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Is formocresol effective on periodontal pathogens in periodontal endodontic lesions ?

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ÖZET

Periodontal endodontik lezyonlarda formokrezol kullanımı periodontopatogenlere karşı etkili mi dir ?

Amaç: Bu çalışmanın amacı primer periodontal sekonder endodontik lezyonlarda formokrezol kullanarak/kullanmadan yapılan endodontik tedavinin periodontal cep florası üzerine etkilerinin değerlendirilmesidir.

Materyal ve Metodlar: Çalışmaya klinik ve radyolojik olarak periodontal endodontik lezyonu bulunan 12 hasta dahil edilmiştir. Hastaların periodontal patolojik ceplerinden kök kanal tedavisi (KKT) öncesi, ikinci seans öncesi ve üçüncü seans öncesi, bir hafta aralıklarla olmak üzere gracey küretler yardımıyla plak örnekleri alındı. KKT sırasında ilk seansta pulpa odası boş bırakılırken ikinci seansta pulpa odasına formokrezol emdirilmiş pamuk pelet yerleştirildi. Alınan örneklerde Fusobacterium nucleatum, Campylobacter rectus, Tannerella Forsythia, Provetella intermedia, Porpyhromonas gingivalis, Aggregatibacter actinomicescomitans Polymerase Chain Reaction yöntemiyle değerlendirildi.

Bulgular: Tedavi süresince elde edilen örneklerde mikroroganizmalar açısından hiçbir fark bulunmadı ($p>0.05$).

Sonuç: KKT işlemleri ve formokrezol kullanımı kısa dönemde periodontal cep florasına etki etmemektedir.

Anahtar Kelimeler: *Periodontal endodontik lezonlar, Periodontal mikrobiyoloji, Formokrezol*

SUMMARY

Objective: This study aims to examine the effects of endodontic therapy with/without formocresol on periodontal pocket flora in primary periodontal secondary endodontic lesions.

Study design: A total of 12 patients with clinically and radiologically diagnosed periodontal-endodontic lesions were enrolled to the study. Periodontal pathologic pockets of the patients were sampled as 36 aliquots using Gracey curettes, before the initiation of the root canal therapy (RCT), before the second session in which the RCT was continued and, again, before the third session in which the RCT was continued, by one-week intervals. During the RCT, steril cotton was put in the pulp chamber in the first session and formocresol-impregnated cotton pellet was put in the pulp chamber in the second session. Quantitative values of Fusobacterium nucleatum, Campylobacter rectus, Tannerella Forsythia, Provetella intermedia, Porphyromonas gingivalis, Aggregatibacter actinomicescomitans were examined using Polymerase Chain Reaction methodology.

Results: In the samples obtained during the therapeutic sessions, no statistically significant difference was observed for the changes seen in the number of microorganisms ($p>0.05$).

Conclusion: The procedures of RCT and the use of formocresol had no short-term effect on the number of periodontopathogens present in periodontal pocket flora.

Key words: *Periodontal endodontic lesions, Periodontal microbiology, Formocresol*

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Introduction

Periodontal-endodontic lesion is a commonly encountered condition in the clinics, which requires a complex therapeutic approach. Generally, while periodontal therapy targets the repair and the regeneration of the structures that support the teeth, endodontic therapy firstly considers pulp and periapical tissues. However, periodontal and endodontic structures are in a relation, leading to an interaction in both healthy condition and function, and in a disease (1,2). The procedures performed on periodontal tissues may lead to some degenerative alterations of the pulp (3,4). Similarly, the procedures performed during the endodontic therapy may affect periodontal wound healing or regeneration, by acting on periodontium of the tooth (5,6).

The success of periodontal and endodontic therapies depends on the elimination of disease factors, in individual or combined lesions. In periodontal therapies, many studies were conducted for the use of systemic or local drugs to eliminate the microorganisms (7,8), but limited success could be obtained. On the other hand, in endodontic therapy, only the success of root canal therapy with “bio-mechanic” preparation remained limited (9). Therefore, it was reported that, various volatile drugs placed in the pulp chamber or in root canals to achieve an optimal success affect root canals and lateral canals via direct contact or by evaporation (10). However, it is known that these drugs put in the pulp chamber don’t affect only these regions, but also periodontal tissues via aforementioned pathways (11).

Most commonly used volatile drugs used in intracanal drug therapy were 2 % glutaraldehyde, iodine and formocresol, due to minimal toxicity and minimal irritation (12-14). In the studies performed, it was reported that antibacterial effect of formocresol was quite higher compared to that of other intracanal drugs (12). Nevertheless, there are also the studies that show that it leads to a decrease of fibroblast counting and a delay in wound healing in periodontal tissues (11,15). However, it is of interest that these studies showed no or little periodontal tissue destruction and investigated the effects of formocresol in apical tissues.

