
Ferric sulphate and formocresol pulpotomies in baboon primary molars: histological responses

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ABSTRACT. *Aim* To compare pulpal reactions to ferric sulphate and formocresol pulpotomies in primary molar teeth with inflamed pulps. *Study design* An experimental study in 15 juvenile baboons (*Papio ursinus*). *Materials and methods* Pulpitis was induced with fresh human carious dentine or *Streptococcus mutans* placed into occlusal cavities in 57 primary molars; after 14 days a pulpotomy was performed on the same primary molars with the two pulp medicaments randomly allocated; the pulp was covered with IRM and the cavity filled with amalgam. After 90 days specimens were harvested and examined under the light microscope with the examiner blind to the treatment. *Results* Reaction frequencies in the ferric sulphate-treated and formocresol-treated teeth were: recognisable pulp 52% and 50%, dentine bridges 16% and 12%, internal root resorption 12% and 4%, external resorption 28% and 31%, bacteria 12% and 23%, peri-apical abscesses 32% and 38%. *Statistics* Fisher's exact probability test showed no statistically significant differences between reaction frequencies in the two treatment groups. *Conclusion* A pulpotomy in a primary tooth may be clinically successful in the presence of adverse histological reactions.

KEYWORDS: Pulpotomy, Primary tooth

Introduction

Clinicians' choice of dental pulp treatment for primary teeth today should be evidence-based, but is still largely empirical. Two excellent reviews of pulp therapies for primary and young permanent teeth have shown that while there has been contradictory experimental work, tradition plus clinical signs and symptoms are the main influencing factors in choice of treatment [Ranly, 1994; Ranly and Garcia-Godoy, 2000]. Thus, formocresol (FC) has remained the standard pulpotomy medicament in primary teeth. Ferric sulphate (FS) has become more popular, having been first suggested as a pulpotomy medicament some 24 years ago [Landau and Johnson,

1988], although the original rationale for its introduction has been forgotten [Ranly and Garcia-Godoy, 2000].

Clinical outcome studies of FS and FC pulpotomies in primary teeth are quite numerous [Ranly and Garcia-Godoy, 2000], but experimental investigations in animal models are relatively uncommon and in non-human primates they are rare. Regarding FS, one study in baboon primary teeth compared reactions to FS and FC four and eight weeks after pulpotomy in non-inflamed teeth [Fuks et al., 1997]. This study showed normal pulps in 60% of FS-treated teeth compared with 48% of the FC-treated teeth.

As the pulpotomy procedure is indicated for the coronal amputation of affected, or infected dental pulp when the remaining radicular pulp is judged to be vital [American Academy of Pediatric Dentistry, 1999], experimental assessment of pulpotomy medicament effects should be done on inflamed pulps. The aim of the current study was to compare tissue responses of inflamed baboon primary molar pulps to ferric sulphate and formocresol pulpotomies.

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Materials and methods

Prior to beginning the study, ethics approval was obtained from the Animal Ethics Screening Committee of the University of the Witwatersrand (Clearance 98/36/4).

Study design. Fifteen juvenile baboons (*Papio ursinus*) of unknown age, weighing 3-4 kg, with erupted primary molars and first permanent molars were the study animals. They were housed in the University's Central Animal Service in a purpose-built primate facility with controlled environment, feeding and daily professional care. The sample size was determined by a combination of budget and to provide approximately 30 teeth in each of four treatment groups, two of which are reported in this study.

Randomization and blinding. FS and FC were allocated to teeth using a randomization block with eight holes, marked 55 to 85 to represent the primary molars in the four quadrants, and eight pegs, two marked for each of four treatments (two pulpectomy treatments were a separate experiment). For each baboon, the pegs were shuffled then placed into the numbered holes in clockwise sequential order from 55 to 85.

Operative method. Each baboon was immobilized with an intramuscular injection of ketamine hydrochloride after which anaesthesia was induced with intravenous thiopentone followed by insertion of a nasal endotracheal tube to maintain the airway. Anaesthesia was maintained with inhalation of halothane and oxygen in a semi-closed circuit with carbon dioxide absorber. Vital signs were electronically monitored throughout the anaesthesia.

Pulpitis was induced based on two reported methods [Mjör and Tronstad, 1972; Fuks et al., 1997] and was checked in a study of two baboons [Cleaton-Jones et al., 2001]. The primary molars were isolated with rubber dam and an occlusal cavity was cut with a water-cooled high speed bur. A small pulpal exposure was made in the floor of the cavity with a 1 mm diameter round bur. The cavity was then swabbed with 37% phosphoric acid and dried with cotton wool pellets, and a small piece of fresh carious dentine or a piece of nutrient agar with a colony of *Streptococcus mutans* was placed over the exposure. The cavity was restored with a light-cured composite resin without etching.

