Original Article

Comparison of the clinical, radiographic, and histological outcomes of pulpotomy of deciduous teeth in dogs with formocresol, Gutta-percha, and mineral trioxide aggregate

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ABSTRACT

The aim of this study was to compare the outcomes of pulpotomy of deciduous teeth in dogs with the use of formocresol, Gutta-percha, and mineral trioxide aggregate (MTA). In this experimental study, 24 deciduous premolar teeth, without internal and external resorption, in two hybrid Iranian dogs, aged 6-8 weeks, were divided into 3 groups, using a simple random technique. In the three study groups, diluted Buckley formocresol, Gutta-percha, and MTA were placed on canal orifices, respectively, and the crowns were restored with zinc oxide eugenol paste and glass ionomer. The teeth underwent a radiographic procedure and extracted after 1-month. The histological samples were evaluated in relation to inflammatory reactions and formation of a hard tissue barrier. Data were analyzed with SPSS version 20 (SPSS Inc., Chicago IL, USA), using ANOVA, ANCOVA, Kruskal–Wallis test, and Spearman's correlation coefficient ($\alpha = 0.05$). Kruskal–Wallis test did not reveal any significant differences in clinical signs between the study groups at 2-week interval ($P = 0.416$) and clinical and radiographic signs 1-month after application of the materials ($P = 0.503$ and $P = 0.122$, respectively). ANCOVA did not reveal any significant differences in grading between the groups over time ($P = 0.927$). There were no differences between materials in the clinical, radiographic, and histological outcomes.

Key words: Formocresol, Gutta-percha, Mineral Trioxide Aggregate, Pulpotomy

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INTRODUCTION

The health of the tooth depends on the preservation of the integrity of its hard structures and the function of pulpal and periodontal tissues to nourish the tooth and protect its vitality.[1] Dental caries, trauma, and dental procedures might result in the exposure of the vital pulp, inflammation, and necrosis of the pulp and disturbances in the tooth root maturation, finally leading to tooth loss.[2,3] Indirect pulp capping, pulpotomy, and pulpectomy are the standard procedures for the treatment of deciduous teeth with deep carious lesions. Pulpotomy is one of the most common treatment procedures carried out in pediatric dentistry.[2,4,5]

The formation of a dentin bridge by a number of materials, including calcium hydroxide and mineral trioxide aggregate (MTA), has been shown in previous studies.[6,7] Based on the results of studies on animals and clinical findings in man, efforts are now underway in pediatric dentistry to find an appropriate alternative for the gold standard material, formocresol. Despite the super

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clinical success of formocresol from 1930 until date, there has been an increase in controversies surrounding it. Despite the fact that it is a standard material, there has been an increase in concerns about its toxicity and carcinogenic potential.[2] This material is absorbed and distributed systemically and can induce a humoral immune response. In addition, it can induce hypoplastic effects on permanent teeth.[5] On the other hand, formocresol can induce pulp necrosis and inflict injuries on the adjacent tissues, including gingiva.[8] Furthermore, a relationship has been shown between formocresol and the dentigerous cyst.[9] Caicedo *et al*.[10] reported in 2006 that the pulp of deciduous teeth mounts a proper response to pulpotomy with MTA, so it is a suitable material for pulpotomy procedures, but it is expensive. Kakehashi *et al*.[11] showed the capacity of the exposed pulp cells to deposit dentin in a germ-free environment, without any need for a pulp capping material.

Therefore, it might be hypothesized that application of a neutral material, such as Gutta-percha, might prove successful on the condition that there is proper seal in the deciduous teeth, and the stem cells in the pulp can repair the pulp of the deciduous teeth. The majority of pulpotomy studies have been carried out on human deciduous teeth that should have carious lesions and pulp inflammation to be included in the study due to ethical considerations. However, in this study, the deciduous teeth of dogs, without any carious lesions, were evaluated in order to evaluate the effect of therapeutic agents on healthy pulp without any inflammation. Therefore, the aim of the present study was to compare the clinical, radiographic, and histopathological outcomes of pulpotomy procedures of deciduous teeth in dogs with the application of formocresol, Gutta-percha, and MTA.

MATERIALS AND METHODS

In the present experimental study, 24 deciduous premolars, at least with two-thirds of the root length present, in two hybrid female Iranian dogs, 6-8 weeks of age and 3-5 kg in weight, were divided into 3 groups using a simple random technique. The teeth had no internal or external resorption. All the animal care procedures and tests in the present study conformed to the guidelines issued by the Isfahan University of Medical Sciences in relation to animal studies.