In the literature, in addition to the lack of a study that examined the effect of endodontic therapy on periodontal pocket flora, there was no study that examined the effects of formocresol on periodontal pocket flora. Therefore, this study aimed to examine the changes of periodontal pocket flora during the sessions of endodontic therapy and to investigate the effects of formocresol on periodontal pocket flora.

Material and methods

A total of 12 patients with clinically and radiologically diagnosed periodontal-endodontic combined lesions, who presented to Oral and Dental Health Center with several complaints, were enrolled to the study. Inclusion criteria of the study included being systemically healthy, the absence of periodontal therapy and antibiotic therapy within the last 6 months, being caries-free with intact root canal, having non-vital pulp test, no history of trauma, and the presence of radiographically and clinically determined combined periodontic lesion and endodontic lesions. The study was approved by Gülhane Military Medical Academy (GATA)'s Ethic Board. In addition, all participants gave written and verbal consent.

Clinical Method and Sampling of Periodontal Pocket

The clinical periodontal evaluation was based on the Plaque Index (PI), Gingival Index (GI), Probing Depth (PD), and Clinical attachment level (CAL). Probings were carried out using a Williams probe calibrated in millimeters and were assessed at deepest periodontal pocket in each tooth. All clinical evaluations and therapies were performed by the same investigator. Microbiological samples were obtained from periodontal pocket of the patients in the first session before the initiation of the root canal therapy, just before the second session and just before the third session, by one-week intervals, on three different occasions. Prior to sampling, supragingival plaque was gently removed with sterile cotton pellets. Sample sites were isolated with cotton rolls and air-dried. Subgingival specimens were collected by means of a sterile Gracey curette (Hu Friedy, Chicago, USA). Probing in the tooth with periodontal-endodontic lesion was achieved by pulling the curette in one movement from the deepest region. The samples were placed in saline-containing test tubes and were stored at -20 C until the time of study. The labeled test tubes were then transported to Department of Microbiology in GATA.

In the first session, as a procedure of root canal therapy, cavitation was performed under local anesthesia. The pulp tissue was extirpated from the canal using barbed broaches and K files, and canals were irrigated using physiologic saline solution. Canals were enlarged for a No. 35 Hendstrom file (Maillefer, Ballaigues, Switzerland) to be introduced 1 mm from the radiographic apex. During this first session, a temporary restoration was performed by putting a sterile cotton pellet in the pulp. During the second session, which was planned for one week later, temporary filling material was removed, another dressing was performed using physiologic saline solution, and formocresol-impregnated cotton pellet was placed in pulp chamber. For the patients, who were invited for a visit of one week later after the restoration with temporary filling material, samples were obtained and thereafter, routine therapeutical procedures were continued.

DNA extraction and Real-time TaqMan assay procedures

DNA was extracted from the samples by treatment with 1% SDS and 100 mg proteinase K (Sigma Chemicals, St. Louis, Missouri, USA) in a buffer containing 50 mM Tris (pH 8.0), 50 mM EDTA, 100 µM NaCl. After 2 hours of incubation at 55ºC in a waterbath, the DNA was purified by repeated extraction with phenol/chloroform/isoamyl alcohol (25:24:1). DNA was washed in 75% ethyl alcohol at 10,000 g for 5 min at 4°C, air-dried at 37°C, and dissolved in 100 mL distilled water (16).

The multiplex real-time PCR (TaqMan) method was performed by using a 7500 ABI Prism Sequence Detector (Applied Biosystems, Foster City, Calif., USA). In brief, 2 □l of the extracted nucleic acid solution was added to 23 µl of reaction

Table I: PCR primers used for the identification of periodontopathogens

Microorganism			Base Position Amplicon length in bp
Porphyromonas Gingivalis	Forward Reverse Probe	5'-TGGGACTTGCTGCTCTTGCTATG-3' 5'-GATGGCTTCCTGCTGTTCTCCA-3' FAM-5'-CAAAGACAACGAGGCAGAACCCGTTA-TAMRA-3'	194
Provetella Intermedia	Forward Reverse Probe	5'-AGACGGCCTAATACCCGATGTTG-3' 5'-TTACCCGCACCAACAAGCTAATCAG-3' JOE-5'-TGGCATCTGACGTGGACCAAAGATTC-TAMRA-3'	105
Tannerella Forsythia	Forward Reverse Probe	5'-GCG TAT GTA ACC TGCCCGCA-3' 5'-CCGTTACCTCACCAACTACCTAATG-3' FAM-5'-AGGGATAACCCGGCGAAAGTCGGA-TAMRA-3'	149
Campylobacter rectus	Forward Reverse Probe	5'-CACCCGATAACCCTACTCCTCCTA-3' 5'-GATCCGTTCCATCAGTACCCACTA-3' FAM-5'-CCGGTACCGAATCCTGAGGAACCA-TAMRA-3'	132
Aggregatibacter actinomicescomitans	Forward Reverse Probe	5'-CGCTTACCGTTATGACCGTGTGA-3' 5'-GCCCGGAATGCTTTGCTATATTTC-3' FAM-5'-AGGCAAGACGGGAAGCTAACGCAAA-TAMRA-3'	288
Fusobacterium Nucleatum	Forward Reverse Probe	5'-GCGGAACTACAAGTG TAGAGGTG-3' 5'-GTTCGACCCCCAACACCTAGTA-3' JOE-5'-AATGCCGATGGGGAAGCCAGCTTA-TAMRA-3'	175

