeger W. Direct comparison of inhaled nitric oxide and aerosolized prostacyclin in acute respiratory distress syndrome. Am J Respir Crit Care Med 1996;153:991-6.

174. Zwissler B, Kemming G, Habler O, Kleen M, Merkel M, Haller M, et al. Inhaled prostacyclin (PGI_2) versus inhaled nitric oxide in adult respiratory distress syndrome. Am J Respir Crit Care Med 1996;154:1671-7.

175. Zwissler B, Welte M, Habler O, Kleen M, Messmer K. Effects of inhaled prostacyclin as compared with inhaled nitric oxide in a canine model of pulmonary microembolism and oleic acid edema. J Cardiothorac Vasc Anesth 1995;9:634-40.

176. van Heerden PV, Blythe D, Webb SAR. Inhaled aerosolized prostacyclin and nitric oxide as selective pulmonary vasodilators in ARDS - A pilot study. Anaesth Intensive Care 1996;24:564-8.

177. Troncy E, Collet J, Shapiro BA, Guimond J, Blair L, Charbonneau M, et al. Should we treat acute respiratory distress syndrome with inhaled nitric oxide? Lancet 1997;350:111-12.

178. Haraldsson A, Kieler-Jensen N, Nathorst-Westfelt U, Bergh C, Ricksten S. Inhaled prostacyclin compared to inhaled nitric oxide in the evaluation of heart transplant candidates with elevated pulmonary vascular resistance. Br J Anaesth 1996;76:(Suppl 1)14.

179. Booke M, Bradford DW, Hinder F, Harper D, Brauchle RW, Traber LD, et al. Effects of inhaled nitric oxide and nebulized prostacyclin on hypoxic pulmonary vasoconstriction in anesthetized sheep. Crit Care Med 1996;24:1841-8.

180. Sitbon O, Brenot F, Denjean A, Bergeron A, Parent F, Azarien R, et al. Inhaled nitric oxide as a screening vasodilator agent in primary pulmonary hypertension. A dose-response study and comparison with prostacyclin. Am J Respir Crit Care Med 1995;151:384-9.

181. Ubatuba FB, Moncada S, Vane JR. The effect of prostacyclin (PGI_2) on platelet behaviour. Thrombus formation in vivo and bleeding time. Thromb Haemost 1979;41:425-35.

182. Bindl L, Fahnenstich H, Peukert U. Aerosolised prostacyclin for pulmonary hypertension in neonates. Arch Dis Child Fetal Neonatal Ed 1994;71:F214-F216.

183. Webb SAR, Stott S, van Heerden PV. The use of inhaled aerosolized prostacyclin (IAP) in the treatment of pulmonary hypertension secondary to pulmonary embolism. Intensive Care Med 1996;22:353-5.

184. Schranz D, Huth R, Wipperman C, Ritzerfeld S, Schmitt F, Oelert H. Nitric oxide and prostacyclin lower suprasystemic pulmonary hypertension after cardiopulmonary bypass. Eur J Pediatr 1993;152:793-6.

185. Haraldsson A, Kieler-Jensen N, Nathorst-Westfelt U, Bergh CH, Ricksten S. Comparison of *inhaled nitric oxide and inhaled aerosolized prostacyclin in the evaluation of heart transplant* candidates with elevated pulmonary vascular resistance. Chest 1998;114:780-6.

186. Eichelbronner O, Reinelt H, Wiedeck H, Mezody M, Rossaint R, Georgieff M, et al. Aerosolized prostacyclin and inhaled nitric oxide in septic shock - different effects on splanchnic oxygenation? Intensive Care Med 1996;22:880-7.

187. Habler O, Kleen M, Zwissler B, Pusch R, Welte M, Vogelmeier C, et al. Inhalation of prostacyclin (PGI_2) for 8 hours does not produce signs of acute pulmonary toxicity in healthy lambs. Intensive Care Med 1996;22:426-33.

188. Habler O, Kleen M, Takenaka S, Leiderer R, Pusch R, Welte M, et al. Eight hours' inhalation of prostacyclin (PGI_2) in healthy lambs: effects on tracheal, bronchial, and alveolar morphology. Intensive Care Med 1996;22:1232-38.