The samples were coded after a period of quarantine. The animals underwent a general anesthesia procedure using an intramuscular injection of 10 ml of ketamine hydrochloride and 8 ml of sodium pentobarbital. Agfa films (Agfa-Gevaert N.V., Mortsel, Belgium) were used to provide radiographs from the teeth, followed by cleaning the teeth and the adjacent structures with 0.2% of chlorhexidine. All the calculi were removed with an ultrasonic tip. Access cavity was prepared in each tooth and the pulpal tissue in the pulp chamber was completely removed with the use of a sharp excavator and a round bur in a slow-speed handpiece. After locating the root canal orifices, cotton pellets impregnated with physiologic serum were used to achieve hemostasis, followed by directly placing the materials on the canal orifices based on the study group as follows:

In Group 1, 1/5 dilution of Buckley formocresol (Sultan, New York, USA) was placed on all the root canal orifices for 4 min. After fixation was achieved the tooth was restored with a layer of KemDent Zonalin paste (KemDent Works, Swindon, England) a light-cured glass ionomer (GC Fuji IX, Fuji Oyama Factory, Fuji, Japan).

In Group 2 (the control group), after hemostasis was achieved with cotton pellets impregnated with normal saline solution, Gutta-percha (Dentsply, Philadelphia, USA) was prepared in the form of paste and placed on canal orifices as a neutral material. Then the teeth were restored with light-cured glass ionomer. Given the evidence in relation to the effect of Zonalin on the histological reactions subsequent to pulpotomy this material was not used in this group.

In Group 3, after achieving hemostasis with cotton pellets impregnated with normal saline, MTA (ProRoot, Dentsply, Philadelphia, USA) was used as a pulp capping material and the teeth were restored with light-cured glass ionomer. Based on the results of previous studies,[7,12] it was hypothesized that the pulp moisture was sufficient for the final setting of MTA because it was not possible to achieve general anesthesia again and carry out a two-visit procedure to made sure of setting of the material due to ethical considerations.

Postoperative care

After general anesthesia, all the animals received 5 mg/Kg of medicine intramuscularly and 50% of analgesin for 3 days. In addition, a plaque was controlled postoperatively with 0.2% of chlorhexidine twice a week by a blinded examiner in relation to the study procedures for 1-month. A checklist was used to record the criteria for clinical success and failure. The clinical success criteria consisted of the following: Absence of swelling, pulp polyps, fistulae, and the pathologic tooth mobility. The presence of the criteria above was considered the clinical failure of the procedure.

Postoperative examinations

After 1-month,[13,14] the teeth once again underwent a radiographic procedure under general anesthesia and

then extracted and fixed in 4% of paraformaldehyde. The teeth were decalcified in 10% of buffered formic acid and immersed in paraffin. For histopathological evaluations 5-μm sections were prepared along the tooth roots and the samples taken from the center of each root canal were separately stained with H and E and evaluated under a light microscope under constant light intensity by a pathologist in relation to inflammatory reactions and formation of a dentin bridge. Table 1 presents the criteria used for histological evaluations. In order to quantify the histological criteria and make it possible to compare the samples, each condition or state was given a score, and the sum of the scores was defined as the histological response. Table 1 presents the scoring system of histological responses.[7]

Analysis of data

Data were analyzed with SPSS version 20 (SPSS Inc., Chicago IL, USA), using ANOVA, ANCOVA, Kruskal-Wallis test, and Spearmen's correlation coefficient (α = 0.05).

Table 1: The scoring system of histological responses

State	Score
Evaluation of inflammation and inflammatory cell response	
Absence of inflammatory cells	0
A few inflammatory cells, consisting of PMNs and leukocytes	1
A moderate number of inflammatory cells	2
Evaluation of tissue structure (tissue disorganization)	
Normal tissue beneath the pulpotomy area	0
Absence of normal tissue beneath the pulpotomy area but presence of normal tissue in deeper layers of the pulp (50% disorganization)	1
Loss of the general structure of the pulp (total disorganization)	2
Pulp necrosis	3
Hard tissue formation	
Absence of hard tissue formation	0
Incomplete and partial hard tissue formed	1
Formation of a thick hard tissue layer	2

PMNs: Polymorphonuclear

RESULTS

In the present study, 24 premolars underwent pulpotomy procedures in two dogs. After 2 weeks, only one tooth in the MTA group exhibited mobility, and the remaining teeth had no problems. Therefore, the clinical success of formocresol and Gutta-percha was 100%, and that of MTA was 87.5% after 2 weeks. Kruskal-Wallis test did not show significant differences in clinical signs between the study groups at 2-week postoperative interval (P = 0.416).

