The Evaluation Of Antimicrobial Efficacy Of Antibiotic Canal Pastes Used In Regenerative Endodontic Treatments

Rejeneratif Endodontik Tedavilerde Kullanılan Antibiyotikli Patların Antimikrobiyal Etkinliklerinin Değerlendirilmesi

Seçil ÇALIŞKAN*, Mustafa AYDINBELGE**

*PhD, DDS, Department of Pediatric Dentistry Kayseri, Turkey.

**PhD, DDS, Assist Prof, Erciyes University Faculty of Dentistry , Department of Pediatric Dentistry Kayseri, Turkey.

Abstract

In the review of literature, there was no study giving a comparative evaluation of the effectiveness of the most popular ones of antibiotic combinations ((Triple Antibiotic Paste- (Ciprofloxacin-Metronidazole-Minocycline Mixture (TAP) (1: 1: 1 ratio), Double Antibiotic Paste- (Ciprofloxacin-Metronidazole (DAP)) (1: 1 ratio), and Modified Triple Antibiotic Paste - (Ciprofloxacin-Metronidazole-Cefalexin (mTAP)) (1: 1: 1 ratio)) on microorganisms. The aim of this study was evaluate the efficacy of several antibiotic paste combinations on pathogenic microorganisms that would be isolated from oral microflora and root canal in our study. Microorganisms were used in this study; Candida albicans (C. albicans ATCC 10231), Enterococcus faecalis (E. faecalis ATCC 29212), Escherichia coli (E. coli ATCC 25922), Streptococcus mutans (S. mutans ATCC 25175), Staphylococcus aureus(S. Aureus ATCC 25923), Pseudomonas aeruginosa (P. aeruginosa ATCC 15442). In each experimental period, the same microorganism suspension was planted in ten different media, the test discs were placed and the plates were elevated to the oven for incubation at 37 °C. After a 24-hour incubation, plaques were removed from the oven and the inhibition zones around the discs were noted with help of transparent ruler, measured in millimeters.One-way analysis of variance was used for independent groups in the comparison of the groups, and Tukey test was used in multiple comparison tests (p <0,05). No statistically significant differences were found between medicaments in antimicrobial activity against bacteria other than E.faecalis.

Key words: Antibiotic Canal Pastes, Antimicrobial Efficacy, Regenerative Endodontic Treatments.

Özet

Yapılan literatür taramasında antibiyotik kombinasyonlarından en populerleri olan Üçlü Antibiyotik Patı-(Ciprofloksasin-Metronidazol-Minosiklin Karışımı (TAP) (1:1:1 oranında), İkili Antibiyotik Patı-(Ciprofloksasin-Metronidazol(DAP))(1:1 oranında) ve Modifiye Üçlü Antibiyotik Patı -(Ciprofloksasin-Metronidazol-Cefaleksin)(1:1:1 oranında) ' nın mikroorganizmalar üzerindeki etkinliklerini karşılaştırmalı olarak değerlendiren çalışmalara rastlanmamıştır. Bu çalışmanın amacı oral mikrofloradan ve kök kanalından izole edilebilecek patojen mikroorganizmalar üzerine çeşitli antibiyotikli pat kombinasonlarının etkinliklerinin değerlendirilmesidir. Çalışmamızda kullanılan mikroorganizmalar Candida albicans (C. albicans ATCC 10231), Enterococcus faecalis (E. faecalis ATCC 29212), Escherichia coli (E. coli ATCC 25922), Streptococcus mutans (S. mutans ATCC 25175), Staphylococcus aureus(S. Aureus ATCC 25923), Pseudomonas aeruginosa (P. aeruginosa ATCC 15442) suşlarıdır. Her deney periyodunda aynı mikroorganizma süspansiyonundan on farklı besiyerine ekim yapılmış, test edilen diskler yerleştirilmiş ve plaklar 37°C inkübe edilmek üzere etüve kaldırılmıştır. İnkübasyonun 24. saatlerinde plaklar etüvden çıkarılmış, şeffaf bir cetvel yardımı ile disklerin çevresindeki inhibisyon zonları milimetrik olarak ölçülerek not edilmiştir.Grupların karşılaştırılmasında bağımsız gruplarda tek yönlü varyans analizi kullanıldı. Çoklu karşılaştırma testlerinde Tukey testi kullanılmıştır (p<0,05). Enterococcus Faecalis dışındaki bakterlere karşı olan antimikrobiyal etkinlikte medikamanlar arasında istatistiksel olarak anlamlı farklılık tespit edilmemiştir.

Anahtar Kelimeler: Antibiyotikli Kanal Patları, Antimikrobiyal Etkinlik, Rejeneratif Endodontik Tedaviler.

İletişim Adresi
Dr. Seçil ÇALIŞKAN
Erciyes Üniversitesi Diş Hekimliği Fakültesi
Pedodonti Anabilim Dalı, KAYSERİ
Email: sclctn@hotmail.com

INTRODUCTION

The complex internal structure of root canals provides the environment for reproduction and proliferation of microorganisms that play a role in pulp diseases (1, 2).