mixture containing 0.8 µM of each primer and 0.4 µM each fluorophore probe (final concentration), and mixed with 12,5 µl of TaqMan Universal PCR Master Mix (Applied Biosystems). The TaqMan cycling conditions included a 10 min degradation of the preamplified templates at 95ºC and then 40 cycles of denaturation at 95ºC for 15 s and annealing and extension at 60ºC for 60 s. All experiments were repeated at least twice for testing the reproducibility of the assay. Table I lists the nucleotide sequence of the PCR primers and probes.23

Statistical analysis

Statistical evaluation was performed using the SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA). Descriptive data were reported in mean and standard deviation for scale variables. Comparisons between groups were performed using Wilcoxon's Signed Rank test. The correlation between the number of microorganisms and clinical indexes was evaluated using Spearman rho correlation test. P values ≤0.05 were considered statistically significant.

Results

Periodontal pocket samples were obtained from each tooth in three consecutive visits, wherein 'A', 'B', and 'C' represent the 1st, 2nd, and 3rd visit, separated by 7-day intervals. All of the samples obtained from the teeth with periodontal endodontic lesions on different time points showed F nucleatum (100%). While the most commonly seen microorganisms, following F nucleatum, were respectively C rectus (83.3-91.6%), T forsythia (83.3%), P intermedia (75%) and P gingivalis (58.3-66.6%), Aa (33.3-50%) had the lowest quantity in the sample (Table-II).

Microbial identification and microbial counts in PCR study at different time intervals 'A', 'B', and 'C' for each microorganism were recorded as shown in Table III and these values were analyzed using the Wilcoxon's Signed Rank test. None of the differences between time intervals showed any statistical significance (p>0.05).

In different sessions, the correlation between the counted number of microorganisms and PI, GI, PD and CAL values was calculated using Spearman Brown correlation coefficient. Accordingly, while, in the first session, statistically significant correlations were observed between P gingivalis and PI, PD and CAL, and between the number of F nucleatum and GI and PD (p<0.05), no correlation was observed between other microorganisms and clinical parameters (p>0.05).

Discussion

Periodontal diseases and therapies may lead to pulpal inflammation, which occurs via lateral canals, accessory canals and exposed dentin canals, even when the apical tooth is not affected (17). Therefore, intracanal region and periodontium are in connection via apical foramen or aforementioned pathways. Intracanal or intrapulpal volatile canal disinfectants may pass to periodontal pocket, through these same ways, likely to the passage of microorganisms, and thereby, may act on periodontologic microorganisms. Nescovic et al (5). performed root canal therapy alone to 24 teeth with periodontal endodontic problems, and based on the results obtained from the clinical and radiological examination performed 12 months later, they reported a success rate of more than 91 %. In the same study, they also reported that even the root canal therapy alone might provide a healing of the lesion in the teeth with periodontal endodontic lesions, regardless of whether the origin is periodontal or endodontic. Furthermore, positive outcomes could be obtained in periodontal clinical parameters in the teeth with periodontal endodontic lesions using an effective root canal therapy before an advanced periodontal therapy (18). Therefore, in our study, we aimed to examine the changes of pocket flora using extirpation and canal widening alone or using formocresol-impregnated cotton pellet placed in pulp chamber.

There are few studies in the literature to show the number and the species of the microorganisms present in the teeth with periodontal endodontic lesions (19,20). Kurihura et al (21). reported that spiroquets, rods and motile microorganisms were predominant in the periodontal pocket samples obtained from 5 patients with primary periodontal secondary endodontic lesions. Zehnder (22) reported that black pigmented anaerobic species were predominant in the periodontal pocket flora of a patient endodontically infected due to localized aggressive periodontitis. On the other hand, Kerekes et al (23) noted that similar microorganisms were present in deep periodontal pocket and in the pulp, and they mostly observed bacteriodes, fuso-

Table II: Distribution of microorganisms detected on different time points.