At 1-month postoperative interval, 89.6% of the teeth were asymptomatic clinically, with 50% exhibiting physiologic mobility. Table 2 presents the clinical signs of teeth at 1-month postoperative interval separately in each group.

Based on the results, the clinical success of all the three materials tested was 87.5% after a month. Kruskal-Wallis test did not reveal any statistically significant differences in clinical signs between the study groups at 1-month postoperative interval (P = 0.503).

Table 3 presents the frequency percentages of radiographic signs at 1-month postoperative interval.

As table shows the radiographic success rates of formocresol, Gutta-percha, and MTA at 1-month postoperative interval were 100%, 75%, and 62.5%, respectively, with no statistically significant differences between them based on the results of Kruskal-Wallis test (P = 0.122).

The histological samples were evaluated for inflammation, soft tissue disorganization, and formation of a dentin hard tissue barrier. Tables 4-6 present the frequency

Table 2: The frequency percentages of clinical signs of the teeth at 1-month postoperative interval separately in each group

Group	Abscess	Pulp polyp	Exfoliation	Physiologic mobility	Without clinical signs	Total
Formocresol	0	0	12.5	37.5	50	100
Gutta-percha	0	0	12.5	12.5	75	100
MTA	0	0	12.5	62.5	25	100

MTA: Mineral trioxide aggregate

Table 3: The frequency percentages of radiographic signs at 1-month postoperative interval

Group	Furcal radiolucency	Periapical radiolucency	Pathologic root resorption	Internal resorption	Physiologic root resorption (>2/3 of the root length)	Absence of radiographic signs	Total
Formocresol	0	0	0	0	42.9	57.1	100
Gutta-percha	6.25	12.5	6.25	0	12.5	62.5	100
MTA	12.5	6.25	0	18.75	0	62.5	100

MTA: Mineral trioxide aggregate

percentages of these three variables separately in each group.

Kruskal–Wallis test did not show any significant differences in inflammation, soft tissue disorganization, and hard tissue formation (dentin bridge) between the three study groups (P = 0.93, P = 0.426, and P = 0.077, respectively).

Subsequently, the dimensions of the dentin bridges formed were evaluated. Table 7 presents the dimensions of the dentin bridges formed and grading in each group separately.

ANCOVA did not reveal any significant differences in the dimensions of the dentin bridges between the study groups at different time intervals (P = 0.114).

ANCOVA did not demonstrate a significant differences in the grading of the study groups at different time intervals (P = 0.927).

Spearman's correlation coefficient showed a significant correlation between inflammation and soft tissue disorganization (r = 0.731, P < 0.001), and between dentin bridge formation and inflammation (r = 0.340, P = 0.010). Therefore, an increase in inflammation was associated with an increase in soft tissue disorganization and a decrease in dentin bridge formation.

Figures 1-3 present histological findings of Gutta-percha, formocresol, and MTA.

DISCUSSION

Pulpotomy is still the most common dental procedure in children. The aim of this procedure is to preserve the vitality of the root pulpal tissue, avoid pain, and swelling and finally preserve the tooth through the extirpation of the coronal pulp and prevention of root canal pulp inflammation.[2,5]

Table 4: The frequency percentages of inflammation in the histological samples separately in each group

Group	None	Mild	Moderate	Severe	Total
Formocresol	25	25	50	0	100
Gutta-percha	50	14.3	14.3	21.4	100
MTA	57.1	0	0	42.9	100

MTA: Mineral trioxide aggregate

It is very important during a pulpotomy procedure to eliminate the irritants, infection control, and use biocompatible materials.[8] However, the final aim of pulp treatment with a capping material is to induce dentinogenesis by pulp cells[9] to form a dentin bridge beneath the pulpotomy procedure area because it is a prerequisite for the long-term preservation of the pulp vitality.[4] The nature of the hard tissue formed is still unknown, and this hard tissue has been described as dentin-like,[9] bone-like,[15] and reparative dentin bridge.[16]

Ideal pulpotomy material is not been identified. Formocresol is a popular material for the pulpotomy procedures of deciduous teeth.[17,18] Despite its popularity, there are ever-increasing concerns about its toxicity and potential carcinogenicity.[19]

MTA has yielded good results in the pulpotomy of deciduous teeth.[20] In addition, due to its high antimicrobial properties and biocompatibility, MTA can induce the formation of hard tissues.[5] Various studies have shown that MTA induces thicker dentin bridges and less pulpal inflammation compared to calcium hydroxide.[21-24]

A study showed that the pulp has the capacity to form dentin and repair itself in a germ-fine environment even in the absence of pulp capping materials.[10] Therefore, the reparative potential of the pulp depends on the presence

