Effects Of Antibacterial Agents On Dental Pulps Of Monkeys Mechanically Exposed And Contaminated.

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Objective: *The purpose of this study was to compare the effectiveness of antibacterial agents and mineral trioxide aggregate in the healing of bacterial contaminated primate pulps.* ***Study Design:*** *The experiment required four adult male primates (Cebus opella) with 48 teeth prepared with buccal penetrations into the pulpal tissues. The preparations were performed under general anesthesia and the exposed pulps were exposed to cotton pellets soaked in a bacterial mixture consisting of microorganisms normally found in human pulpal abscesses obtained from the Endodontic Clinic of UNESP. Following bacterial inoculation (30 minute exposure), the pulpal tissue was immediately treated with either sterile saline, Cipro HC Otic solution (12), diluted Buckley' formecresol solution (12) or Otosporin otic solution (12) for 5 minutes. After removal of the pellet, hemostasis was obtained and a ZOE base applied to the DFC treated pulps and the non-treated controls (12). After hemostasis, the other exposed pulps were covered with mineral trioxide aggregate (ProRoot). The pulpal bases were all covered with a RMGI (Fuji II LC). The tissue samples were collected at one day, two days, one week and over four weeks (34 days).* ***Results:*** *Following perfusion fixation, the samples were demineralized, sectioned, stained and histologically graded. After histologic analysis, presence of neutrophilic infiltrate and areas of hemorrhage with hyperemia were observed. The depth of the neutrophilic infiltrate depended on the agent or material used. The pulpal tissue treated with Otic suspensions demonstrated significantly less inflammation (Kruskal Wallis non parametric analysis, H=9.595 with 1 degree of freedom; P=0.0223) than the formocresol and control groups. The hard tissue bridges formed over the exposure sites were more organized in the MTA treatment groups than in the control and ZOE groups (Kruskal Wallis non parametric analysis, H=18.291 with 1 degree of freedom; P=0.0004).* ***Conclusions:*** *Otic suspensions and MTA are effective in treating bacterial infected pulps and stimulate the production of a hard tissue bridge over the site of the exposure.*

Keywords: *pulp exposures, pulp response, bacteria, primates*
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INTRODUCTION

In preserving the severely carious dentition, the dental profession has attempted various treatments for cariously compromised dental pulps that are often controversial.[1] Occasionally a practitioner will incorporate treatment of exposed dental pulps that has been suggested by only one successfully treated clinical case.[2] In other dental practices, clinicians routinely resort to full endodontic treatment, ignoring current research suggesting more conservative treatment. For example, indirect pulp capping and stage caries removal is not widely utilized and yet is well supported by research.[3] In addition, there appears to be little consensus on direction as dentists in different countries have adopted various conservative treatment techniques for cariously exposed dental pulps. One such technique (from Brazil) is the application of otic solutions in performing coronal pulpotomies of cariously exposed teeth.[4] The objective of this initial research project was to determine if there existed any validity to the concept of utilizing otic solutions with either a calcium hydroxide or mineral trioxide aggregate dressing. Ninety primary molars were selected that showed clinical and radiographic indications for pulpotomy treatment. The pulpotomies were performed in two treatment visits with a corticosteroid/antibiotic solution (Otosporin Otic Solution) as interim therapeutic medicament. The

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experimental groups were designated as two groups of 45 teeth, in which the pulpal remains were treated with either $Ca(OH)^2$ paste (Group 1) or MTA, mineral trioxide aggregate (Group 2). Radiographs were taken immediately post-treatment and at 3, 6, and 12-month follow-up appointments. After submitting to clinical and radiographic criteria for successful treatment, three teeth in Group 1 were determined to have failed after three months, while two cases failed after six months and one more failed at one year. In Addition, two failures were found in Group 2 at the 12-month follow-up. These results would indicate that both materials may be utilized for pulpotomy bases after otic solution application in primary teeth. The success rate of using Otic Solution was considered by the authors to be comparable to dilute formocresol. As such, the utilization of Otosporin Otic Solution for pulpotomy treatment is reportedly practiced by a number of pediatric dentists in Brazil. As of this date, no histological studies have been published determining the healing capability of Otic solutions on pulpal tissues.

