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The role of intracanal medicaments in inhibition of bacteria isolated from root canals of infected primary molars

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Abstract

Microbes are considered as the primary etiologic agents in endodontic diseases. Disinfection of the root canal is obtained by the combined effect of biomechanical preparation, irrigation and intra canal medicament. The aim of the present study was to assess the antimicrobial activity of intracanal medicaments (formocresol and Endosepton) against two micro organisms (Streptococcus mutans and staphylococcus aureus) isolated from 15 necrotic pulps of primary molars indicated for pulpectomy procedure. The samples were cultured, and purified using microbiological evaluation.

Broth dilution test was performed in our study by preparing test tubes containing 10 ml of BHI broth (pH. 7) which then inoculated with strains of the tested bacteria and incubated at 37 C° for 24 h. After over night incubaction, ten fold dilution were made in test tubes containing 9 ml of normal saline by adding 1 ml of the inoculum to the first tube . Then from dilution 10^{-1} , 0.1 ml of cell suspension was added to 9.9 ml of formocresol and endosepton, then 0.1 ml was taken and spread on duplicates of BHI agar plates at different intervals and incubated aerobically for 24 h. at 37 C°. Colonies on the plates were counted after incubation and CFU/mL (colony forming unit) was calculated. Our results indicating that there were no significant differences between the intracanal medicaments, but there were high significant differences between the intervals time of the study. We concluded that both materials had great antibacterial effect against the pathogens commonly isolated from necrotic pulpal tissue of primary teeth.

Keywords: Endodontic diseases, intra canal medicament, pulpectomy, endodontic pathogens.

Introduction

Endodontic treatment of primary teeth with necrotic pulp is routine in dental practice, and its success depends on many factors and the reduction or elimination of bacterial infection is the most important one. Pulpectomy is indicated in teeth that show evidence of chronic inflammation or necrosis in the radicular pulp, with or without periapical or furcation pathology[(1)].

The persistence of bacteria in the root canal system after endodontic treatment may cause persistent inflammation in the periradicular tissue and often leads to failure, so the control of infection is fundamental

because the ample medullary bone spaces favor dissemination of infection and also because the developing permanent tooth germ is very close to the roots of the primary teeth[2,3].

Numerous measures have been introduced to reduce the infection from the root canal system, including various mechanical instrumentation techniques, irrigation regimes, and intracanal medications[4].

Chemical disinfection is necessary to remove microorganisms, their byproducts, pulp tissue remnant, and other debris from the root canal because it is difficult to eliminate all microorganism from an infected root canal system by mechanical instrumentation alone[5],Thus, for optimal success, it becomes imperative to place intracanal medicaments within the pulp chamber or canals, which exert their antimicrobial effect by direct contact with the organisms or by way of vapor action of the volatile components that reaches all the irregularities within the canals to eliminate the endodontic infection and the microbial proliferation in the root canal system and neutralize the bacterial endotoxin in teeth with pulp necrosis[6]. Of these, formocresol, is reported to have excellent antimicrobial activity and vapor-forming effect with minimal toxicity and tissue irritation, This medication acts both by direct contact and at distance[7] . Endosepton is a solution for root canal disinfection and intracanal medication which offers a triple-action bactericidal, sedative and anti-inflammatory [8]

Hence the present study was aimed at evaluating and comparing the antibacterial efficacy of these intracanal medicaments in primary teeth against two bacterial strains commonly found in endodontic infections and obtained directly from necrotic root canals of primary teeth using aerobic methodology.

Material and method

In this clinical laboratory study, 15 necrotized primary teeth of consecutive patients whose ages ranged from 5-7 years were selected after approval from institutional review board, College of Dentistry/ Al- Mustansiria University, who gave ethical clearance to conduct the study.

Inclusion Criteria

*Healthy children without any systemic illness.

* None of these patients were treated with antibiotics during or prior to sampling.

*Presence of abscess, sinus tract or obvious radiolucency.

*Maxillary or Mandibular second primary molar was selected.