Two weeks later the baboons were anaesthetised as before, the primary molars were isolated with rubber dam and the coronal pulp chamber was opened with a high speed, water-cooled bur. The coronal pulp was removed with a low speed round bur and haemostasis was obtained with saline and cotton-wool pellets. Ferric sulphate (15.5% - Astringent Ultradent

Products Inc., Utah, USA) was applied for 15 seconds to the stumps of the root pulps in 29 teeth with a cotton pellet. Formocresol (20% - Buckley's Formocresol, Sultan Chemists Inc., New Jersey, USA) was placed on the pulp stumps for two minutes using a cotton-wool pellet. All cavities were dressed with IRM and sealed with amalgam. Postoperatively the baboons were fed a soft diet of maize porridge plus veterinary protein-vitamin-mineral mix for seven days then returned to the standard laboratory diet of baboon cubes, protein-vitamin-mineral mix plus fruit of the day. A narcotic analgesic, buprenorphine, was available for any baboon that might need this.

Ninety days later, each baboon was immobilized with ketamine and humanely killed with an intravenous overdose of pentobarbitone. The thoracic aorta was cannulated and the right atrium of the heart was opened, followed by retrograde perfusion of the head with 1L of 0.9% saline then 1L of 10% buffered formol saline. When perfusion was completed the head was removed, soft tissue dissected off and the maxilla and mandible cut away from the skull with a motorized band saw. Each jaw was then trimmed with the band saw anterior to the canine and distal to the first permanent molar to produce a jaw quadrant block containing the experimental teeth. The jaw quadrants were then radiographed from the lateral aspect on occlusal films. Decalcification was in a mixture of sodium citrate (0.03M), formic acid (1.6N) and hydrochloric acid (0.65N), or in Shandon TBD-1 rapid decalcifier (Shandon Inc., Pittsburgh, Pennsylvania, USA) over approximately one month with the end point monitored with radiographs. The specimens were processed into wax and serial sections of the quadrant block cut at 5 to 6 Φ m. Every 10th section was stained with haematoxylin and eosin and representative sections stained with the Brown-Brenn method. Cut sections were labelled with a sequential code and quadrant but not the treatment to ensure blinding of the examiner during microscopy. The code was broken after assessment of the tissue responses.

Assessment of responses. The following tissue responses were looked for:

- within the root canal: recognizable pulp plus the proportion in thirds of the root canal that this occupied, pulp necrosis, pulp inflammation, a continuous secondary dentine bridge across the root canal opening, secondary dentine on the walls, internal and external root resorption (other than that associated with normal tooth exfoliation), and bacteria stained with the Brown-Brenn method;
- at the peri-apex: any abscess was defined as a recognizable collection of acute or chronic

inflammatory cells of any size. The radiographs of the jaw quadrant blocks were examined for periapical radiolucencies.

Statistical analysis. Fisher's exact test was applied for comparison of frequency data using InStat (Version 3.02, GraphPad Software Inc., San Diego, California, USA). A P value of <0.05 was considered to be statistically significant.

Results

All the pulps treated in this experiment were vital at the time of pulpotomy as indicated by bleeding on opening of the coronal pulp chamber. Each baboon gained approximately 1 kg during the 90 days post-pulpotomy, which is the expected normal weight gain for their size, and all ate the standard laboratory diets without obvious discomfort; no baboon required postoperative analgesia.

Six of the 57 pulpotomised teeth were not included in the histological assessment. There were two furcation cuts and two exfoliations in the FS-treated teeth, and one furcation cut and one exfoliation in the FC-treated teeth.

Frequencies of tissue responses are listed in Table 1. Secondary dentine bridges such as the example shown in Figure 1, were uncommon. Recognizable pulp was absent from approximately half the teeth in both

groups. In each such instance the canal was either empty or had scraps of necrotic tissue on the root canal walls or at the apex. Even when recognisable tissue was present some necrosis was seen as well, so the frequency of necrosis was 100%. Within the FS group a quarter of the specimens had recognizable pulp tissue for at least 2/3 of the root length. Inflammation in recognizable pulp tissue was confined to the apical 1/3 of the canal and was associated with the periapical abscesses (Fig. 2). Secondary dentine on the root canal walls was more common in the FC group, but two FS-treated teeth in the same baboon obliterated the root canal down to the apex. Internal root resorption was uncommon, while external root resorption was present in approximately a third of all specimens (Fig. 3). Bacteria were seen in the root canals of 3/24 FS-treated teeth and 6/26 FC-treated teeth. In one root canal in each group bacteria were seen without a periapical abscess. When seen, the bacteria were small numbers scattered along the root canal wall as far as one third above the apex, except for one tooth in which bacteria were seen as far down as the apex. Apical abscesses were slightly more common in the FC than the FS teeth in a ratio of 1.25 to 1, respectively. The diameter of the abscesses varied from 0.25 mm to 4 mm.

No statistically significant differences were found for any of the reactions between the two pulpotomy treatments.

