*DİYOT LAZER, FERRİKSÜLFAT VE FORMOKREZOL SÜT DİŞİ AMPUTASYONLARININ HİSTOLOJİK SONUÇLARI

HISTOLOGICAL OUTCOME OF PULPOTOMY IN PRIMARY MOLARS USING DIODE LASER, FERRIC SULPHATE AND FORMOCRESOL

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Özet

Pulpa amputasyonu enfekte kuronal pulpanın çıkartılması ile radiküler pulpanın canlılık ve fonksiyonelliğini devam ettirmeye yönelik bir tedavidir.

Bu invivo tedavinin amacı Diyod lazer (DL) amputasyonunun histolojik etkilerini Formokrezol (FK) ve Ferriksülfat (FS) amputasyonları ile karşılaştırmalı olarak sunmaktır.

58 çocukta 120 diş (57 birinci süt molar ve 63 ikinci süt molar) diş konvansiyonel amputasyon tekniğiyle tedavi edilmiştir. Kuronal pulpa amputasyonunu takiben hemostaz işlemi için DL(800nm) 1.5 W, 30 Hz, 50 mJ, 1/5 lik dilue FK VE 15.5%lik FS solüsyonları kullanılmıştır. Daha sonar tedavi edilen tüm dişler paslanmaz çelik kuron ile kaplanmıştır. Hastalar klinik ve radyografik olarak 1,3,6,9,12 takip edilip, 12. ayın sonunda ortodontik nedenlerle çekilmesi uygun görülen 12 diş histolojik olarak incelenmek üzere çekilmiştir. Işık mikroskobik incelemesi için, dişler 10% formalinde fikse edilmişlerdir.

Fibrotik dejenerasyon, internal rezorpsiyon alanları, pulpa nekroz alanları, iregüler iritasyon dentin alanları hem FK hem FS gruplarında saptanmıştır. Ayrıca FK grubunda orta şiddetli iltihabi hücre cevabı, FS grubunda da şiddetli iltihabi cevap gözlenmiştir. En düzgün odontoblastik tabaka ve en az histopatolojiye sahip tabala ise DL grubunda belirlenmiştir. Sonuç.Histolojik sonuçlara gore; DL amputasyonu süt dişlerinde FK amputasyonlarına bir alternatif olarak önerilebilir.

Anahtar Kelimeler: Histopatoloji, amputasyon, süt dişleri.

Abstract

Pulpotomy is the amputation of infected coronal pulp to maintain radicular pulp vitality and function.

The purpose of this in vivo study was to compare the histological effects of Diode laser (DL) to Formocresol (FC) and Ferric Sulphate (FS) in pulpotomized vital human primary molars.

120 molars (57 primary first molars and 63 primary second molars) in 58 children treated by a conventional pulpotomy technique. The teeth were randomly assigned to DL, FS and FC groups. After conventional coronal pulpotomy, haemostasis of remaining pulp in the groups was achieved by exposure to DL (810 nm) at 1.5 W, 30 Hz, 50 mJ, by applying 1:5 dilution of FC and by % 15.5 FS solution. All pulpotomized teeth were restored with stainless steel crowns. Subjects were monitored clinically and radiographically at 1,3,6,9 and 12 months. At the end of the 12th month, 12 teeth were chosen among the teeth which were previously planned for serial extraction and removed. For light microscopic investigation, teeth were fixed in 10% formalin solution.

Fibrotic degeneration, internal resorption areas, necrosis in pulp and irregular irritation dentin were observed in both FC and FS group. While a mild inflammatory cell response was detected in the FC group, the FS group revealed a severe inflammatory cell response. The most organized odontoblastic areas and least histopathological score for degeneration were noticed in the DL group.

Our results implicate that nonpharmacological DL pulpotomy technique may be recommended as a suitable replacement for formocresol.

Key words: Histopathology, pulpotomy, primary teeth.