189. Bianco S, Robuschi M, Grugni A, Ceserani R, Gandolfi C. Effect of prostacyclin on antigen induced immediate bronchoconstriction in asthmatic patients. Prostaglandins Med 1979;3:39-45.

190. Doyle P, Swart M, Kong A. Inhaled prostacyclin as a selective pulmonary vasodilator. Anaesth Intensive Care 1996;24:514-5.

191. Bein T, Keyl C, Pfeifer M, Metz C. Administration of aerosolized prostacyclin in patients with severe respiratory failure and pulmonary hypertension. Che (1995;108:119S.

192. Olschewski H, Walmrath D, Schermuly R, Ghofrani A, Grimminger F, Seeger W. Aerosolized prostacyclin and iloprost in severe pulmonary hypertension. Ann Intern Med 1996;124:820-4.

193. Stricker H, Dominghetti G, Waldispuehl B. Transbronchial vasodilator therapy with aerosolized prostacyclin (PG12) in patients with pulmonary hypertension associated with ARDS. Intensive Care Med 1996;22:(Suppl 3)S408.

194. Kieler-Jensen N, Milocco I, Kirno K, Houltz E, Ricksten S. Effects of prostacyclin on myocardial hemodynamics and metabolism after coronary artery bypass grafting. J Cardiothorac Vasc Anesth 1996;10:741-7.

195. Haraldsson , Kieler-Jensen N, Ricksten S. Inhaled prostacyclin for treatment of pulmonary hypertension after cardiac surgery or heart transplantation: A pharmacodynamic study. J Cardiothorac Vasc Anesth 1996;10:)864-8.

196. Santak B, Schreiber M, Kuen P, Lang D, Radermacher P. Prostacyclin aerosol in an infant with pulmonary hypertension. Eur J Pediatr 1995;154:233-5.

197. Bein T, Metz C, Keyl C, Sendtner E, Pfeifer M. Cardiovascular and pulmonary effects of aerosolized prostacyclin administration in severe respiratory failure using a ventilator nebulizing system. J Cardiovasc Pharmacol 1996;27:583-6.

198. Royston D. Inhalation agents for pulmonary hypertension. Lancet 1993;342:941-2.

199. Soditt V, Aring C, Groneck P. Improvement of oxygenation induced by aerosolized prostacyclin in a preterm infant with persistent pulmonary hypertension of the newborn. Intensive Care Med 1997;23:1275-8.

200. McGinn PV, Black AJR. Use of nebulised prostacyclin to ascertain reversibility of secondary pulmonary hypertension. Aust N Z J Med 1996;26:234-5.

201. Mikhail G, Gibbs SR, Richardson M, Wright G, Khagani A, Banner N, et al. An evaluation of nebulized prostacyclin in patients with primary and secondary pulmonary hypertension. Eur Heart J 1997;18:1499-1504.

202. Zapol WM. Nitric oxide inhalation in acute respiratory distress syndrome: It works, but can we prove it? Crit Care Med 1998;26:2-3.

203. Turanlahti MI, Laitinen PO, Sarna SJ, Pesonen E. Nitric oxide, oxygen, and prostacyclin in children with pulmonary hypertension. Heart 1998;79:169-74.

204. Wong WK, Lachenbruch PA. Designing studies for dose response. Stat Med 1996;15:343-59.

205. van Heerden PV, Gibbs NM, Michalopoulos N. The effect of low concentrations of prostacyclin on platelet function in vitro. Anaesth Intensive Care 1997;24:343-6.

206. Modig J. Adult respiratory distress syndrome. Pathogenesis and treatment. Acta Chir Scand 1986;152:241-9.

207. Knaus WA. The ongoing mystery of ARDS. Intensive Care Med 1996;22:517-18.

208. Jenkinson SG. Oxygen toxicity. New Horiz 1993;1:504-11.

209. O'Brian-Ladner A, Pingleton SK. Oxygen toxicity. Pulmonary Perspectives 1996;13:4-5.

210. Kleen M, Habler O, Hofstetter C, Pusch R, Mueller M, Welte M, et al. Efficacy of inhaled prostanoids in experimental pulmonary hypertension. Crit Care Med 1998;26:1103-9.