Variable	Ferric sulphate		Formocresol	
	n	%	n	%
Number of teeth	25		26	
Recognizable pulp:				
none	13	52	13	50
0-1/3	5	20	10	39
1/3 - 2/3	1	4	1	4
>2/3	6	24	3	12
Secondary dentine bridge	4	16	3	12
Secondary dentine on walls	8	32	11	42
Secondary dentine obliterating pulp chamber	2	8	0	0
Internal root resorption	3	12	1	4
External root resorption	7	28	8	31
Bacteria present	3	12	6	23
Periapical abscess	8	32	10	38
Periapical abscess on radiograph	3/8		4/10	
Periapical abscess and bacteria in pulp	2/8		5/10	
Periapical abscess and no bacteria in pulp	6/8		5/10	

TABLE 1 - Frequencies of observations of tissue responses to formocresol or ferric sulphate in pulpotomies



FIG. 1 - Dentine bridge above a pulpotomised pulp. Haematoxylin and eosin x50.



FIG. 2 - Abscess present in remaining pulp tissue at the peri-apex. Haematoxylin and eosin x50.

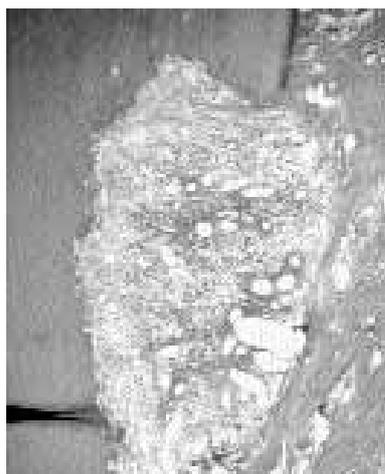


FIG. 3 - External root resorption. Haematoxylin and eosin x50.

Discussion

This study has shown that baboon primary molar inflamed pulp reactions to FS pulpotomy are similar to those of FC pulpotomy. Having said that, both pulpotomy treatments were associated with pulps empty of recognisable tissue and containing necrotic pulp. These reactions may be due to the pulpotomy treatments of the induced pulpitis. In an earlier study, in two baboons to test the pulpitis induction method used in the current study, necrosis of the pulp was present in all 16 experimental teeth and apical abscesses in half of the teeth [Cleaton-Jones et al., 2001]. Also, the empty pulps may be so because the pulp had been destroyed or perhaps had fallen out during sectioning due to

alteration of its normal attachment to the dentine by the treatment or induced inflammation.

The current study on inflamed pulps has shown lower rates of recognisable pulps and dentine bridges, as well as a higher rate of apical abscesses, compared with the similar study in baboon primary pulps without pre-pulpotomy inflammation [Fuks et al., 1997]. This suggests that testing of responses to pulpotomy medicaments should be on inflamed pulps rather than uninfamed pulps, in order to mimic reality and to avoid drawing too favourable a conclusion.

That both FS and FC pulpotomies produce favourable clinical results in human primary teeth is clear from clinical studies [Fei et al., 1991; Fuks et al., 1997; Farooq et al., 2000; Chien et al., 2001]. However, there may be deleterious effects such as enamel defects in permanent tooth successors [Rølling and Poulsen, 1978] or cysts associated with the pulpotomised teeth [Lustig et al., 1999]. In the latter instance, inflammation and prolonged irritation are believed to be causal [Grundy et al., 1984; Benn and Altini, 1996]. This suggests that a treatment associated with inflammation should not be done. Primary teeth have a limited period in the mouth so that inflammation should disappear once the tooth is lost. The current study has shown a high prevalence of apical abscesses and lack of normal pulp that may be present when there is clinical success. Search of the literature and standards organisation recommendations has not produced guidelines that define histological success after experimental pulpotomy. In everyday practice clinical signs and symptoms determine success. In baboons this is not possible to determine in the same way but the

normal weight gain, normal eating, an apparent lack of pain suggest that there is clinical success. Of the 18 abscesses found on microscopy, only 6 were clinically visible on radiographs. Perhaps the combination of a histological abscess and a radiographic change are reasonable signs of experimental success? What was surprising was that of the 18 periapical abscesses seen only 7 had identifiable bacteria in the root canal.

Are experimental observations in animal models directly applicable to humans? While it may be argued that this is questionable when the experimental animals are rodents [Cotes et al., 1997], it is possible when they are dogs [El-Meligy et al., 2001] and unclear if they are lambs [Özata et al., 1987]. But Pascon et al. [1991] consider non-human primates as the best animal model for endodontic studies. We agree with the latter view.

Healing in primary pulps should be good; the pulps of primary teeth have been shown to be quite resilient [Ranly and Garcia-Godoy, 2001] and are cell rich and fibre poor [Mjör, 1996]. An important part of the healing of an exposed pulp is the formation of a dentinal bridge between healing pulp and the exposed area; these dentinal bridges were uncommon in the current study. That is not surprising as formocresol should destroy cells that would produce the healing and the effect of ferric sulphate other than its haemostatic properties is unclear.

Conclusion

This study has shown that both ferric sulphate and formocresol produced apparently successful vital pulpotomies in experimentally inflamed primary molar pulps in a group of baboons, but both were associated with pulp necrosis and apical abscesses at a histological level. No histological criteria for success have been described. We suggest that there is success in primary teeth even if there are histologically recognisable adverse changes provided that the treated tooth appears clinically functional, and there is no associated radiographic change.

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