When pulp vitality test completed anaesthetization, Rubber dam isolation was carried out and the operative fields were cleaned with 30% hydrogen peroxide followed by a 2.5% NaOCl[9]

An access cavity was created using a high speed hand piece and turbine diamond bur, after opening the pulp chamber, the tooth was dried using sterile absorbent cotton and a sterile k-file was introduced inside the canal to made an access to the root canal, then two samples were taken of each canal with sterile paper points proportionate to the canal size up to the working length or to the level of physiological root resorption and kept in place for 60 seconds one after the other. The root canal samples were dropped immediately into a screw capped tube containing thioglycolate broth and submitted to microbiological laboratory for incubation at 37 C° for 24-48 hours[10]

In the present study mutans streptoccoi and staphylococcus aureas

isolates purified and diagnosed according to the morphological characterstic culture and biochemical test, morphological examination of microbial cells was done by using gram stain.

Culture characterstics:

Mitis Salivarius Agar (MSA) was used as the selective media for Stre. Mutans , while Mannital salt agar and blood agar for Staph. Aureus.

Idntification by biochemical test (catalase production test, carbohydrate fermintation test, and coagulase test), then after purification the isolation of pure culuture were done by streaking on brain infusion agar (BHI) and incubated for 24 h. at 37 C°, and the several replicates of each isolated bacteria were subcultured for the conducted expermint.

Broth dilution test was performed in our study by preparing test tubes containing 10 ml of BHI broth (pH. 7) which then inoculated with strains of the tested bacteria and incubated at 37 C°for 24 h. After over night incubaction, ten fold dilution were made in test tubes containing 9 ml of normal saline by adding 1 ml of the inoculum to the first tube . Then from dilution 10^{-1} , 0.1 ml of cell suspension was added to 9.9 ml of the tested formocresol and endosepton, then 0.1 ml was taken and spread on duplicates of BHI agar plates at different intervals and incubated aerobically for 24 h. at 37 C°. All the experment were performed in triplicates.The viable cell count was estimated by counting the number of colonies on the agar plate multiplied by the dilution factor.

Results

The results shown in the figure (1) indicates that the effect of Endoseptone on the both tested bacteria was nearly the same during the first 30 minutes then complete reduction of Strep. after 60 min.,while the cell count of Staph. was completely reduced after 24 hours.

The result in the Fig. (2) showed the effect of formocresol which demonstrates that in the first 30 min. there was a good reduction in the bacterial count, with a little more effect on Strep. than on Staph. after 60 min., but the complete reduction occur after 24 hours.

Tab.(1) showed that there were high significant effectiveness of form. on Sterp. and Staph. growth between the intervals. In case of Endosepton the analysis showed the same results (tab.2)

The comparison between the effects of the materials used demonstrated that there were non significant difference on the tested bacteria during the time of the experiment, except at 60 min. on Strept.the difference was high significant (tab.3)

Disscussion

Microorganisms and their byproducts are considered to be the primary etiologic agents in endodontic diseases. True endodontic pathogens or those associated with therapy-resistant cases were selected as test bacteria for this experiment [11,12] Failure, during and after endodontic treatment are linked to the presence of bacteria in the root canal. This result hence emphasizes the importance of completely eliminating the bacteria from the root canal system. The most effective ways to achieve this aim are by means of instrumentation, irrigation, and use of intracanal medication [13].

The bacteriologic samples were collected with the help of sterile paper points as per the sampling technique described by Grossmanin[14] ,in which the transfer from the canals to the test

tubes took a few seconds and most strains recovered from infected root pulps are known to tolerate substantial oxygen exposure. The time that elapsed between sampling of the root canals and culturings in the laboratory was always less than 8 hours since this period is considered adequate for maintaining the viability of the microorganisms[15].

Formocresol (FC) was first used as a root canal medication by Buckley in 1904. It is widely used in dentistry because of its antibacterial properties in root canal disinfection, it contains formaldehyde, an effective alkylating agent, and cresol, a protein-coagulating phenolic compound. Its action is believed to be due to the release of formaldehyde vapors which act as a germicidal agent [16, 17].In the present study, a significant reduction in the bacterial counts was verified in the FC group, in agreement with studies conducted by Ohara et al.