211. Jacobs BR, Brilli RJ, Ballard ET, Passerini DJ, Smith DJ. Aerosolized soluble nitric oxide donor improves oxygenation and pulmonary hypertension in acute lung injury. Am J Respir Crit Care Med 1998;158:1536-42.

212. Brilli RJ, Krafte-Jacobs B, Smith DJ, Passerini DJ, Moore L, Ballard ET. Aerosolization of novel nitric oxide donors selectively reduce pulmonary hypertension. Crit Care Med 1998;26:1390-6.

213. Adrie C, Ichinose F, Holzmann A, Keefer L, Hurford WE, Zapol WM. Pulmonary vasodilation by nitric oxide gas and prodrug aerosols in acute pulmonary hypertension. J Appl Physiol 1998;84:435-41.

214. Brilli RJ, Krafte-Jacobs B, Smith DJ, Roselle D, Passerini DJ, Vromen A, et al. Intratracheal instillation of a novel NO/nucleophile adduct selectively reduces pulmonary hypertension. J Appl Physiol 1997;83:1968-75.

215. Ichinose F, Adrie C, Hurford WE, Bloch KD, Zapol WM. Selective pulmonary vasodilation induced by aerosolized zaprinast. Anesthesiology 1998;88:410-16.

216. Doering EB, Hanson CW3, Reily DJ, Marshall C, Marshall BE. Improvement in oxygenation by phenylephrine and nitric oxide in patients with adult respiratory distress syndrome. Anesthesiology 1997;87:18-25.

217. Moncada S, Vane JR. Prostacyclin and its clinical applications. Ann Clin Res 1984;16:241-52.

218. Herman F, Hadhazy P, Magyar K. In vivo measurement of the disaggregatory action of prostacyclin. A methodological study. Thromb Res 1986;44:575-85.

219. Czer GT, Moser KM. Dissociation between hemodynamic and platelet antiaggregant effects of prostacyclin in dogs determined with a rapid platelet isolation technique. J Lab Clin Med 1983;102:133-9.

220. Herman F, Magyar K, Filep JG. In vivo antiaggregatory action of platelet-activating factor in Beagle dogs: role for prostacyclin. Thromb Haemost 1991;65:296-9.

221. Mallett SV, Cox DJA. Thromboelastography. Br J Anaesth 1992;69:307-13.

222. Mileikowsky GN, Nadler JL, Huey F, Francis R, Roy S. Evidence that smoking alters prostacyclin formation and platelet aggegation in women who use oral contraceptive. Am J Obstet Gynecol 1988;159:1547-152.

223. Gibbs NM. The effect of anaesthetic agents on platelet function. Anaesth Intensive Care 1991;19:495-505.

224. van Heerden PV. Systemic levels of 6-keto-prostaglandin $F_1\alpha$ following administration of inhaled aerosolized prostacyclin. Anaesth Intensive Care 1997;25:701-3.

225. Michael JR, Barton RG, Saffle JR, Mone M, Markewitz BA, Hillier K, et al. Inhaled nitric oxide versus conventional therapy: effect on oxygenation in ARDS. Am J Respir Crit Care Med 1998;157:1372-80.

226. Dellinger P, Zimmerman JL, Taylor RW, Straube RC, Hauser DL, Criner GJ, et al. Effects of inhaled nitric oxide in patients with acute respiratory distress syndrome: results of a randomixed phase II trial. Inhaled Nitric Oxide in ARDS Study Group. Crit Care Med 1998;26:15-23.

227. Meyer J, Thielmeier G, Van Aken H, Bone HG, Busse H, Waurick R, et al. Inhaled prostaglandin E1 for treatment of acute lung injury in severe multiple organ failure. Anesth Analgesia 1998;86:753-8.

228. Schutte H, Grimminger F, Otterbein J, Spriestersbach R, Mayer K, Walmrath D, et al. Efficiency of aerosolized nitric oxide donor drugs to achieve sustained pulmonary vasodilation. J Pharmacol Exp Ther 1997;282:985-94.

Author Van Heerden P V
Name of thesis Inhaled Aerosolised Prostacyclin As A Selective Pulmonory Vasodilator Van Heerden P V 2000

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.