Histological evaluation of Ankaferd blood stopper, ferric sulfate and formocresol as pulpotomy agents in rat molars

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ABSTRACT

The aim of this study is to evaluate the use of Ankaferd blood stopper (ABS) as a pulpotomy agent in rat molars and to compare it to ferric sulfate (FS) and formocresol (FC). Pulpotomies of 72 rat molar teeth were performed with ABS, FS and FC. Access cavities were sealed with Intermediate Restorative Material. Histological evaluations were conducted at 7, 15 and 30 days post-operatively. Statistical analysis was performed using the Kruskal-Wallis and Dunn's multiple comparison tests. There was no significant difference in inflammatory cell response between groups at 7 and 15 days ($P > 0.05$). However, at 30 days, a higher density of inflammatory cells was observed in the FC group when compared with the other groups ($P < 0.05$). No significant differences in hard-tissue formation were observed between groups at any time tested. Based on the histological findings of this study, ABS may be considered an acceptable alternative to FC and FS for primary teeth pulpotomies. Further, clinical research is needed to confirm this finding.

Key words: Ankaferd Blood Stopper, Ferric Sulfate, Formocresol, Pulpotomy

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INTRODUCTION

Pulpotomy is a common procedure consisting of surgical amputation of inflamed coronal pulp that is often used to treat acutely inflamed primary teeth. The wound surface of the radicular pulp is then treated with a medicament or dressing agent to promote healing or fixation of the underlying tissue.[1] For more than a century, formocresol (FC) was the most commonly used medicament for primary tooth pulpotomy; however, the use of FC has been challenged for its deleterious effects, potential carcinogenicity, immune sensitization, mutagenicity and cytotoxicity.[2-5]

Concerns over FC safety have led to various suggestions for alternatives in pulp therapy, the most recent of which is ferric sulfate (FS).[6] FS is commonly used as a coagulative and hemostatic agent for crown and bridge impressions.[7] Although the exact mechanism of action of FS is still a matter of debate, it involves the agglutination of blood proteins as a reaction of blood to contact with ferric and sulfate ions, with the agglutinated proteins forming a plug to occlude the capillary orifices.[8] The proposed use of FS as a pulpotomy agent is based on the theory that this hemorrhage-control mechanism will minimize the likelihood of inflammation and internal resorption associated with physiologic clot formation. Moreover, the metal-protein clot on the pulp stump surface is expected to form a passive barrier that will prevent the penetration of irritating components in the sub-base.[9] Clinical studies have reported more favorable results with FS pulpotomies when compared with FC pulpotomies;[10-12] however, reports of radiographic and histological results of FS pulpotomies are scarce. A study by Casas *et al*.[13] reporting on radiological success of pulpotomies found periapical radiolucencies and internal resorption in 55% of molars treated with FS within 2 years of treatment. One histological study of FS pulpotomies in rat molar teeth showed moderate inflammation and widespread pulp necrosis[14] and

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Vargas and Packham[15] reported that FS caused chronic pulpal degradation that led to a higher incidence of premature exfoliation and abscess formation.

Alternative medicaments for use in primary molar pulpotomies, including other hemostatic agents, continue to be explored. Ankaferd blood stopper ([ABS]; Ankaferd Sağlık Ürünleri AC, Istanbul, Turkey) is a newly introduced hemostatic agent that combines herbal extracts obtained from five different plants — *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum* and *Urtica dioica* — that have historically been used as hemostatic agents in Turkish traditional medicine. ABS has been approved by the Turkish Ministry of Health for use in the management of dental surgery and external hemorrhage.[16] The basic mechanism of action of ABS is through the formation of an encapsulated protein network that provides focal points for the aggregation of vital erythrocytes. ABS induces the formation of this protein network without affecting the physiology of individual coagulation systems.[16,17] ABS has been safely used to control bleeding in various types of medical[18-20] and dental[21-25] treatment. The aim of this study was to evaluate the use of ABS as a pulpotomy agent in rat molars and compare it with FS and FC.