Introduction

Pulpotomy is a common therapy for cariously exposed pulp in primary molar teeth. With this method, the functional tooth is retained in the oral cavity, without pain and swelling, until it exfoliates (1-4). Over the last 70 years, formocresol (FC) has been a popular material used in the pulpotomy procedure, mainly because it is easy to use and it ensures high clinical success rates (5). However, several studies have shown that FC has hazardous adverse effects, such as mutagenicity and cytotoxicity (6,7). Therefore, a variety of medicaments and non-pharmacologic alternatives have been proposed in the literature to replace FC, such as glutaraldehyde

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(GH), calcium hydroxide (CH), freeze-dried bone, ferric sulphate (FS), mineral trioxide aggregate (MTA), electro-surgery and lasers (2-5,8-18). However, note that there has not been any consensus published on the ideal pulpotomy technique (19).

FS (15.5%) has been investigated widely and reported in animal and human studies as a haemostatic agent in pulpotomy procedures. On contact with blood, a ferric ion-protein complex is formed, and the membrane of this complex seals the cut vessels mechanically, producing haemostasis, and the agglutinated protein complex forms plugs which occlude the capillary orifices, preventing blood clot formation (20). Based on the available evidence so far, FS and FC produce similar treatment outcomes but FS requires much technique sensitivity (21,22). It is worth mentioning that FS has been proposed as a substitute for FC, which some would consider as a new gold standard (1,2,20).

Recent advances in laser technology have made lasers more attractive in endodontic applications, where they can be used as an adjunct or alternative method to traditional pulp therapy procedures. Laser treatment has advantages with respect to control of haemorrhage, sterilization and stimulation effects on the dental pulp cells. Since the effects of ruby laser irradiation on dental pulp tissue were reported (23), other studies using CO_2, Nd:YAG, Er:YAG, Er,Cr:YSGG, diode laser (DL) and argon laser (AL) pulpotomies have been published (18, 24-26)

DLs have been applied widely in oral surgery procedures involving soft tissue. Since these lasers are relatively poorly absorbed by the tooth structure, soft tissue surgery can be performed safely in close proximity to enamel, dentin and cementum. DLs can be used for ablation, incision and excision (cutting, vaporization, curettage, coagulation and haemostasis) (26). Furthermore, the laser has the advantages of being portable and compact with a minimum setup time (26). Based on these characteristics and previous studies (18,24-27), the DL could be an alternative for pulpotomy therapy.

The purpose of this study was to investigate, histologically, the effect of diode laser (DL), formocresol (FC) and ferric sulphate (FS) on human dental pulp as pulpotomy agents.

Materials and Methods

The proposed approach was a randomized cohort study approved by the University of Marmara Health Sciences Ethics Committee (Document number MAR-YC-2008-0131). The participants were 58 children-32 males and 26 females aged between 5 and 9 years-at the University of Marmara, Department of Paediatric Dentistry, in Istanbul. Prior to the clinical procedures, informed consent was obtained. All pulpotomies were performed by the same trained and experienced pediatric dentist (B.D).

Inclusion criteria:

Clinical criteria:

1. Vital primary molar teeth in children that required pulpotomy treatment due to pulpal exposure to caries and lacked excessive haemorrhage (i.e.controllable within 2 min).
2. Teeth had neither spontaneous pain nor tenderness to percussion.
3. Teeth were deemed to be restorable with stainless steel crowns.

Radiographic criteria:

1. A modified Ekstrand *et al*.(28) criterion was used in the visual examination. The teeth were scored as 3: demineralisation involving the middle 1/3 of dentin and 4: demineralisation involving the inner 1/3 of dentin.

2. Absence of furcal or periapical radiolucency and widened periodontal ligament spaces.

3. Absence of a pathologic internal or external resorption.

4. Teeth with physiologic root resorption of less than 1/3 of their roots.

Clinical procedures:

One-hundred and twenty primary molars were randomly assigned into 3 groups (n=40), according to the pulpal therapy technique:

Group I: Diode laser (LaserSmile™, Biolase Technology, Inc., Irvine, California, USA)

Group II: Formocresol (Buckley's Formo Cresol®, SultanHealthcare, Hackensack, NJ, USA)

Group III: Ferric sulphate (Astringedent™ Ultradent Products Inc., Salt Lake City, UT, USA)