The fact that the numbers and diameters of the dentin canals in crown and root portions of the teeth are capable of allowing the passage of microorganisms or microorganism byproducts, this reveals the relationship of tissues to periapical pathology (3). Pathogenecity of bacterial infection in periapical tissues; together with the resistance of the organism, depends on the activations of microorganism in the dentin canals for the continuation of the infection, and the transport of this microorganisms through the root canals (4).

In addition the diffuculties in controlling endodontic persistent infections require the application of intracanal medicaments so as to stop infection following biomechanical preparation (5).

Today there is no ideal medicine for root canal treatment, but the most commonly used intracanal agent is calcium hydroxide (6, 7). Calcium hydroxide is found suitable for use by investigators because it has antibacterial properties due to high ph, also it has tissue – solubilizing properties; it stops root resorption, accelerate the repair process, and stimulates hard tissue formation (8)

Since the ph of calcium hydroxide is 12.5, most of the bacterial species frequently found in infected root canals become ineffective shortly after they have been directly contacted with this substance. The antibacterial activity of calcium hydroxide is associated with the release of hydroxyl ions in aqueous media. Hydroxyl ions affect bacteria by causing cytoplasmic membrane damage, protein denaturation and dna damage. However, the bacteria housed in root canals which are difficult to reach due to complex canal structures, such as deep dentin canals, lateral canals, stenosis, and irregularities, neutralize the alkaline ph in such teeth and proliferate over time in these areas which remain free from the effect of calcium hydroide (9). For this reason, recently, antibiotic compounds which can not only reach the periodotal tissues and the periapical lesions but also diffuse through cement and dentin, have begun to be used in canal treatments (10, 11)

In the review of literature, however, there was no study giving a comparative evaluation of the effectiveness of the most popular ones of antibiotic combinations((Triple Antibiotic Paste- (Ciprofloxacin-Metronidazole-Minocycline Mixture (TAP) (1: 1: 1 ratio), Double Antibiotic Paste- (Ciprofloxacin-Metronidazole (DAP)) (1: 1 ratio), and Modified Triple Antibiotic Paste - (Ciprofloxacin-Metronidazole-Cefalexin (mTAP)) (1: 1: 1 ratio)) on microorganisms.

From this point, it is aimed to evaluate the efficacy of several antibiotic paste combinations on pathogenic microorganisms that would be isolated from oral microflora and root canal in our study.

MATERIAL AND METHOD

Microorganisms used in study

The strains used in this study were sourced from National Collection of Type Cultures, Central Public Health Laboratory, and obtained from culture collection of Ankara Refik Saydam Hıfzısıha Institute.

The following lyophilized microorganisms were used in this study; Candida albicans (C. albicans ATCC 10231), Enterococcus faecalis (E. faecalis ATCC 29212), Escherichia coli (E. coli ATCC 25922), Streptococcus mutans (S. mutans ATCC 25175), Staphylococcus aureus(S. Aureus ATCC 25923), Pseudomonas aeruginosa (P. aeruginosa ATCC 15442)

Preparation of Experimental Materials

In our study, the agents used for evaluating antimicrobial effects were placed in sterile conditions in a safety cabinet according to the manufacturer’s instructions and placed in round sterile polyethylene molds with an internal diameter of 5 mm and a thickness of 2 mm, disc-shaped medicament groups were obtained. Just before each experiment period, 3 fresh medicinal discs were prepared and used from each material for each microorganism.

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Implementation of Medicament Applications and Agar Diffusion Test

20 µl samples for each microorganism were prepared from stock bacterial suspensions prepared in accordance with the routine production of lyophilized bacterial strains to be in Mcfarland 0.5 standard blend in sterile 0,9% saline, S. mutans and S. aureus, E. faecalis, E. coli, and P. aeruginosa were transferred to Mueller Hinton agar medium, C. albicans RPMI agar medium on 5% sheep blood agar media, and the sterilized media was uniformly spread over the entire surface of the media with a sterile buoyancy rod.

Following these procedures, the covers of petri dishes that contain the media were closed, after plates were left at room temperature for 15 minutes, different medicament discs to be tested were placed in groups of 3, equidistant from each other, and the plates were placed in previously opened wells with diameters of 5 mm and depths of 2 mm.

In each experimental period, the same microorganism suspension was planted in ten different media, the test discs were placed and the plates were elevated to the oven for incubation at 37 °C. After a 24-hour incubation, plaques were removed from the oven and the inhibition zones around the discs were noted with help of transparent ruler, measured in millimeters (figure 1, 2, 3, 4, 5, 6). The study was repeated three times using four batches for each standard bacterium each time.