MATERIALS AND METHODS

A total of 12 Wistar rats weighing between 250 g and 300 g were used in this study. Animals were housed 2 per cage, kept in a room with a constant temperature of 22°C ± 1°C and a 12/12-h light-dark cycle and fed rat chow and water *ad libitum*. All experiments were performed in the surgical research laboratory of the Ondokuz Mayıs University Faculty of Medicine. All procedures were conducted according to guidelines approved by the Animal Ethical Committee of Ondokuz Mayıs University.

Experimental procedure

Caries-free maxillary first and second molars and mandibular first molars (72 teeth) in 12 animals were pulpotomized and assigned to one of three groups according to the material used in the pulpotomy procedure (ABS, FS [Astringedent, Ultradent Products, Inc., Salt Lake City, UT, USA], FC [Sultan Chemists Inc, Englewood, NJ, USA]). In all animals, right maxillary first and second molars were treated with ABS, left maxillary first and second molars were treated with FC and left and right mandibular first molars were treated with FC.

Prior to treatment, animals were anesthetized with 60 mg/100 kg intra-peritoneal ketamine- *hydrochloride* (Ketalar, Eczacıbaşı İlaç Sanayi ve Ticaret AŞ., Istanbul, Turkey). Antisepsis of the oral cavity was achieved by rinsing with 0.2% chlorhexidine digluconate solution (Orasept, Biofarma Pharmaceutical Sec. and Trade. Inc., Istanbul, Turkey) for 60 s. Animals were positioned in dorsal decubitus and immobilized on a surgical table and a holding device was used to maintain their mouths in an open position.

Class I cavities were prepared in first and second maxillary and first mandibular molars with a 012 round diamond bur under copious sterile saline irrigation (Hager and Meisinger GmbH, Heisinger, Germany). Every effort was made to ensure consistency of the size of pulpal exposure. Pulpotomies were performed, after which pulp chambers were irrigated with sterile saline and gently dried with sterile absorbent paper points. The following procedures were then performed:

- ABS group: Radicular pulp stumps were covered for 15 s with a cotton pellet moistened with ABS solution, after which the ABS was flushed away from the pulp chamber with sterile saline.
- FS group: Radicular pulp stumps were covered for 15 s with a cotton pellet moistened with FS solution, after which the FS was flushed away from the pulp chamber with sterile saline.
- FC group: Radicular pulp stumps were covered for 5 min with a cotton pellet moistened with a 1:5 dilution of Buckley's FC.

Coronal cavities of teeth were then filled with Intermediate Restorative Material (IRM, Dentsply International, Inc., Milford, USA). Animals were sacrificed with an overdose of ether inhalation anesthesia at 7, 15 and 30 days post-operatively.

Histological procedure

Following euthanasia, soft-tissue around the jaws was removed using a surgical blade and the treated teeth and surrounding tissue were retrieved using cutting disks. Teeth and surrounding bone were placed in individual containers containing 10% formalin and labeled. Samples were stored in 10% formic acid for 15 days for decalcification, dehydrated in an ascending series of alcohol rinses, embedded in paraffin, sliced into 6-μm sections, stained with hematoxylin and eosin (H and E) and examined under a light microscope (Nikon Eclipse E 600, Nikon Corp., Tokyo, Japan). Histological evaluation criteria are given in Table 1.

Statistical analysis

Histological data was statistically analyzed using the Kruskal-Wallis and Dunn's multiple comparison tests, with the level of significance set at $P < 0.05$. Statistical analysis was performed using the SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The surgical procedure was well-tolerated by the experimental animals, with no apparent adverse events occurring during an observation period of 7-30 days.

Of the 72 pulpotomized teeth, some teeth were excluded from the study due to loss of the restoration, impossibility of using the histological sections (e.g., absence of a clearly identifiable material/pulp interface); or furcal perforation during preparation of the access cavities. After discarding the teeth that met any of these exclusion criteria, other teeth were randomly eliminated in order to have all groups with the same number of specimens, thus totalizing nine exclusions and a final sample size of 63 teeth distributed in the three groups (n = 21).