For many decades, pulpotomy treatment of primary teeth was based on the theory of devitalizing the remnant pulpal tissue, mainly utilizing formocresol for fixation of the pulpal stumps. However, the application of this chemical does not produce true fixation of the pulp, but only creates a state of chronic inflammation.[5] Because of this property, it cannot be considered a biological medication as it does not promote pulpal tissue healing. In addition, it is toxic, mutagenic, and considered carcinogenic, with great capability of diffusion, being able to reach the periapical tissues[6] and the permanent tooth follicle.[7]

The ideal material for pulp treatment would be bactericidal, creating no injury to the pulp or adjacent oral structure, promote regeneration of the pulp tissue and not interfere in the physiological process of root resorption. New substances, considered to be more biologically kind, such as dehydrated bone, collagen, morpho-genetic proteins and allogenic dentin matrix have been proposed, as well as non-pharmacological techniques for the treatment of the exposed pulp, including laser and electro-surgery. However, the results confirming the long term success of these materials and techniques are not yet available, therefore, the ideal agent for pulp therapy in primary teeth has not yet been identified.[8]

Calcium hydroxide, considered biocompatible, has a highly alkaline pH and demonstrates capability of inducing a mineralized barrier while also conferring a bactericidal effect, is considered to be a pulp tissue regenerating material.[9] While there are controversies with regarding its application in primary tooth pulpotomies due to the occurrence of internal resorption,[10] a review of scientific papers published between 1955 and 1984, reported a clinical success rate between 31% and 100%.[6] Calcium hydroxide has been recommended for application to traumatically exposed dental pulps and its successful outcome is very well documented.[11,12, and 13] The bactericidal effects of calcium hydroxide allow for successful treatment of the bacterially contaminated pulp after surgical amputation of the exposed pulpal tissue.[14] The amputation of the exposed, contaminated pulpal tissue is often credited with a significant contribution to the successful outcome.[15] Other studies have shown dentin bridge formation after a direct pulp cap with VLC Dycal®, a resin based light cured calcium hydroxide formulation. Pitt Ford and Roberts[16] studied the pulpal response to mechanical exposure and capping either immediately or after 24 hours in 64 teeth of four cynomolgus monkeys with the use of Dycal, VLC Dycal, or Prisma-Bond®. Dentin bridges were present in almost all teeth filled with Dycal or VLC Dycal, and pulpal inflammation was observed in only one tooth that showed evidence of infection. The success rate of pulp capping delayed for 24 hours was as high as that for immediate capping.

A product referred to as mineral trioxide aggregate (MTA®) was proposed by the University of Loma Linda, California, to seal communications between the root canal system and the external surfaces of teeth at different levels.[17] MTA is made up of fine hydrophilic particles (powder) that harden in the presence of water. Its main components are tricalcium aluminate, tricalcium silicate, tricalcium oxide and silicate oxide, all found in Portland cement, with the addition of bismuth oxide to confer radio-opaque characteristics. Electronic micro-analysis revealed that the main molecules present in MTA are calcium and phosphate ions, and as these are also components of the dental tissues, they may confer biocompatibility to this material. After mixing with water, the pH progresses from 10.2 to 12.5 in three subsequent hours, providing evidence, among other properties, of an antimicrobial effect on some facultative anaerobic bacteria, due to its highly alkaline pH.[18]

This material is characterized by demonstrating excellent sealing capacity[17,19, and 20] biocompatibility[21,22, and 23] not having mutagenic potential,[24] low cytotoxicity[25] and for stimulating the activation of cellular response,[26] the deposition of cementum, which may facilitate the regeneration of the periodontal tissue[27] and the formation of mineralized tissue.[28,29] Holland *et al.*[29] observed that MTA has a similar mechanism to that of calcium hydroxide, with calcium oxide as one of its main components which, after mixing the powder with water, would be converted into calcium hydroxide. After contacting the tissue fluids the calcium hydroxide dissociates itself into Ca and OH ions. The calcium ions react with carbon dioxide in the tissues giving rise to the calcite granulations. Together with these granulations there would be an accumulation of fibronectine, which would allow cellular adhesion and differentiation. In time a hard tissue bridge is formed.[30] This review of the literature would indicate that the pulpal reaction of MTA would be at least as compatible as calcium hydroxide.