Based on the clinical/radiographic assessment and carious pulp exposure in affected molars, coronal pulpotomies were performed. In each treatment group, coronal pulpotomies were achieved with small and medium slow-speed round burs under local analgesia (*Ultracain*® DS Ampul - Sanofi Aventis Ltd., Istanbul, Turkey) and rubber dam isolation. The pulpotomy site was cleaned with a sterile spoon excavator, and the initial haemorrhage was controlled using a dry sterile cotton pellet applied with pressure. In cases with excessive and persistent haemorrhage, pulpectomy was performed, and the tooth was eliminated from the study. Complete haemostasis then was achieved as follows, depending on the group assignment.

- In the FC group, the sterile cotton pellets were placed in 19% formaldehyde Buckley's FC solution. They were immediately blotted dry using a sterile cotton roll. The cotton pellet was placed directly over the radicular pulp stumps and left for 5 min for fixation.
- In the FS group, a 15.5% FS solution with a plastic syringe and a cotton-tipped needle were utilized. FS was applied by wiping the cotton tip on the pulp stumps for 15 sec. The pulp cavity was washed with saline to remove any blood clot particles.
- In the DL group, a DL beam at a wavelength of 810 nm was transmitted. The DL fibre tip was kept 1-2 mm from touching the tissue. The pulp at canal orifices was exposed with parameters of a frequency of 30 Hz and energy of 50 mJ, with a power of 1.5 W for 10 sec with air-cooling operation mode without water.

In each group, a zinc oxide eugenol dressing (ZOE) (Kalzinol®, Dentsply, Konstanz, Germany) was placed directly on the radicular pulpal stumps and sealed by a second layer of glass-ionomer restorative material (Ketac™ Molar, Easy Mix™, 3M ESPE™, Seefeld, Germany). Final crown restorations were completed immediately with stainless steel crowns (SSC) (3M™ Unitek™ Stainless Steel Crowns, Primary, Neuss, Germany).

Subjects were monitored clinically and radiographically at 1, 3, 6, 9 and 12 months. At the end of the 12th month, 12 teeth were chosen among the teeth which were previously planned for serial extraction and they were neither sensitive to palpation nor to percussion. These were removed at the end of 12 months follow-up and histologically evaluated by light microscopy.

Histopathological examination: Following extractions, the teeth were immediately fixed in 10% buffered formalin solution and decalcified in decalcification solution (Shandon TBD-1 Decalcifier, Thermo Fisher Scientific, USA) for light microscopic investigation. After decalcification, the roots which were slightly resorbed were chosen and sectioned gently without damaging the pulpotomy material in sagittal direction. Root samples were dehydrated in ascending alcohol series, cleared in toluene and embedded in paraffin. Paraffin sections (5 µm) were stained with Masson's trichrome and examined under light microscope (Olympus-BX51, Tokyo, Japan) by two microscopists who were unaware of the treatment. All sections were evaluated according to the following criteria (29,30,31): Inflammatory cell response, pulp vitality, hard tissue formation (dentin bridge formation), odontoblastic layer change, internal resorption, pulp calcification and fibrotic degeneration (Table 1).

Criteria	Score: 0	Score:1	Score:2	Score:3
Inflammatory cell response	None or a few scattered inflammatory cells present in the pulp area corresponding to the pulp exposure, characteristic of normal tissue.	Slight inflammatory cell infiltrate with polymorphonuclear (PMNs) or mononuclear (MNLs) leukocytes	Moderate inflammatory cell infiltrate involving the coronal third of the radicular pulp	Severe inflammatory cell infiltrate involving the coronal third of the radicular pulp or characterizing abscess
Odontoblastic layer change	regular	irregular	absent	
Pulp vitality	vital pulp	partial necrosis	total necrosis	
Fibrotic degeneration	absent	present		
Pulp calcification	absent	present		
Internal resorption	absent	present		
Hard tissue formation (dentin bridge formation)	absent	present		

Table 1. Scoring system used for histological investigation

Results

Fibrotic degeneration, internal resorption area, necrosis in pulp and irregular irritation

dentin were observed in both FC and FS groups (Figure 1A-D).

