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2004

http://hdl.handle.net/10539/20270

Cleaton-Jones,P., Duggal,M., Parak,R., et al. 2004. Pulpitis induction in baboon primary teeth using carious dentine Streptococcus mutans. Journal of the South Africa dental association;59(3):119-122

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SADJ April 2004
Vol 59 No 3 pp 119 – 122

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SUMMARY

The objective of this equivalency study was to see if a colony of Streptococcus mutans placed into cavities in primary molar teeth produced pulpitis similar to an established pulpitis induction method using carious dentine. In two juvenile baboons (Papio ursinus), occlusal cavities were cut in all 16 primary molar teeth, followed by making a small pulpal exposure after which the cavity was swabbed with 37 per cent phosphoric acid. In one half of the teeth, fresh soft human carious dentine was placed over the pulpal exposure; in the remaining teeth the exposure was covered with a colony of Streptococcus mutans in agar. All the cavities were restored with unlined light-cured composite resin. After 14 days specimens were harvested and examined under the light microscope with the examiner blind to the induction method. In both groups of teeth there was recognisable pulp, hyperaemia, micro-abscesses in the pulp and peri-apical abscesses. Reactions to soft caries were more severe than to Streptococcus mutans. The results show that Streptococcus mutans placed in a cavity with an exposure produces comparable pulpitis to fresh soft human carious dentine in the same type of cavity and that both methods produce pulpitis suitable for testing pulpotomy or pulpectomy treatments.

INTRODUCTION

Ideally, choice of dental pulp treatment for primary teeth today should be evidence-based, yet it is still largely traditional although influenced to some extent by signs and symptoms.[1,2] Evidence from clinical outcome studies of pulpotomies in primary teeth are quite numerous, but experimental investigations in animal models are relatively uncommon and in non-human primates they are rare.

Since 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,[3] experimental assessment of reactions to pulpotomy or pulpectomy should be on inflamed pulps. Pulpal inflammation has been induced with infected dental tissue and with chemical irritants. For example, to produce such inflammation in permanent teeth, Lundy and Stanley[4] used unlined silicate restorations in experimental cavities in human permanent teeth, a method that became a standard positive control for evaluation of the pulpal effects of restorative materials.[5] Later, Mjör and Tronstad[6] produced pulpal inflammation in monkey permanent teeth by placing soft carious human dentine into buccal class V cavities and covering this with amalgam, or by filling a cavity with gutta percha. As regards primary teeth, soft carious dentine has been used in baboons.[7]

Nowadays, fresh soft human carious dentine is difficult to come by in research institutions due to declining prevalence of carious teeth. The current equivalency study was done to see if pulpitis induced in baboon primary teeth by a colony of Streptococcus mutans is similar to that produced with soft carious dentine. The rationale was that bacterial cultures are readily available and of particular importance and the bacterial load may be standardised. This is something that is not possible with carious dentine.

MATERIAL AND METHODS

Experimental animals

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). Two juvenile baboons (Papio ursinus) of unknown age, weighing 3 to 4 kg, with erupted primary molars as well as 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.

Cavity preparation and induction

Each baboon was immobilised with an intramuscular injection of ketamine hydrochloride (Anaket-Vet®, Centaur Labs, Bayer (Pty) Ltd, Isando, South Africa) after which anaesthesia was induced with intravenous thiopentone (Bodene Intramed, Woodmead, South Africa) 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 a carbon dioxide absorber. Vital signs were electronically monitored throughout the anaesthesia.

The two primary molars in each jaw quadrant were isolated with rubber dam and an occlusal cavity was cut in each using a water-cooled high-speed diamond bur (Hi-Di®, Ash Instruments Division, Dentsply Ltd, Weybridge, United Kingdom). After this, a small pulpal exposure was made in the floor of the cavity with a 1 mm diameter, slow-running round steel bur (Ash Instruments Division, Dentsply Ltd, Weybridge, United Kingdom), followed by swabbing of the cavity with 37 per cent phosphoric acid (AnalaR, Merck KGaA, Darmstadt, Germany) to remove the cavity smear layer. The cavity was then dried with cotton wool pellets. The same test material was placed over the exposure in both pri-