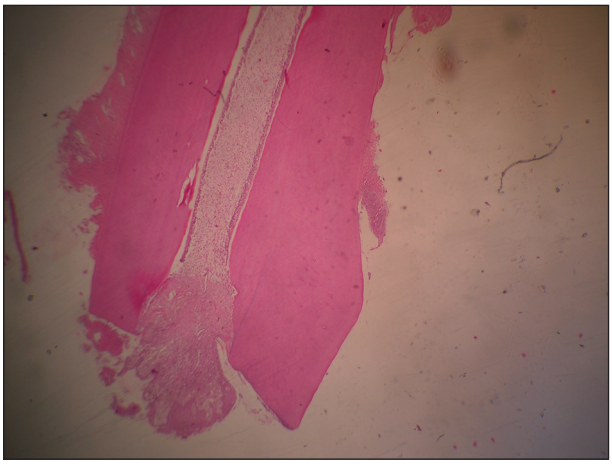

Figure 1: Normal pulp tissue with absence of inflammatory cells and absence of hard tissue formation beneath the pulpotomy area in Gutta-percha (control) group (×100)

Table 5: The frequency percentage of soft tissue disorganization in the histological samples separately in each group

Group	Normal	50% disorganization	Total disorganization	Necrosis	Total
Formocresol	50	25	0	25	100
Gutta-percha	50	7.1	14.3	28.6	100
MTA	42.9	14.3	14.3	28.6	100

MTA: Mineral trioxide aggregate

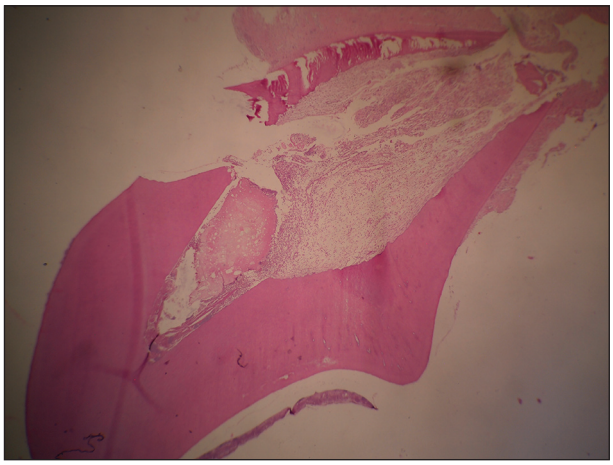

Figure 2: Absence of normal tissue beneath the pulpotomy area but presence of normal tissue in deeper layers of the pulp with absence of inflammatory cells in formocresol group (×100)

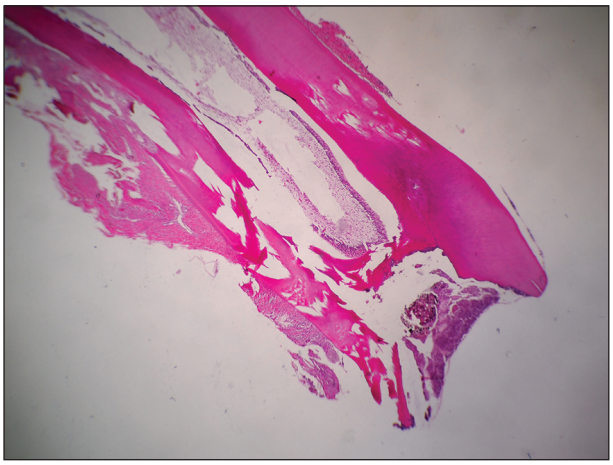

Figure 3: Normal pulp tissue with absence of inflammatory cells and formation of a thick hard tissue layer beneath the pulpotomy area in mineral trioxide aggregate group (×100)

Table 6: The frequency percentage of hard tissue formation in the histological samples separately in each group

Group	Absence of dentin bridge	Incomplete dentin bridge	Thick dentin bridge	Total
Formocresol	100	0	0	100
Gutta-percha	92.9	0	7.1	100
MTA	57.1	0	42.9	100

MTA: Mineral trioxide aggregate

Table 7: The dimensions of the dentin bridges formed and grading in each treatment group

Group	Dentin bridge dimensions (μm^2)		Grading	
	Mean	SD	Mean	SD
Formocresol	0.00	0.00	4.25	1.11
Gutta-percha	53.57	14.16	4.14	0.66
MTA	146.43	15.41	3.71	1.21

MTA: Mineral trioxide aggregate, SD: Standard deviation

of a suitable environment free of microorganisms in associative with proper coronal seal. Therefore, in the present study in one group Gutta-percha was used to cap the pulp after a pulpotomy procedure as a neutral material to evaluate the reparative potential of the pulp in dogs without the use of therapeutic materials, so that the role of pulp and its stem cells could be evaluated without the effect of therapeutic compounds.