However, some studies have suggested a superiority of MTA to calcium hydroxide materials. Pitt Ford *et al.*[31] evaluated the reaction of pulp tissue after capping with MTA and Dycal® and found the presence of a dentin bridge in all the pulps treated with MTA after six months, whereas in the group in which Dycal was used, all showed severe chronic inflammation and in only 2 the formation of a dentin bridge

was detected. Abedi *et al*.[27] found less inflammatory reaction and greater formation of calcified tissue after the use of MTA in comparison with Dycal in direct capping of tooth pulp in dogs and monkeys.

Is this more favorable response true for the primary dentition? Eidelman *et al.*[32] compared the effect of MTA to that of formocresol in primary tooth pulpotomies and found that with the 32 teeth followed up for 6-30 months after pulp treatment, not one case of the experimental group (MTA) showed clinical or radiographic lack of success, while in the control group (formocresol), one case of failure with internal re-absorption was detected after 17 months. The obliteration of the root canal was observed in 7 of the 17 teeth treated with MTA and in 2 of the 15 in which formocresol was used.

Earlier studies have shown the susceptibility of bacteria in infected pulp to antibiotics. Sato *et al*.[33] reported a study to clarify the antibacterial efficacy of mixed antibacterial drugs on bacteria of carious and endodontic lesions of human primary teeth *in vitro*. They demonstrated that a carious or endodontic lesion can be sterilized by an antibiotic mixture. Yoshiba *et al*.[34] also investigated the effects of antibacterial drugs on bacterially contaminated dental pulps but in monkeys. They reported that when antibiotics were added to the pulp capping agent (alpha-tricalcium phosphate) it effectively disinfected pulpal lesions, without destroying any of the sound pulp tissue. However, hard tissue barrier formation was delayed by this mixture as compared with $Ca(OH)^2$. The use of topical antibiotics in pulp therapy is therefore not without precedence. The additional advantage of using an antibiotic solution instead of a more caustic medicament, such as, formocresol, is the ability to do more than one pulpotomy at a time without risking the patient to possible mutagenic side effects.[35]

Holan, Eidelman and Fuks[36] have also suggested that MTA may be beneficial for use in primary molar pulpotomies. The ability of MTA to stimulate the formation of a dentin bridge is one such advantage over formocresol. Maroto *et al* [37] reported apparent radiographic evidence of dentin bridge formation when using MTA instead of formocresol. One can conclude from the literature that antibiotic solutions and MTA would have positive properties and beneficial effects on pulpal tissue. The use of otic solutions for pulpotomy treatment has been proposed by Dr. Celio Percinoto in Brazil[4] and by members of the American Academy of Pediatrics in the United States of America (personal communications).

MATERIALS AND METHOD

The experiment required four primates, young adult *alpha* male Capucin *Cebus Opella*, who were randomly selected from the primate population of the Araçatuba, Sao Paulo, Monkey Research facility. The research project was presented to and approved by the Animal Research Committee of Sao Paulista State University, UNESP, Araçatuba, Sao Paulo, Brazil. The Capucin *Cebus Opella* primate was utilized for this study because of international agreements protecting other primates and the study requirement that the samples would be reviewed at both institutions, UNESP and Northwestern University. This required international transportation of samples and compliance with CITES, the Convention on International Trade of Endangered Species of Wild Fauna and Flora. Throughout the research project the animals were cared for according to International Standards for Animal Care. All procedures were performed in the Primate Operating Rooms at the Monkey Research Facility.

The primates' premolars were chosen for the study due to their anatomical similarity to human molars. In addition, these primates have three premolars in each quadrant, allowing for a larger sample size of similar teeth. Many previous primate studies have utilized all the animal's teeth, introducing additional variables. On the other hand, these primates' primary molars were not considered as research candidates as they are very difficult to pulpotomize due to their very small size. The preparations were performed under general anesthesia administered by an experienced animal anesthetist utilizing Thiopental 30mg/kg IV along with Diazepam 0.17cc IM and with as sterile conditions as practical. The teeth were isolated with rubber dam, pumice prophylaxis performed and the operative area disinfected with providone-iodine. Small pediatric size high speed handpieces (Dabi Atlante) were utilized to create the preparations. The handpieces were autoclaved prior to each use. A new sterile small round bur was used to make the preparation with sterile saline as the coolant. Cavity preparations bordered by enamel but extending into the mesial/distal surfaces of the teeth were created and a pulpotomy of the coronal tissue was accomplished from the center of the cavity. A buccal approach was utilized due to UNESP previous studies' experiences with the occlusal access preparations. A greater number of operator errors were noted when using the occlusal approach in performing pulpotomy procedures on small primates and the loss of temporary restorations after treatment was noted to be more common. After the preparations were performed, the pulp chamber was rinsed with sterile saline to remove operative debris and hemostasis was obtained (Figure 1).