Inflammatory cell response

The ABS group exhibited mostly mild inflammation on the day 7, with inflammation decreasing over time. However, differences in inflammatory cell response over time were not statistically significant ($P > 0.05$).

In the FS group, inflammatory cell response varied significantly over time ($P < 0.05$), with the highest inflammatory cell response observed on day 7.

In the FC group, differences in inflammatory cell response did not vary significantly over time ($P > 0.05$).

When the inflammatory responses of the groups were compared, no significant differences were found at day 7 or day 15 ($P > 0.05$); however, at day 30, intense inflammatory response was significantly greater in the FC group when compared with the ABS and FS groups ($P < 0.05$) [Table 2].

Hard-tissue formation

No significant differences were observed in hard-tissue formation between groups at any of the experimental periods ($P > 0.05$). Although hard-tissue formation scores increased with time, differences between days were not significant for any of the groups ($P > 0.05$) [Table 3].

Figures 1-3 show histological appearance of groups.

Table 1: Histological evaluation scores

Inflammatory cell response
None or few inflammatory cells beneath the exposure site
Mild inflammatory cell (e.g., mono-or poly-morphonuclear leukocytes) infiltration beneath the exposure site
Moderate inflammatory cell infiltration involving up to a third of the coronal radicular pulp
Severe inflammatory cell infiltration involving a third or more of the coronal radicular pulp
Hard-tissue formation
No hard-tissue formation
Modest hard-tissue deposition beneath or lateral to the exposed area
Moderate hard-tissue deposition beneath or lateral to the exposed area
Complete dentin bridge formation beneath the exposed area

DISCUSSION

Given the many positive reports on the use of ABS in medicine and dentistry,[18-25] this study aimed to assess the histological characteristics and suitability of ABS as an alternative to FC and FS as a pulpotomy agent in rat teeth.

FC was selected as a control pulpotomy dressing because it is still considered by many to be the standard therapeutic agent for primary teeth pulpotomies. FS was also tested

Table 2: Distribution of inflammatory cell response scores by group

	Inflammatory cell response											
Group	7 days				15 days				30 days			
	0	1	2	3	0	1	2	3	0	1	2	3
ABS	2	5	–	–	4	2	1	–	7	–	–	–
FS	1	4	–	2	6	–	1	–	6	1	–	–
FC	4	–	2	1	1	5	1	–	3	2	–	2

ABS: Ankaferd blood stopper, FS: Ferric sulfate, FC: Formocresol

Table 3: Distribution of hard-tissue formation scores by group

	Hard-tissue formation											
Group	7 days				15 days				30 days			
	0	1	2	3	0	1	2	3	0	1	2	3
ABS	4	–	3	–	–	4	1	1	–	3	1	3
FS	2	4	1	–	2	4	1	–	–	–	5	2
FC	3	2	2	–	4	1	2	–	2	3	–	2

ABS: Ankaferd blood stopper, FS: Ferric sulfate, FC: Formocresol

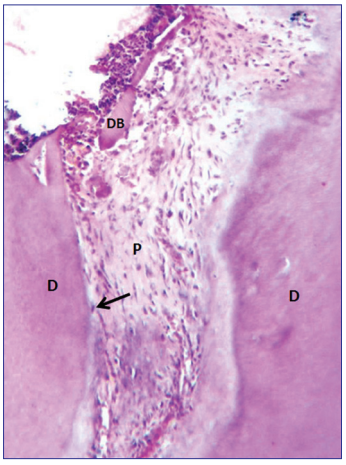

Figure 1: Pulpotomy with Ankaferd blood stopper at 30 days shows moderate hard-tissue deposition without inflammation (D: dentin, DB: Dentin bridge, P: pulp,*: odontoblast) (H and E, ×10)

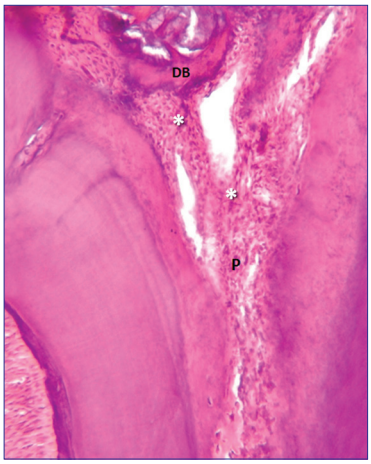

Figure 2: Pulpotomy with ferric sulfate at 30 days shows moderate hard-tissue deposition with mild inflammation (D: dentin, DB: Dentin bridge, P: pulp,*: inflammation) (H and E, ×10)