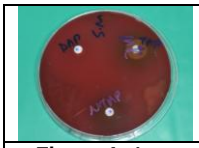

Figure 1. Agar diffusion test 24 h (Streptococcus mutans)

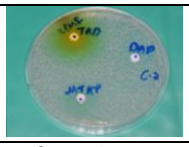

Figure 2. Agar diffusion test 24 h (Candida albicans)

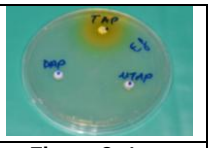

Figure 3. Agar diffusion test 24 h (Enterococcus faecalis)

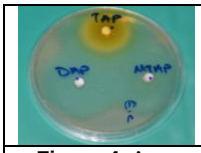

Figure 4. Agar diffusion test 24 h (Escherichia coli)

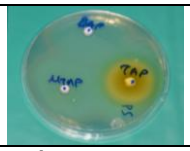

Figure 5. Agar diffusion test 24 h (Pseudomonas aeruginosa)

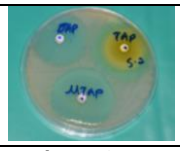

Figure 6. Agar diffusion test 24 h (Staphylococcus aureus)

Data Analysis

One-way analysis of variance was used for independent groups in the comparison of the groups, and Tukey test was used in multiple comparison tests ($p < 0,05$).

RESULTS

In our study, the averages of the diameters of the inhibition areas of antimicrobial solutions on microorganisms are shown in Table 1.

	GROUPS			
BACTERIA	TAP $\bar{x} \pm ss$	DAP $\bar{x} \pm ss$	MTAP $\bar{x} \pm ss$	P
Pseudomonas aeruginosa	40,0±0,0	40,0±0,0	40±0,0	-
Escherichia coli	43,0±0,0	43,0±0,0	40±0,0	-
Staphylococcus aureus	36,0±0,0	30,0±0,0	42±0,0	-
Streptococcus mutans	46,0±0,0	48,0±0,0	46±0,0	-
Enterococcus faecalis	37,0±0,0	30,0±0,0	35,1±0,2	0,004
Candida albicans	23,0±00	0,0	10±0,0	-

Table 1. Table of antimicrobial activity values of the groups. (Values are expressed as standard deviation and arithmetic mean)

No statistically significant differences were found between medicaments in antimicrobial activity against bacteria other than E.faecalis. Activity sequencing for E. faecalis is TAP> mTAP> DAP (p=0.004). C. albicans, no activity was observed for DAP while the highest value was again in the TAP group.

DISCUSSION

Various materials and combinations have been tested for the 3D elimination of microorganisms from the root canal due to the polymicrobial nature of endodontic infections. In their study of 2014 in which they assessed the antimicrobial activity of calcium hydroxide clorhexidine and TAP against E. faecalis, Mozayani et al. (12), reported that they obtained the highest inhibitory effect with TAP. This finding, which is is in agreement with that of our study, has been associated in studies where extracted human teeth have been used with TAP's penetrating dentin better than calcium hydroxide and chlorhexidine gluconate (12). Similar to our study, Ald et al. (13), who used the agar diffusion method, reported similar results.

In another study in which propolis, TAP, chlorhexidine gluconate and , calcium hydroxide evaluated antifungal effects using the infected dentin model, at the end of day 1, all medicaments with the highest effect chlorhexidine gluconate and subsequent calcium hydroxide were reported to have a high antifungal effect (14). At the end of day 7, although the highest value was chlorhexidine gluconate, there was no significant difference with another group (14). In view of this situation, we can come to conclusion that with the increase of time, medicaments show similar efficacy.

Although antibiotics do not have antifungal activity, metronidazole and ciprofloxacin exhibit antimicrobial activity by contributing to the synthesis of extracelluler matrix and the collagen of fibroblasts by inhibiting the protein synthesis on the ribosomes due to minoxycline in TAP (15, 16).

It has been proven that TAP penetrates dentin tubules and is effective against anaerobic, gram-positive and gram-negative microorganisms (17). In addition to creating an aseptic environment, Tap also accelerates the functional development of the pulp-dentin complex by contributing to periapical healing and repair (18).

Alizera et al (13) reported that TAP against E. faecalis exhibited better antimicrobial activity than calcium hydroxide, as in our results. And another study showed that TAP was significantly more effective against E. faecalis than DAP (19). Also according to the study published in 2017 used different experimental method, TAP group showed a moderate antibacterial effect (20). In addition, strong antimicrobial efficacy against E. faecalis has been observed in all three antibiotic patstes (TAP, DAP and mTAP) in view of the results of our study. When we evaluate the results for C. albicans, the best antifungal activity is TAP followed by mTAP. In the DAP group, strains were resistant to C. albicans, and we think that this difference is caused by minocycline in TAP. An important study done by Sato et al. (21) to evaluate antimicrobial activities is published reports that the efficacy against endodontic pathogens was also preserved when testing TAP and DAP concentrations at 1mg/ml less.

Also in our study, unlike the other studies, TAP, DAP and mTAP's antimicrobial activities against P. Aeruginosa, E. coli, S. aureus, S. mutans's strains were evaluated and similar positive results were obtained in all groups.

We are of the opinion that our study findings, while making an important contribution to the study of the antimicrobial efficacy of various antibiotic combinations used today in regenerative endodontic applications, should be supported with more comprehensively planned studies done using more extensive bacterial strains and different methods in ex-vivo and in-vivo conditions.

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