The pulp chambers were then filled with cotton pellets soaked in a bacterial mixture consisting of microorganisms normally found in human pulpal abscesses. The microorganism solution consisted of anaerobic and aerobic bacteria, obtained from the Endodontic Clinic of UNESP. The bacterial mixture contained *Porphyromonas gingivalis* and *Fusobacterium nucleatum* as both are known pathogens responsible for acute dental pulpitis and alveolar abscesses. Following bacterial inoculation (30 minute exposure to allow for absorption into the pulpal circulation and binding to the collagen of the dentin as per established protocol at the Monkey Research Facility) a sterile cotton pellet soaked with Cipro HC (Alcon Laboratories, Fort Worth, TX, USA) Otosporin Otic solution (Sao Paulo, Brazil) diluted formocresol (Buckley's 1:5 dilution) or sterile saline was applied to the inoculated pulp for five minutes.

Exposure of the pulpal cavity to the oral environment to

encourage contamination of the pulpal tissues as was previously performed would not be allowed by the International Standards of Animal Care (Appendix D: International Guiding Principles for Biomedical Research Involving Animals 1985). This is due to the pain the experimental animal would suffer if a pulp is left exposed. Subjecting an intelligent animal to pain and suffering is strictly forbidden and is un-ethical. Therefore, the half hour exposure to known human abscess bacteria was suggested by the Microbiology Department at Children's Memorial Medical Center and has been adopted by the Monkey Research Facility in Araçatuba. This is a reasonable amount of time to keep the animal under general anesthesia while allowing for the thorough contamination of the pulps by a standardized set of known pathogens. In addition, due to the organisms and exposure time being a set amount, it reduces the variability that would certainly occur if the pulps were simply exposed, as each animal may have different levels of various pathogens in their oral microflora. After removal of the contamination pellets, the pulpal chamber were inspected, debris removed and the pulp treating agents were applied with sterile cotton pellets.

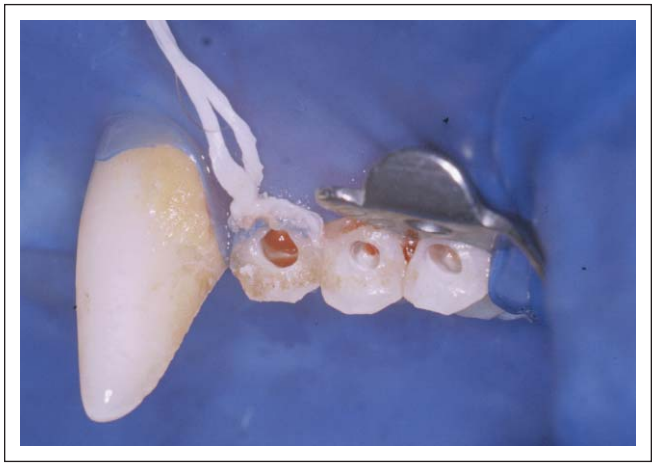

Figure 1. Isolation, disinfection and preparation of primate premolars demonstrated above. Pulpotomy of coronal pulpal tissue performed and hemostasis achieved.