mary molars in a quadrant in a crossover design. In one baboon it was a small piece of soft fresh human carious dentine in the each primary molar in the top right and lower left quadrants; with single 24-hour colonies of Streptococcus mutans in blood agar in each of the primary molars of the remaining quadrants. In the second baboon the order of quadrants was reversed. Each cavity was restored with a light-cured composite resin (Z100, 3M Dental Products, St Paul, Minnesota, USA) without etching. The 24-hour colonies of Streptococcus mutans were grown at 37 °C under anaerobic conditions in Tryptone Soy agar (Biolab Merck, Midrand, South Africa) containing 5 per cent horse blood in a gas mixture of 10 per cent carbon dioxide, 9,7 per cent hydrogen and 80,3 per cent nitrogen.

This was an equivalency study to see if a more convenient material (Streptococcus mutans colony in blood agar) would produce similar pulpitis to a method (carious dentine) already shown to induce pulpitis. In this type of study a control (cavity without induction material) is not required.

Post-operatively the baboons were fed a soft diet of maize porridge plus veterinary protein–vitamin–mineral mix (PVM Products Ltd, Silverton, South Africa) for seven days then returned to the standard laboratory diet of baboon cubes, protein–vitamin–mineral mix plus fruit of the day. A single dose of an intramuscular narcotic analgesic, buprenorphine (Temgesic®, Shering-Plough, Isando, South Africa) was given once the baboons had emerged from the general anaesthetic. No further doses were required. The need for analgesia is indicated by poor eating or depression.

Preparation of tissue samples

Fourteen days later, each baboon was immobilised with ketamine and humanely killed with an intravenous overdose of pentobarbitone (Euthanaze®. Centaur Labs, Bayer (Pty) Ltd, Isando, South Africa). 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 per cent saline then 1L of 10 per cent buffered formol saline. Unless otherwise specified, perfusion and decalcification solutions were made in our laboratory using AnalaR reagents (Merck KGaA, Darmstadt, Germany). When perfusion was completed the head was removed, soft tissue dissected off and the maxilla and mandible cut away from the skull with a motorised band saw (Crown OKTO, Crown Mills Equipment, Johannesburg, South Africa). 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 primary molars. The jaw quadrants were then radiographed from the lateral aspect on occlusal films and examined for peri-apical radiolucencies.

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) depending on available supplies, 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 μm. Every tenth section was stained with haematoxylin and eosin. Representative sections were stained for bacteria with the Brown-Brenn modification of the Gram stain.[8] Cut sections were labelled with a sequential code and quadrant, but the induction method was kept unknown to ensure blinding of the examiner during microscopy. The code was broken only after assessment of the tissue responses.

Each step serial section was examined and categorical responses noted as follows for the coronal pulp, root pulp, apical 2 mm of root, and at the peri-apex; the worst appearance per specimen was the one recorded and the maximum possible frequency for any of the categories recorded was 8 per experimental group:

- recognisable pulp
  - none = no pulp tissue present for assessment. This could be an artefact but is usually indicative of pulp necrosis with liquefaction.
  - strands = pulp cells and stroma separated by large oedematous spaces.
  - substantial = congruent amount of pulp tissue whether inflamed or not.
- inflammation
  - hyperaemia = dilated blood vessels and oedematous spacing between cells.
  - abscess = a recognisable collection of acute inflammatory cells and pus; if of very small size it was termed a micro-abscess.
- Bacteria = presence of bacteria stained with Brown-Brenn method.

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). $P<0.05$ was considered to be statistically significant.

RESULTS

All the pulps in this experiment were vital at the time of pulpal exposure indicated by bleeding on opening the coronal pulp chamber. Within the two-week post-operative period the baboons behaved as usual and ate the laboratory diet as they did before the operation. Intra-oral examina-

Table I. Frequencies of pulp responses by site and experimental group. The maximum possible frequency per response in each experimental group is 8.