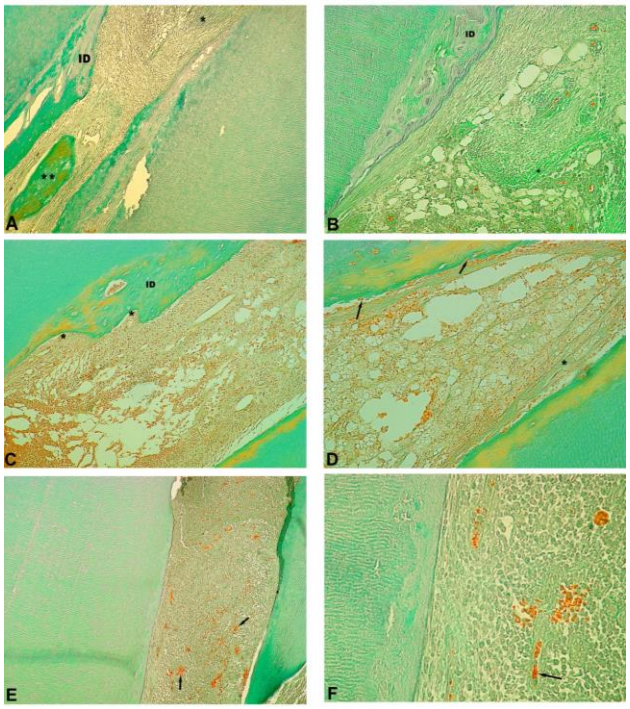

Figure 1. (A) Irregular irritation dentin (ID), fibrotic degeneration (*), calcific degeneration (**) and moderate inflammatory response in pulp were observed in FC group. **(B)** Irregular irritation dentin (ID), fibrotic degeneration (*) and moderate inflammatory response in pulp were noticed in FC group. **(C)** Internal resorption areas (*), severe pulp inflammation and irregular irritation dentin layer (ID) were observed in FS group. **(D)** Odontoclast cell in the internal resorption areas (arrows), fibrotic degeneration (*) and severe inflammatory response in pulp were observed in FS group. **(E)** Regular dentin tubules and small hemorrhagic areas (arrows) in pulp were noticed in DL group. **(F)** Slight inflammatory response and small hemorrhage areas (arrow) in pulp in DL group. Masson's thrichrome stain, A,C,E:original magnification x 100; B,D: original magnification x 200; F: original magnification x 400.

While the FC group revealed a mild inflammatory cell response, a severe inflammatory cell response was detected in the FS group (Figure 1A-D). In the laser group, minimal inflammatory cell response, fibrotic degeneration, and moderate internal resorption area were observed (Figure 1E-F). Moreover, minimal necrosis and scattered hemorrhagic area were observed in the pulp of the laser group (Figure 1E-F). On the other hand, total necrosis, dentin bridge formation and calcific degeneration were not detected in the laser group. Irritation dentin structure was thinner and more regular in the laser group as compared to the other two groups.

According to the histological scoring, inflammatory cell response in pulp of the all teeth was found to be in varying degrees. However, score 3 was detected only in the FC and FS groups While pulpa necrosis was observed in all groups, total necrosis (Score 2) appeared in the FC group. Hard tissue formation was analyzed according to the presence or absence of dentin-bridge beneath the pulpotomy dressing. One dentin bridge formation was present in the FC and FS groups, whilst it was not seen in the DL group. Regular odontoblastic layer which reflects the least degree of degenerative changes in the odontoblastic layer was most prominent in the DL group. Odontoblastic degeneration was observed in all teeth in the FC and FS pulpotomy groups. Internal resorption was mostly seen in all teeth of the FS group. However it was equal to each other in FC and DL groups and found to be present only in half of teeth. Pulp calcification was observed one in FC and two in FS groups while none was seen in the DL group. Fibrotic degeneration was seen all specimens of the FS group (Table 2).

Discussion

An ideal pulpotomy material or drug must preserve radicular pulp tissue healthy, be highly biocompatible, and prevent bacterial micro leakage. Another criterion is the ability of material to promote healing (13). This investigation presented a long-term follow-up study of the histological outcomes of pulpotomy utilizing DL, FC and FS.