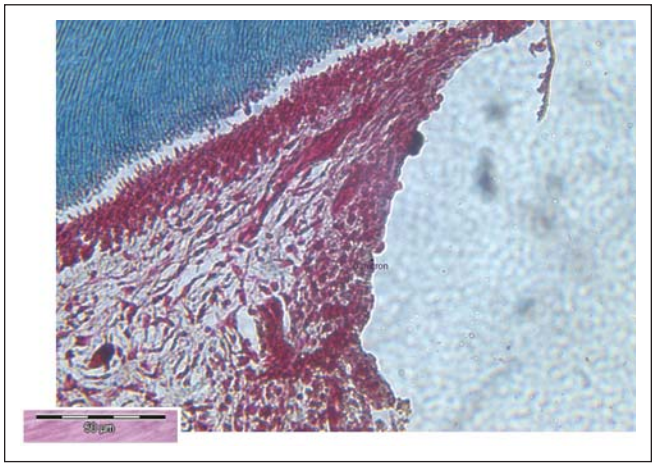

Figure 2: Microphotograph of 48-hour sample treated with MTA and sterile saline. Hyperemia and severe inflammation are evident.

The diluted Buckley's formocresol treated pulps were covered with a zinc oxide eugenol (ZOE) base. The Cipro HC, Otosporin and control pulps were covered with a base of MTA (ProRoot, Dentsply, Milford DE, USA) mixed and applied according to manufacturer's direction. The pulp capping bases were then covered with a RMGI (Fuji II LC GC America).

The monkeys were cared for according to International Standards for Animal Care. The primates were observed for any changes in eating habits or signs of inflammation or suppuration in the oral tissues. The primates were to be medicated with analgesics if determined necessary by the care givers. The primates' behavior was closely monitored and recorded. There were no behavioral changes noted in any of the animals.

Table 1. Four Treatment Groups- each consisting of 12 teeth, primate premolars

• Sterile saline and MTA (control)
• Buckley's formocresol and ZOE (clinical standard)
• Cipro HC Otic and MTA
• Otosporin Otic and MTA

Table 2. Distribution of Treatment Groups

• Monkey 1 (M24A)	
• UR-Cipro HC MTA • LR- Control MTA	• UL-Otosporin MTA • LL-Formocresol ZOE
Monkey 2 (M48B)	
• UR- Otosporin MTA • LR-Formocresol ZOE	• UL-Cipro HC MTA • LL –Control MTA
Monkey 3 (M7DC)	
• UR-Control MTA • LR-Otosporin MTA	• UL-Formocresol ZOE • LL-Cipro HC MTA
Monkey 4 (M34DD)	
• UR-Formocresol ZOE • LR-Cipro HC MTA	• UL-Control MTA • LL-Otosporin MTA

The tissue samples were collected during animal sacrifice. The monkeys were sacrificed with the following schedule; M24A after 24 hours, M48B after 48 hours, M7DC after 7 days and M34DD after 34 days. The animals were sedated and given an overdose of general anesthesia. Following vascular perfusion with fixation, block dissection of the maxilla and mandible was accomplished (Figure 2) and the retrieved blocks placed in 10% formalin for 48 hours. Decalcification of the tissue blocks was done by placing the samples into formic acid-sodium citrate solution. After dehydration, the samples were embedded in paraffin for sectioning. The tissues were cut into serial sections of 6 µm with a Leica BM 2025 microtome. The sections were then stained using the following methods: Hematoxylin and Eosin, and Masson-Trichrome.

The 48 samples (stained, sectioned and slide mounted as *per* governmental standards) were analyzed at Paulista State University (UNESP, Araçatuba, Brazil) and then transported to Northwestern University (Chicago, Illinois) for a second independent histological evaluation utilizing a Leitz Dialux 20 microscope. The evaluators (UNESP and Northwestern University) were unaware of the materials and technique utilized as all the samples were assigned identification by location only. The samples were evaluated at both 63X magnification and at 160X magnification. The histological analysis consisted of the following parameters: necrosis, hyperemia, quantity and quality of hard tissue bridging, presence of odontoblastic-like cells, other calcifications, presence of giant cells, particles of capping agent, and a ranking of the inflammation. The amount of bridge formation was analyzed and ranked along with the characteristics of the bridge, such as, completeness, regularity, tubule presence and amount of odontoblastic like cells. The thickness of the hard tissue bridge was also measured with the phase contrast microscope using three randomly chosen points on two separate sections of each sample (results to be submitted for future publication). The data was statistically analyzed with the assistance of a statistician unaware of the sample groups' constituents.