Site	Response		Soft carious dentine N	Streptococcus mutans N
Coronal pulp	Recognisable pulp	None	1/8	1/8
		Strands	5/7	0
		Substantial	2/7	7/7
	Inflammation	Hyperaemia	6/7	7/7
		Micro-abscess	3/7	5/7
	Bacteria		8/8	8/8
Root Pulp	Recognisable pulp	None	4/8	3/8
		Strands	3/4	0/5
		Substantial	1/4	5/5
	Inflammation	Hyperaemia	4/4	5/5
		Micro-abscess	2/4	1/5
	Bacteria	None	2/8	0/8
Apical 2 mm of root	Recognisable pulp	Strands	4/8	1/8
		Substantial	4/4	2/7
		Hyperaemia	0	5/7
	Inflammation		4/4	5/7
	Bacteria		0	0
Peri-apical Area	Inflammation	Abscess	5/8	2/8
	Bacteria		0	0



Figure 1: Normal primate molar pulp. Haematoxylin and eosin. X256.



Figure 2: Experimental pulpitis – the pulp adjacent to the dentine has been compressed into strands by marked oedema. Haematoxylin and eosin. X100.



Figure 3: The root canal is empty due to pulp necrosis with liquefaction. A peri-apical abscess is clearly evident. Haematoxylin and eosin. X64.



Figure 4: High power view of external root resorption at the peri-apex of the specimen in Figure 3. Haematoxylin and eosin. X450.

tion at euthanasia showed no pointing or draining abscesses. One peri-apical radiolucency was seen on the radiographs of the excised jaw specimens. Frequencies of tissue responses in the three pulp areas are listed in Table I. In the table, frequencies are given per pulp that could be assessed. For inflammation this means pulps with recognisable pulp; for bacteria this is all the teeth.

Coronal pulp

The normal baboon pulp is very cellular (Figure 1). In the experimental teeth recognisable pulp was seen in 13/16 teeth but the arrangement of this tissue varied between the two induction method groups. In the soft caries group, strands of pulp predominated compared with substantial amounts of pulp that almost filled the coronal site in the Streptococcus mutans group. Hyperaemia and oedema were seen in 13/13 teeth with sufficient recognisable pulp for assessment. Where oedema was pronounced, the pulp was reduced to strands of tissue separated by spaces (Figure 2). Micro-abscesses were noted in 8/13 assessable pulps and were more common in the Streptococcus mutans group. Bacteria were seen in 16/16 pulps.

Root pulp

Eleven of the sixteen teeth had recognisable pulp in the root that could be assessed for inflammation. Hyperaemia and oedema were common; present in 9/11 teeth. Bacteria were seen in the root in 2/8 teeth in the soft caries group and not at all in the Streptococcus mutans group.

Apical 2 mm of root

All but one of the teeth in the Streptococcus mutans group had recognisable pulp, mostly substantial amounts, compared with only strands of recognisable pulp in half of the teeth in the soft caries group. Hyperaemia was common in both groups.

Peri-apical area

Peri-apical abscesses with associated external root resorption (Figures 3 and 4) were more than twice as common in the soft caries group compared with the Streptococcus mutans group. No bacteria were seen in this tissue in either of the two groups.

Statistical analysis showed no significant differences in frequency of any of the responses between the two pulpitis induction groups.

DISCUSSION

This study has shown that both soft carious dentine and a colony of Streptococcus mutans, placed in an experimental cavity in baboon primary molars produced a pulpitis that was slightly more severe in the soft carious dentine group at 14 days than in the Streptococcus mutans group. No statistically significant differences were found in the current study using the Fisher's exact test, a test that is suitable to find statistically significant differences between small samples such as in the current study. The absence of such a difference, using this test, however, merely indicates that a difference is not large

and does not preclude finding a statistically significant difference in a larger sample.
That only one peri-apical radiolucent area was recognised on the very clear specimen radiographs may seem surprising. An explanation is that the peri-apical abscesses seen in the histological sections were generally small and there was considerable overlapping of structures because of the developing permanent teeth. Also, 14 days is not long enough for peri-apical bone to be lost and replaced with sufficient granulation tissue to be seen on a radiograph.

Is 14 days a suitable time to assess pulpitis induction is a natural question. An answer is that there is no generally accepted timing for this, nor is there a single accepted pulpitis induction method. Mjör and Tronstad6 examined induced pulpitis at 8 days in 45 permanent teeth of 4 monkeys. They found that carious dentine and amalgam in buccal Class V cavities produced a localised, severe pulp reaction, whereas gutta-percha gave a slight to moderate reaction. Cavities left open produced slight, moderate and severe reactions without a systematic pattern. Their conclusion was that both carious dentine and gutta-percha produced inflammation suitable for studies of pulp healing, but the variable inflammation of the open cavities was unsuitable.