In the present study, two female dogs were used in order to eliminate the effect of gender, tooth type, and the dental arch on the results as confounding factors and the teeth were divided into three groups using a simple randomization method.

Given the similarity between the sequences of eruption of deciduous and permanent teeth in dogs and man and since the aim of the present study was to evaluate the effect of therapeutic materials on the inflammation-free vital pulp, the study was carried out on deciduous teeth in dogs.

Akcay *et al.*[25] carried out a histological study to evaluate the response of pulp in human deciduous teeth to calcium hydroxide and MTA with and without irrigation with 5% of sodium hypochlorite solution. The results showed that physiologic serum and sodium hypochlorite solution were not different from each other as irrigants. Therefore, physiologic serum was used for irrigation in the present study.

The clinical and radiographic success of formocresol in the present study was comparable to that in studies by Ibricevic and Al-Jame,[26] Shabzendedar *et al.*,[27] Durmus and Tanboga,[28] and Ruby *et al.*[29] However, the results of the present study in relation to the absence of any significant differences between formocresol and MTA were not consistent with those of studies by Jayam *et al.*,[30] Godhi *et al.*,[31] and Shirvani and Asgary,[5] which might be attributed to differences in follow-up periods between the present study and studies by Jayam *et al.*[30] and Godhi *et al.*,[31] and differences in sample sizes and study designs. In the present study, pulpotomy was carried out on sound caries-free deciduous teeth in dogs and the teeth were followed for 1-month postoperatively; however, in studies by Jayam *et al.*[30] and Godhi *et al.*[31] the teeth were followed for 3, 6, and 12 months postoperatively. In addition, in all the studies above pulpotomy procedures were carried out on carious deciduous teeth in man. Therefore, carious lesions and pulp inflammation might have resulted in responses to the therapeutic materials. It should be pointed that one of the limitations of the present study was the fact that deciduous teeth in dogs are exfoliated at 2 months of age, making it impossible to carry out long-term evaluations. On the other hand, the

results of the present study were consistent with those of studies by Sushynski *et al*.[32] and Marghalani *et al*.[33] in relation to the absence of any differences between formocresol and MTA. Sushynski *et al*.[32] evaluated the outcomes of pulpotomy procedures with formocresol and gray MTA for 2 years and reported that gender, dental arch, and tooth type had no effect on the outcomes of the treatment. In addition, they did not report any significant differences between the clinical outcomes of formocresol and MTA.

Marghalani *et al*.[33] carried out a meta-analysis to evaluate the studies which had compared the effects of MTA and formocresol used for the pulpotomy of deciduous teeth. The results on 377 deciduous teeth in 20 studies showed no significant differences in the percentages of the clinical and radiographic success of formocresol and MTA. The results of this study did not show any significant differences in inflammation, hard tissue formation and grading between formocresol, Gutta-percha, and MTA. In the samples treated with formocresol, no dentin bridge had formed; however, the differences between Gutta-percha and MTA groups and formocresol group were not significant despite the formation of dentin bridges with the use of Gutta-percha and MTA. However, the differences might have become significant with an increase in sample size and longer follow-up periods. In addition, the results of the present study showed that an increase in pulp inflammation resulted in an increase in soft tissue disorganization and a decrease in dentin bridge formation. Therefore, it can be concluded that an increase in inflammation severity is naturally associated with a decrease in dentin bridge formation. The results of this study did not show any significant differences in the clinical, radiographic, and histological outcomes of pulpotomy with the use of Gutta-percha, formocresol, and MTA in the caries-free deciduous teeth of dogs at short-term. Further studies with larger sample sizes are recommended in order to evaluate the effect of other therapeutic materials. In addition, clinical studies are suggested in man. One of the limitations of the present study was carrying out pulpotomy procedure on caries-free teeth and evaluating them after a month. Because of physiologic root resorption and mobility of primary teeth, long-term follow-up was impossible. It is recommended that in future studies the response of infected tooth pulp to therapeutic materials be evaluate at longer follow-up periods.

CONCLUSION

- One month after pulpotomy of deciduous teeth in dogs with formocresol, Gutta-percha, and MTA, no significant differences were found in clinical, radiographic, and histological outcomes.
- An increase in inflammation severity was correlated with an increase in tissue disorganization and a decrease in dentin bridge formation.
- The results of this study showed that Gutta-percha and MTA can be an appropriate alternative for the gold standard material, formocresol.

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Conflicts of interest

There are no conflicts of interest.

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