Table 3. Ranking for Inflammation and Bridge Formation

- Inflammation-
  - 0- few inflammatory cells present
  - 1- slight amount of inflammation
  - 2- moderate inflammation
  - 3- severe inflammation, micro-abscesses
  - 4- necrosis or abscess formation
- Hard tissue bridge-
  - 0- no presence of bridging
  - 1- slight formation, mostly soft tissue
  - 2- moderate amount of bridge, irregular
  - 3- hard tissue bridge, regular and complete
  - 4- hard tissue bridge with apparent odontoblasts, tubules

RESULTS

Sterile saline and MTA (Control)

After 24 hours, the exposed pulp tissues were protected by a coagulum. One of the specimens showed many neutrophils close to the coagulum. The adjacent pulp tissue showed hyperemic vessels, erythrocytes, fibroblasts and a discrete infiltration of chronic inflammatory cells. After 48 hours, it was possible to observe fibroblasts with nucleus and cytoplasm and a discrete infiltration of chronic inflammatory cells (Figure 2).

After 7 days, the exposed pulp tissues were still protected by coagulum. Under the coagulum there were fibroblasts, hemorrhage, hyperemia and a discrete infiltration of chronic inflammatory cells. The specimens showed an intense infiltration of neutrophils and micro abscess formation; more apically, a mild infiltration of chronic inflammatory cells was present. After 30 days, an irregular hard tissue bridge was observed with one sample composed of few dentinal tubules and a unicellular layer of odontoblast like cells. More apically, irregular areas of calcification of different thickness and few chronic inflammatory cells were seen. The other specimens did not show a hard tissue bridge; a coagulum full of nucleus fragments and neutrophils was present. That was followed by an intense infiltration of chronic inflammatory cells and few neutrophils.

Otosporin Otic solution

After 24 hours, the tissue adjacent to the exposure site was characterized a coagulum presenting few chronic inflammatory cells and many nucleus fragments. This was followed by hyperemic vessels and hemorrhage, with erythrocytes displaced into the pulp tissue, few fibroblasts and a discrete infiltration of chronic inflammatory cells. After 48 hours, the morphologic aspect of the specimens was similar to the aspect observed after 24 hours.

After 7 days, the exposed pulp surface was still protected by a coagulum. Hyperemic vessels, hemorrhage and a few fibroblasts were observed. It was also seen an infiltration of chronic inflammatory cells (mainly macrophages). One of the specimens showed the above aspect, however, no fibroblasts could be observed. In another specimen, small and irregular areas of calcification located in the coronal pulp tissue were present. After 34 days, the pulp tissue showed a hard tissue bridge composed of few dentinal tubules and a layer of odontoblasts with a reduced number of cells. The adjacent tissue showed few if any chronic inflammatory cells. One of the specimens did not show a hard tissue bridge; in this case, an irregular layer of eosinophilic tissue was present. Close to this area, an infiltration of chronic inflammatory cells was observed, mainly macrophages and plasmacytes. Also, dentin chips and hard tissue formation were observed.

Cipro HC Otic Solution

After 24 hours, the exposed pulp tissue was protected by a coagulum, presenting few neutrophils and many nucleus fragments. The adjacent tissue presented few neutrophils, chronic inflammatory cells, hyperemic vessels, erythrocytes and fibroblasts. After 48 hours, a reduction of the number of chronic inflammatory cells and more fibroblasts with nucleus and cytoplasm were observed. No neutrophils were observed.

After 7 days, a layer of an eosinophilic tissue was observed on the pulp tissue. The adjacent tissue showed few chronic inflammatory cells and odontoblast-like cells. After 34 days, a complete hard tissue bridge presenting few dentinal tubules and a layer of odontoblasts (almost unicellular) was often observed (Figure 3). This hard tissue bridge was sometimes irregular and of variable thickness. One of the specimens showed dentin chips on the pulp surface. A small

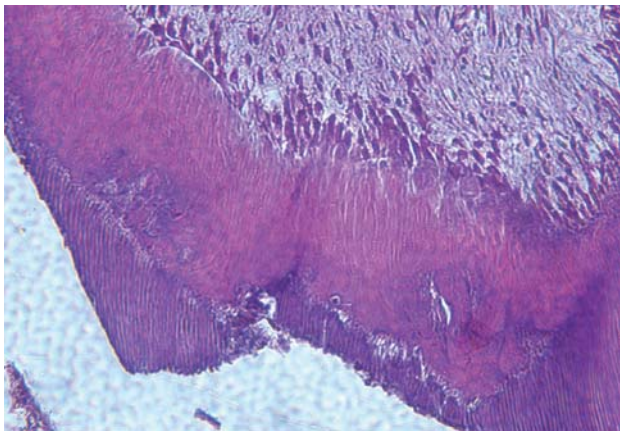

Figure 3. Histological section of specimen treated with Cipro HC and MTA demonstrating the formation of dentin like bridges at 34 days. Also note the odontoblast like cells lining the bridge and the lack of inflammation (160X magnification).