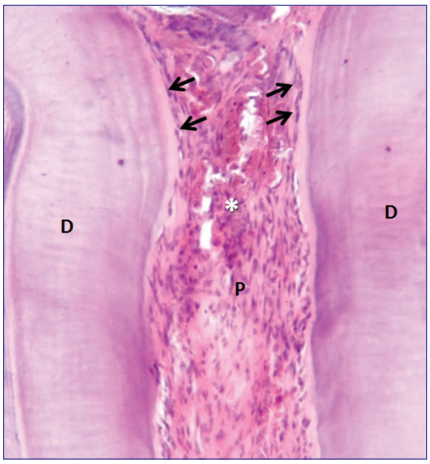

Figure 3: Pulpotomy with formocresol at 30 days shows mild inflammation (D: dentin, P: pulp,*: mild inflammation,*: odontoblast) (H and E, ×10).

due to its increase in popularity as a replacement for FC and calcium hydroxide in pulpotomies.[26] The use of plants and plant extracts for medicinal purposes has experienced remarkable advancement in recent years; similarly, the use of biocompatible substances has become a major area of interest in modern dentistry, especially when direct contact with dental tissue is necessary.[27] As a natural, biocompatible substance,[28] ABS was considered a promising agent for use in pulpotomy treatment of primary teeth.

The success of a pulpotomy may be increased by promoting hemostasis of the remaining pulpal tissue,[29] whereas a blood clot on the wound surface may provoke a chronic inflammatory response and impair the healing process by preventing intimate contact between the capping material and pulp tissue.[30] Various procedures have been used to control pulpal hemorrhaging, including electro surgery and laser irradiation[31,32] as well as hemostatic agents like FS.[8,10,11,13,29,32] ABS has also been tested as a hemostatic agent in primary teeth pulpotomy treatment.[24,25]

The histological response of vital pulp to FC application depends upon the length of contact time and the concentration of the material. Ranly[9] reported that FC treatment leaves pulp chronically inflamed and susceptible to abscess formation and internal resorption. Fuks *et al*.[33] reported 29% of teeth showed severe inflammation 8 weeks after FC pulpotomy treatment; the results of the present study at 30 days post-treatment was in line with these earlier findings. Moreover, in the present study, the inflammatory response of pulp tissue to FC was greater than that of FS and ABS, which appeared to induce an inflammatory response in pulpotomized teeth at 7 and 15 days, but which resolved at 30 days. The unfavorable response to FC may be explained by an inadequate fixation of pulp tissue: if the fixed layer fails to function as an effective barrier, the underlying tissue is affected by FC and responds with inflammation and other pathologic mechanisms.[34]

The presence of a "dentin bridge" at the site of pulpal amputation is one sign of a successful vital pulpotomy.[35] However, the formation of a dentin bridge has also been reported in teeth with irreversible inflammation,[36] and successful pulp capping has been reported without the presence of a reparative dentin bridge over the exposure site.[37] Studies have indicated that exposed pulp possesses an inherent ability to produce dentin in response to operative procedures or trauma, irrespective of the agent applied to the amputation site.[38] In the present study, hard-tissue depositions were observed in all groups, even the FC group, which supports the results of other studies.[14,35,39]

Of the three agents tested in the present study, FS and ABS appear to have promoted less inflammatory cell response than FC. FS and ABS also have the advantage of a short application time (15 s), which is especially important in pediatric dentistry.

CONCLUSION

Alternative vital primary pulp treatment agents must be equally or more effective than FC with a wider margin of safety. According to these criteria, ABS was found to be an acceptable alternative pulpotomy agent. More experimental data and further clinical research with larger sample sizes and longer follow-up periods are needed.

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