Jones-Cleaton et al.(36) stated that there was no defined success criteria for histologically successful pulpotomy therapy. Instead in literature, the success of the pulpotomy treatments was defined as the absence of any clinical and radiographic pathology at the follow-up appointments. However, Karami et al.(37) defined success criteria in their histological study as follows dentin bridge formation, preserving pulp vitality,

not having -internal resorption or interradicular pathology.

Scores		FC N=4	FS N=4	DL N=4
Inflammatory cell response	0	0	0	0
	1	1	0	3
	2	2	3	1
	3	1	1	0
Pulp Vitality	0	0	0	1
	1	2	4	3
	2	2	0	0
Dentin bridge formation	0	3	3	4
	1	1	1	0
Odontoblastic layer change	0	0	0	3
	1	2	4	1
	2	2	0	0
Internal resorption	0	2	0	2
	1	2	4	2
Pulp calcification	0	3	2	4
	1	1	2	0
Fibrotic degeneration	0	2	0	3
	1	2	4	1

Table 2. Histological evaluation of extracted teeth

Although FC has high clinical success rates, radiological and histological success rate are not parallel with these results (32, 33, 34). It was reported that when FC fixation progress through apical part where the healthy pulp tissues preserved, necrotic areas, vasodilation and inflammation occur (33).

Cotes et al.(32) histologically assessed the pulpal reaction after use of FC and FS in maxillary first molars of 120 rats for four weeks. The authors concluded that the teeth treated with FC showed the least pulpal inflammatory response.

Salako et al.(33) histologically evaluated Bioactive glass, MTA, FS and FC pulpotomies in rats. Analysis performed 2 and 4 weeks after the pulpotomies. FS showed moderate inflammation of pulp with widespread necrosis in coronal pulp in 2 and 4 weeks. FC showed zones of atrophy, inflammation, and fibrosis. Fibrosis was more extensive in 4 weeks with evidence of calcification in certain samples.

Eyüpoğlu (38) compared FC, $CaOH_2$, FS and MTA pulpotomies clinically, radiologically and histologically. No statistical difference was obtained between the groups according to pulp necrosis and inflammation penetration. Additionally, odontoblastic layer irregularities, fibrotic and calcific degenerations were seen in all groups. While the least degenerative changes were occurred in MTA group, all specimens of FS showed degeneration. Internal resorption was mostly seen in FS group.

Goyal et al.(39) evaluated the different concentrations of FC efficacy in pulpotomy at children. The study was conducted on 45 primary molars for the Clinical, Radiographic study and 45 premolars orthodontically indicated for extraction for the Histological study. The samples were randomly and equally divided into 3 groups of 15 each for pulpotomy with full strength formocresol, 1:5 diluted formocresol and 1:25 diluted formocresol respectively. The pulpotomized primary molars were clinically evaluated at 1st, 3rd, 6th and 9th month while the pulpotomized premolars were subjected for histological evaluation after extraction. They reported that in all the 3 groups the blood vessels appeared engorged and dilated due to pulp irritation. Also paralel with Salako et al.(33),they observed fibrosis in varying degrees.

In the study of Ratnakumari and Thomas (40), recently developed Indian material, Sree Chitra-Calcium Phosphate Cement (Chitra-CPC) and formocresol were evaluated and compared related to the response of human pulp tissue when they used as pulpotomy agents in deciduous teeth. They concluded that Chitra-CPC gave more favorable results, in respect of pulpal inflammation, dentin bridge formation, quality of dentin-bridge and connective tissue in dentin-bridge. In FC group, pulp tissue subjacent to the exposure site showed a layer of dense homogenous eosinophilic tissue. Heavy inflammation was present in six samples. Also, area apical to the inflammatory zone appeared normal, i.e. healthy, vital pulp tissue in most of the samples. Layers of odontoblasts adjacent to the capping agent were disorganized.