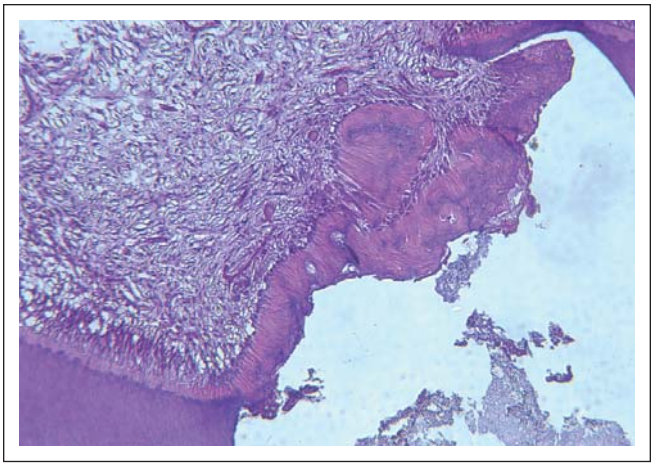

Figure 4. Microphotograph of specimen treated with formocresol and ZOE. This section demonstrated irregular hard tissue bridge formation at 34 days (63X magnification).

layer of odontoblasts and inclusions of particles of dentin by new-formed dentin were present. In one of the specimens, in the inner part of the pulp tissue, dentin fragments and hard tissue deposition were observed. Very few chronic inflammatory cells were observed.

Buckley's Formocresol

After 24 hours, a coagulum was observed on the exposed pulp surface. The adjacent tissue showed hyperemic vessels and hemorrhage with erythrocytes, few fibroblasts, a discrete infiltration of chronic inflammatory cells and few neutrophils. After 48 hours, the surface area showed an intense infiltration of neutrophils followed by a discrete infiltration of chronic inflammatory cells, hyperemic vessels and hemorrhage with erythrocytes displaced into the tissue.

After 7 days, a coagulum with nucleus fragments was observed close to the exposed surface. More apically there were hyperemic vessels and an intense infiltration of chronic inflammatory cells. One of the specimens showed a coagulum followed by a small area of normal pulp tissue; the adjacent tissue showed an eosinophilic tissue with many neutrophils and nucleus fragments; that was followed by a tissue with a reticular aspect. After 34 days, one of the specimens showed an irregular hard tissue bridge presenting cells and a unicellular layer of odontoblasts (Figure 4). The adjacent pulp tissue showed many fibroblasts and very few chronic inflammatory cells. Another specimen showed fibrous tissue with few fibroblasts and neutrophils, inclusions of dentin chips by new hard tissue and an infiltration of chronic inflammatory cells around it. The other specimen showed coagulum followed by a pulp tissue presenting numerous chronic inflammatory cells, mainly macrophages. More apically, there was a fibrous capsule parallel to the pulp surface showing numerous fibroblasts and chronic inflammatory cells followed by a pulp tissue with a discrete infiltration of chronic inflammatory cells.

The inflammation and bridge rankings of M34DD obtained at 34 days by the two evaluators were re-conciled and then subjected to statistical analysis. The pulpal tissue treated with Otic suspensions demonstrated significantly less inflammation (Kruskal Wallis, non-parametric analysis, KW=9.595 with 1 degree of freedom; P=0.022) than the formocresol and control groups. The hard tissue bridges formed over the exposure sites were more organized in the MTA treatment groups than in the control and ZOE groups (Kruskal Wallis analysis, KW=18.291 with 1 degree of freedom; P=0.0004). The Dunn's multiple Comparisons test demonstrated significant differences between the control group and the MTA groups.

Table 4. Results of Inflammation and Bridge Formation Rankings of MM34DD. The higher the inflammation score correlates with a more intense pulpal response. The higher the hard tissue bridge score the more organized and complete the bridge formation appeared.