Endosepton antibacterial effect attributed to its content of camophor, chlorophenol which are derivatives of phenol that considered as an oldest compound for controlling microorganism and is used in the form of vapor forming intracanal medicaments. Phenol is a protoplasmic poison and produces necrosis of soft tissues by its ability to penetrate and disrupt the cell wall of bacteria and subsequently the protoplasm. The results revealed great effect of both intracanal medicaments on Staph. aureus and Strep. mutenus after 1, 30, 60 min. with complete reduction after 24 hours which gives an indication that its maximum effect can reached after 24 hours that means keeping the canals antiseptic for the subsequent appointment.

According to the present results there were high significant difference between the times of our experiment for both medicaments and on both tested bacteria which proves the effectivity of these medicaments over the experiment periods of time, and the difference between the medicaments were non significant during the study, this finding gives a clue that both medicaments were nearly the same in their effect, with exception at 60 min for sterp. Mutans the difference was highly significant .

In general the

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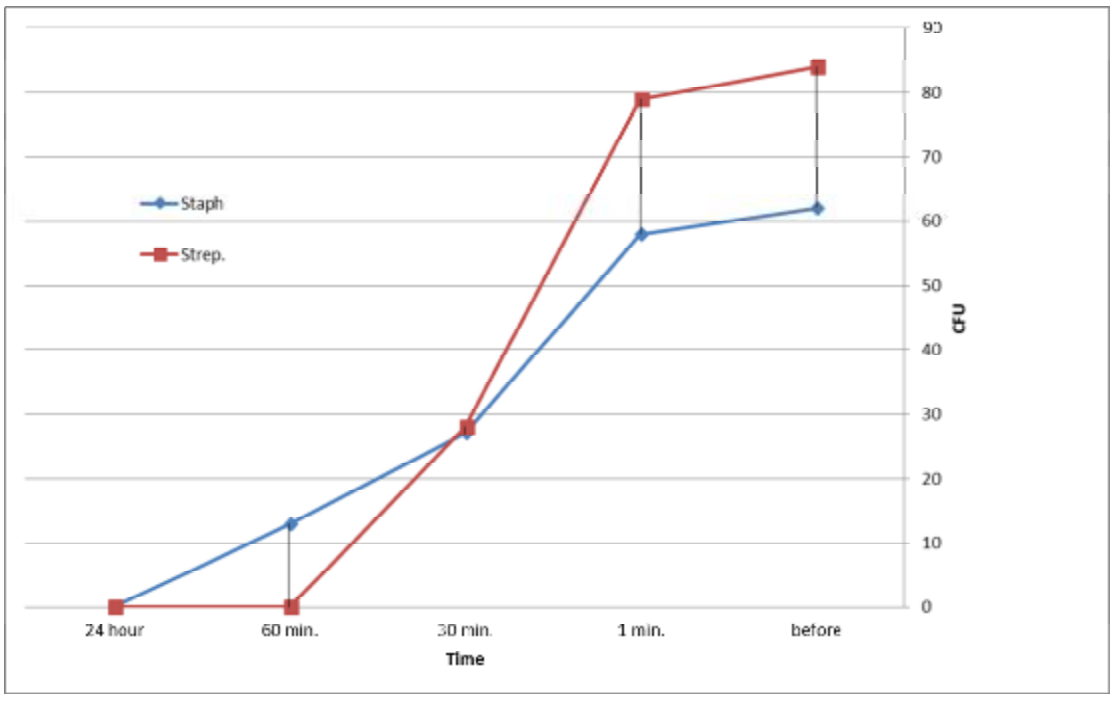

Figure 1. Effect of Endosepton on Staph.aureus and Strept. Mutans represented by CFU counts at different time intervals.