Lervik and Mjör[9] induced pulpitis in 136 permanent teeth from 19 monkeys with soft carious dentine covered with amalgam, or gutta percha and evaluated the outcome at 2 to 3, 4 to 5, 7 to 8, 14, and 82 days. They reported that inflammation severity varied with cavity depth – it was more severe as the depth increased. Increased pre-dentine formation was seen at 7 to 8 days suggesting that healing had begun; while at 14 days 2/16 teeth showed necrosis. Healing occurred in most teeth after 82 days through formation of a barrier in the dentine. Their conclusions were that experimental procedures that often caused severe pulp reactions initially were usually followed by pulp healing without removal of the causative factor, but severe or moderate inflammation delayed or inhibited the formation of secondary dentine. They recommended that to obtain severe pulpitis without healing, 3 to 5 days might be the most appropriate period and that observations longer than 7 to 8 days should not be used.

The fine structure of pulpitis induced in five monkey permanent teeth with carious dentine was studied after 7 days.[10] Four out of the five teeth had inflammation with polymorphonuclear leucocyte infiltration and one had extensive tissue destruction with numerous bacteria present. This indicates that a shorter induction period may be chosen if less damage is desired; such a choice limited the period to 3 to 5 days in an earlier study in non-human primates.[7]

Choice of time, after pulpitis induction, for experimental pulpotomy should be based on clinical reality as well as experimental studies. Discussion with experienced clinicians with paedodontic experience in South Africa indicated that children with inflamed pulps generally present to the dentist about 14 days from first onset of symptoms. Similarly, local experience is that the pulpitis presenting clinically is generally associated with a pulpal exposure. Therefore in this study an exposure was included, as previously used,[7] and the 14 day post-induction time was chosen.

Evidence of pulp healing as described by Mjör and Tronstad[6] was not seen in the current study suggesting that the 14-day post-pulpitis induction period is acceptable.

Is the baboon a suitable experimental model for pulpitis studies? We believe that it is – the baboon (Papio ursinus) was chosen for the experiment because its dentition is similar to that of humans and primates are accepted models for tissue healing that may be extrapolated to humans.[6 7 9 10] Research on induced pulpitis in primate primary teeth is rare. Fuks et al.[7] induced pulpitis in 94 primary teeth in 12 baboons (Papio ursinus) by placing carious dentine in buccal cavities in incisors and canines or occlusal cavities in molars for 3 to 5 days before pulpotomy. They concluded from their evaluation of the pulpal response that the induced pulpitis is suitable to assess pulpotomy.

How severe an experimental inflammation should be, in either permanent or primary teeth, for evaluation of pulpotomy or pulpectomy has not been defined. Logically, inflammation should be severe enough to mimic the clinical situation that would confront a dentist who would choose to use either of these two treatments based on published recommendations.[3] It should, however, not be so severe that all pulp is destroyed in all experimental teeth perhaps coupled with clinical sepsis. In the current study, 11/16 experimental teeth still had recognisable pulp at 14 days, which we feel is a reasonable mimicry of what a dentist is likely to encounter in clinical practice. Is the pulpitis induced so severe that pulpotomy should not be considered? We believe that this is not so and in an experimental study on baboons using the same pulpitis induction methods have shown that pulpotomy can successfully treat the inflamed pulp.[11]

Why should there be a greater inflammation in the soft caries group than the Streptococcus mutans group? A likely explanation is that the bacterial load is greater in this group although we have no evidence to prove this. Regarding bacterial load an advantage of the single colony of Streptococcus mutans is that the load may be reasonably standardized. In the current study we chose 24 hours to ensure that the organisms were in an exponential growth phase.

CONCLUSION

Both induction methods produced a pulpitis that would fit the recommendations for treatment by pulpotomy[3] but using a colony of Streptococcus mutans is more reproducible and produces a less severe pulpitis.

ACKNOWLEDGEMENT

This study was supported by a Wellcome Trust Collaborative Research Initiative Grant. We are most grateful to Professors M Altini and H Coleman for help with diagnoses and photomicroscopy.

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