Average Rank (SD)	Formocresol ZOE	Control (saline) MTA	Otosporin Otic MTA	Cipro HC Otic MTA
Inflammation Rank	1.7 (1.5)	1.25 (0.6)	0.17 (0.17)	0.25(0.18)
Bridge Rank	1.3 (0.7)	0.7 (0.3)	3.52(0.7)	3.1 (0.4)

DISCUSSION

Pulpotomy therapy is one of the most important treatment techniques necessary to maintain and preserve the dentition. For example, pulpotomy therapy may be used in young permanent teeth either in case of traumatic exposure of the pulpal tissue or when an immature tooth with an un-developed root structure has a vital pulp but with a carious exposure. Treatment that preserves the dental pulp vitality is quite desirable and MTA does fulfill that requirement.[38] However, MTA is difficult to place and requires a setting time that is currently much too long.

The use of bacterial contamination is integral in properly

gauging the pulpal response to capping agents. The 30-minute inoculation allowed for the absorption of the bacteria into the pulpal circulation and for dentin invasion with binding to collagen.[6,39, and 40] With good isolation and the same 30 minute bacterial suspension used to contaminate all the exposed pulps and with MTA as the pulp capping agent (considered by many to be "State of the Art"), the treatment agents were the only variable except with the use of zinc oxide eugenol in the diluted formocresol group. The zinc oxide eugenol was used with formocresol because that is considered the "Gold Standard" for many practitioners when considering clinical effectiveness. A future study may compare the effectiveness of MTA versus ZOE when utilizing diluted formocresol in treating contaminated pulpal tissue. Unfortunately, formocresol may not be available in the near future (personal communication, Food and Drug Administration, Director, Dental and Dermatology Section) due to its mutagenic and possibly carcinogenic properties.[41] In addition, a very caustic agent such as formocresol may prevent the more "biologic" MTA healing characteristics from occurring.

The reduction in inflammation found in the Otic Suspension Group is not surprising.[42] The topical steroid effect would be expected, and could lead to less post-operative pain if utilized in humans undergoing endodontic treatment. The inflammation could also be decreased by the antibiotic component of the Otic suspensions.[43, 44] Bacterial contamination increases the pulpal response and the Control Samples, without anti-microbial agents, did present with an increase in inflammation compared to the specimens treat with the Otic suspensions.

The overall amount of "dentin" like bridging initially appeared to be thin in the sections as compared to other studies in the literature. However, the time period for this study was shorter,[45] as one of the principle authors has shown in previous primate studies that a material either succeeds of fails within the first 34 days.[46] A longer study period would most likely present with the same findings as this study, only with relatively thicker dentin-like bridges. Future studies may attempt to retrieve more specimens and, perhaps, after 120 days to further advance the data derived from this initial study. Additional research should be performed on the primary teeth of primates before Otic suspensions are widely accepted in Pediatric Dentistry for the routine treatment of cariously exposed primary molars.

The use of Otic suspensions for pulpal therapy would be considered an off-label use by the Food and Drug Administration. Otic suspensions are often used by podiatrists to treat nail infections and the use of these Otic suspensions by Pediatric Dentistry for pulpal therapy is considered to be a similar situation to the Podiatric. Fluoride varnish is another similar situation, approved as a de-sensitizing agent (in the USA) but utilized as a fluoride therapeutic agent. The main issue with the use of Otosporin is the reportedly high incidence of allergic responses to its application. Cipro HC Otic suspension reportedly has had no reported allergic responses to topical application.

As with all treatment modalities, the pulpotomy technique will progress through an un-ending state of evolution, with slow and unstoppable improvement. Primate studies with more biologic medicaments and pulp capping materials may provide revolutionary advancements provided that the results are replicated in large, well designed and controlled, clinical studies.

CONCLUSION

This initial research project did demonstrate enhanced healing of pulpal tissue mechanically exposed, contaminated with human abscess bacteria, and treated with Otic suspensions as compared to either the control (MTA and sterile saline) or DFC treated primate premolars.

Further research is required before anti-microbial agents (Otic suspensions) are utilized instead of diluted formocresol (DFC) for the treatment of pulpally involved primary teeth.

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