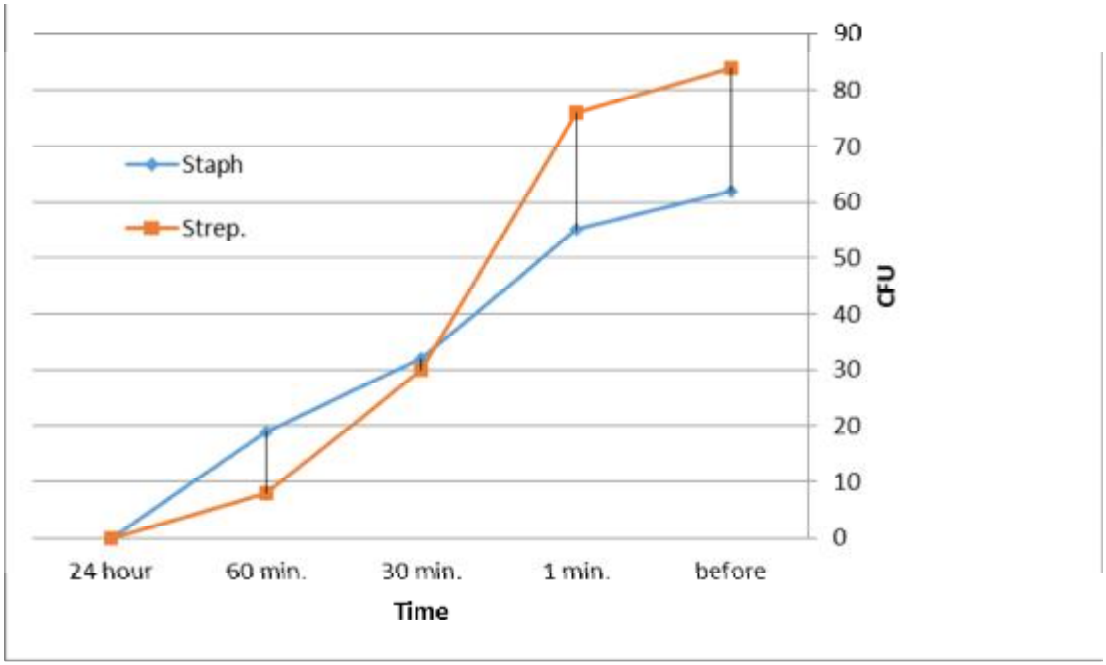

Figure 2. Effect of Formocresol on Staph.aureus and Strept. Mutenss represented by CFU counts at different time intervals.

Table (1) A comparison of the antibacterial effect of Formocresol between the intervals time on the tested bacteria

Time	Staph			Strep.		
	z-test	p-value	Sig.	z-test	p-value	Sig.
1 & 30 min.	4.51	0.000	H.S	7.35	0.000	H.S
1 & 60 min.	6.59	0.000	H.S	10.49	0.000	H.S
1 min. & 24 hours.	9.94	0.000	H.S	11.78	0.000	H.S
30 & 60 min.	2.37	0.009	H.S	4.06	0.000	H.S
30 min. & 24 hours.	6.57	0.000	H.S	6.04	0.000	H.S
60 min. & 24 hours.	4.74	0.000	H.S	2.89	0.002	H.S

N.S: Non Significant difference at level P>0.05.
H.S: Highly Significant difference at level P<0.01.
S: Significant difference at level P<0.05.

Table (3) A comparison between Formocresol and Endosepton on the tested bacteria at time intervals

Time		Staph			Strep.		
		z-test	p-value	Sig.	z-test	p-value	Sig.
1 min.	Formocresol & Endoseptone	0.95	0.171	N.S	0.87	0.1922	N.S
30 min.	Formocresol & Endoseptone	0.90	0.184	N.S	0.32	0.375	N.S
60 min.	Formocresol & Endoseptone	1.23	0.109	N.S	2.90	0.002	H.S
24 hours.	Formocresol & Endoseptone	Non			Non		

Table (2) A comparison of the antibacterial effect of Endosepton between the intervals time on the tested bacteria

Time	Staph			Strep.		
	z-test	p-value	Sig.	z-test	p-value	Sig.
1 & 30 min.	5.99	0.000	H.S	8.18	0.000	H.S
1 & 60 min.	8.17	0.000	H.S	12.21	0.000	H.S
1 min. & 24 hours.	10.44	0.000	H.S	12.21	0.000	H.S
30 & 60 min.	2.69	0.004	H.S	5.79	0.000	H.S
30 min. & 24 hours.	5.88	0.000	H.S	5.79	0.000	H.S
60 min. & 24 hours.	3.81	0.000	H.S	Non		

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24 hours.	Formocresol & Endoseptone	Non			Non		