In the current study, although the teeth with no clinical and radiological pathology extracted for histological analysis, all of the specimens of FC group showed necrosis in varying degrees. Partial necrosis was seen mostly but total necrosis was also detected in one specimen. Mostly observed histological pathologies in FC group are; moderate

inflammation infiltration, internal resorption areas, pulp necrosis and irregularities in irritation dentin. The histologic observations regarding FC in this study revealed results consistent with previous observations (5, 35, 38, 39, 40) differs from Cotes et al. (32). As we used diluted FC in the pulpotomy procedure, it could be the probable reason for thin fixation layer formation. Also using ZnOE cement as a pulpotomy base may be the other reason for inflammation and internal resorption. Observing internal resorption mostly in FS group may be due to the fact that FS does not have a fixation effect. Inflammation infiltration in FS group is more than FC group in the present study which is similar with Salako et al.(33) but as a difference, we did not observe any total necrosis in FS group.

Conflicting results regarding pulp healing following laser pulpotomy have been published.

Shoji et al.(41) investigated the effects of a CO2 laser on amputated dental pulps in dogs. They observed no detectable damage in the radicular pulp in teeth that were treated.

Jukic et al.(42) compared the effects of CO2 and Nd:YAG lasers on amputated vital dental pulps in molars and premolars of dogs at 30 and 45 days. Laser irradiation caused carbonization, necrosis, inflammatory infiltration, edema and hemorrhage in pulpal tissues, and no new dentine formation as was found.

Toomarian et al.(43) histologically evaluated pulpotomies performed using the Er,Cr:YSGG laser in 48 caries-free primary canines versus pulpotomies using formocresol (mixture of 50% formocresol and 50% formaldehyde) in twelve dogs. The investigators found that samples treated with laser showed favourable histological features. Two months after treatment with formocresol or laser, the apical portion of the dental pulp remained vital. The authors concluded that the Er,Cr:YSGG laser system was an acceptable alternative for formocresol pulpotomy in pulpotomy of deciduous teeth based on six carious-free primary cuspids.

In the present study histological findings in the DL group are; minor inflammation in the pulp tissue, fibrotic degeneration and moderate internal resorption. However, total necrosis, dentin bridge formation and calcific degeneration were not observed. These results are similar with Odabas et al.(31), Shoji et al.(41) but differs from Jukic et al.(42) who reported coagulation necrosis and carbonization areas under pulpotomy site.

While some of the studies claiming that dentin bridge formation between pulp medicament and pulp stating the success in vital pulp therapies in primary teeth (36, 37), others indicating that pulp was not staying vital beneath the dentin bridge. In some of the studies no dentin bridge formation was observed following FC and FS pulpotomies, while in some it was observed (32, 44, 45). In our study, while no dentin bridge formation was recorded in DL group, it was observed in FC and FS groups. The results are consistent with some of the previous studies (31, 40, 44, 45).

We assume that the reason for not observing dentin bridge formation in DL group can be attributed to the fact that laser ablation did not effectively stimulate the odontoblastic layer. Although we did not observe any dentin bridge formation in DL group in histological analysis, clinical success rate of this group was higher than FC and FS group. Therefore, we support the idea that dentin bridge formation can be evaluated as one of the success criteria.

Odontoblastic layer organization changes or completely disappear in most of the pulpotomized primary teeth (46). In the present study all pulpotomy groups showed odontoblastic layer irregularities which is compatible with the results of Eyüboğlu and Ratnakumari and Thomas (38,40). Among the pulpotomy groups, ‘regular odontoblastic layer’ was mostly observed in DL group which is similar with Odabas et al.(31) and Toomarian et al.(43). Inflammation and necrosis detected in all study groups could be the result of these odontoblastic irregularities.

Based on overall results, we could conclude that clinical and radiological success are not in good correlation with histological success. Therefore, even histologically unfavourable results are obtained, clinically successful with no radiological pathology pulpotomy therapies may be considered as successful.

Conclusion

Although there is no standardized criteria for histological analysis up to date, DL pulpotomy offers favourable results with preserving pulp vitality in vital pulpotomies in

primary teeth. However, additional studies with a larger sample size and long-term outcomes are required before definitive recommendations can be made.

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