		A		B		C	
	N	Calculated percentage (%)		Calculated percentage (%)		Calculated percentage (%)	
Porphyromonas Gingivalis	12	7	58.3	8	66.6	7	58.3
Provetella Intermedia	12	9	75.0	9	75.0	9	75.0
Tannerella Forsythia	12	10	83.3	10	83.3	10	83.3
Campylobacter rectus	12	10	83.3	11	91.6	10	83.3
Aggregatibacter actinomicescomitans	12	5	41.6	6	50.0	4	33.3
Fusobacterium Nucleatum	12	12	100.0	12	100.0	12	100.0

Table III: Comparison of the number of microorganisms detected in different stages of the therapy.

	Time of measurement	Mean	Standard Deviation			Wilson Z Value	P
	A	1.008.332.286	2.347.530.208	A	B	-,338	0,429
Porphyromonas Gingivalis	B	713.443.114	1.308.954.218		C	-,338	٠,٢٨٦
	C	256.351.486	519.310.436	B	C	-,676	0,429
	A	984.802.511	2.084.859.735		B	-1,244	0,214
Provetella Intermedia	B	1.754.113.711	2.916.839.196	A	C	-,296	0,767
	C	335.913.444	507.125.993	B	C	-,533	0,594
	A	1.547.788.889	2.755.676.189		B	-1,718	0,086
Tannerella Forsythia	B	193.622.222	258.898.985	A	C	-1,784	0,074
	C	126.280.000	230.676.800	B	C	-,059	0,953
	A	361.980.200	1.068.159.041		B	-1,886	0,059
Campylobacter rectus	B	35.148.000	81.131.768	A	C	-1,070	0,285
	C	19.199.440	33.234.245	B	C	-,051	0,959
	A	263.120	359.844	A	B	-,944	0,345
Aggregatibacter actinomicescomitans	B	843.000	1.553.360		C	-1,826	0,068
	C	1.565.000	1.205.363	B	C	-,730	0,465
	A	3.195.758.508	3.246.503.802		B	-,549	0,583
Fusobacterium Nucleatum	B	4.500.833.333	4.782.624.723	A	C	-,471	0,637
	C	3.373.116.750	3.972.312.487	B	C	-,392	0,695

Different stages of the therapy; A: Before the initiation of the root canal therapy, B: Before the second session in which the root canal therapy was continued, C: Before the third session in which the root canal therapy was continued.

bacteria, spiroquets, wolinellas, selenomonas, camplyobacter, and peptostreptococcus. In our study, of all the samples obtained from the periodontal pockets of the teeth with periodontal endodontic lesions in 12 patients, 100% had F nucleatum, 83.3-91.6% had C rectus, 83.3% had T forsythia, 75% had P intermedia, 58.3-66.6% had P gingivalis and 33.3-50% had Aa. Saygun et al (24). reported that, of the samples obtained from periodontal pocket flora of the patients with aggressive periodontitis, 78-83% had P gingivalis, T forsythia, C rectus and 44% had P intermedia and Aa. Furthermore, It is seen that the numbers of the microorganisms detected in our study are partly consistent with these studies.

Intracanal drugs applied to pulp chamber or into the canals to obtain an optimal success may act by showing a direct contact with microorganisms or by reaching irregular intracanal spaces after being evaporized (25,26). It is reported that formocresol and 2% glutaraldehyde, iodine potassium is an excellent antibacterial intracanal drug, which shows its efficacy by being evaporated, with minimal tissue irritation and toxicity (12,13). In the study conducted by Lele et al (12) to investigate the antibacterial features of intracanal drugs during recurrent session of root canal therapy performed on primary molar teeth, the authors reported that formocresol and 2% glutaraldehyde were very efficient to reduce the number of aerobic and anaerobic microorganisms. In addition, there are also studies that report that formocresol is irritant agent for apical periodontal tissues. Breault et al (11) noted that formocresol, which was used as an intracanal antiseptic, might have an adverse effect on periodontal wound healing and regeneration. In the study performed by Yamasaki et al (15) to investigate

the effects of formocresol used after pulpectomy on periapical tissues in rats, the investigators reported a prolonged time to healing and an increased number of inflammatory cells in periapical tissues. In our study, we observed that formocresol used during the root canal therapy did not affect the number and the species of periodontal pathogens. Although formocresol shows an antibacterial effect in the canals, this may be resulting from the lack of an adequate evaporation to affect periodontal pocket flora in the teeth with periodontal-endodontic lesions.

Conclusion

Consequently, this study demonstrated that, in root canal therapy, the procedures performed without the administration of a intracanal drug or the use of intracanal drug, which contained formocresol applied in the pulp chamber, did not provide short-term benefits for periodontal pocket flora. It is thought that long-term microbial studies are needed.

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