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ANTIBACTERIAL ACTIVITY OF INTRACANAL MEDICAMENTS AGAINST BACTERIAL ISOLATES IN CASES OF ACUTE PERIAPICAL PERIODONTITIS (NONEXUDATIVE FORM)

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SUMMARY:

The presence of microorganisms and derivative toxins in the infected root canals determine the changes that occur in the apical periodontium.

The aim in this in vitro study has been to evaluate the antimicrobial activity of the intracanal medicaments against bacterial isolates in cases with acute periapical periodontitis.

The following clinical isolates from cases with acute periodontitis (nonexudative form) were used in the present study: *Streptococcus pyogenes, Streptococcus mitis, Streptococcus bovis, Streptococcus anginosus, Enterococcus faecalis, Staphylococcus aureus.* We tested their sensitivity to some of the most frequently used intracanal medicaments (in vitro), i.e.: Calcium hydroxide paste (Calcident 450® W/P Dental, Germany), Sodium hypochlorite 3% (Switzerland), Metronidazole 0.5% (Troya Pharm, Bulgaria), Chlorhexidine gluconate 0.1% (Troya Pharm, Bulgaria), and a combination of Sodium hypochlorite 3% and Metronidazole 0.5%.

The results show that Calcium hydroxide had the strongest impact in direct contact with the studied microorganisms, followed by Chlorhexidine gluconate 0.1% and Sodium hypochlorite 3%. The combination of Sodium hypochlorite 3% + Metronidazole 0.5% is more active than Metronidazole 0.5% used on its own.

Key words: acute periapical periodontitis, bacterial isolates, intracanal medicaments

INTRODUCTION

The presence of microorganisms and derivative toxins in the infected root canals cause the development of apical periodontitis. A number of studies (2, 6, 8, 10, 12) show that the endodontic infection is poly-microbial, and that in teeth with necrotic pulp it is represented by obligatory and facultative anaerobes, microaerophilic bacteria and fungi.

An important stage in the successful endodontic therapy is the treatment of the microflora with suitable solutions for irrigating the endodont (endodontic irrigants), both during the biomechanical preparation of the root canal and between the visits.

Sodium hypochlorite (NaOCl) in various concentrations is a frequently recommended endodontic irrigant in contemporary endodontics (5, 7, 11, 14).

Chlorhexidine (Chlorhexidine gluconate) is an alternative irrigant. During the last decade it has become widely used in endodonic practice as an effective antimicrobial means for irrigating the endodont (1, 7, 12).

To remove the microorganisms from some root canals that have been subjected to an extensive exposure to infection, long-term treatment medicinal inlays should be used (i.e. Potassium hydroxide, etc).

The aim of the present study has been formulated on the basis of the above sources and previous results of our research.

AIM:

The aim in this study has been to establish in vitro the antimicrobial activity of various intracanal medicaments against microorganisms isolated from cases with acute periapical periodontitis.

MATERIALS AND METHODS:

The following clinical isolates from cases with acute periodontitis (nonexudative form) have been used in the present study: *Streptococcus pyogenes, Streptococcus mitis, Streptococcus bovis, Streptococcus anginosus, Enterococcus faecalis, Staphylococcus aureus.*

We tested their sensitivity to some of the most frequently used intracanal medicaments (in vitro), i.e.: Calcium hydroxide paste (Calcident 450® W/P Dental, Germany), Sodium hypochlorite 3% (Switzerland), Metronidazole 0.5% (Troya Pharm, Bulgaria), Chlorhexidine gluconate 0.1% (Troya Pharm, Bulgaria), and a combination of Sodium hypochlorite 3% and Metronidazole 0.5%.

The agar diffusion method was applied, using Petri dishes with Muller-Hinton blood agar (Merck Darmstadt) that were inoculated with bacterial suspension of the strains (Brucella blend, density 0.5 Mc Farland).

In each agar Petri dish five cavities were made and filled with the respective medicaments. The experimental plates were pre-incubated 30 minutes at room temperature and then incubated at 37ºC for 24 to 48 hours. In order to establish the antimicrobial activity of the tested substances, the zone of bacterial inhibition of each medicament was measured and recorded (in millimeters).

The data was entered and processed with the statistical package SPSS 12.0.1. The significance level for rejecting of the null hypothesis was $p<0.05$.

RESULTS

Diagrams 1-6 present the arithmetic means of the diameters of the activity zones /in mm/ of the studied medicaments in the cases of the different microorganisms.

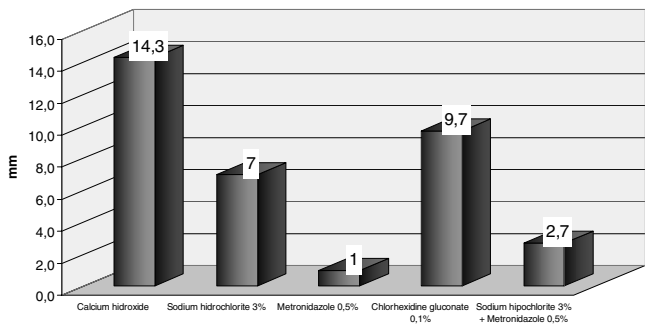

Diagram 1: Means of the diameters of the activity zones /in mm/ with Streptococcus pyogenes

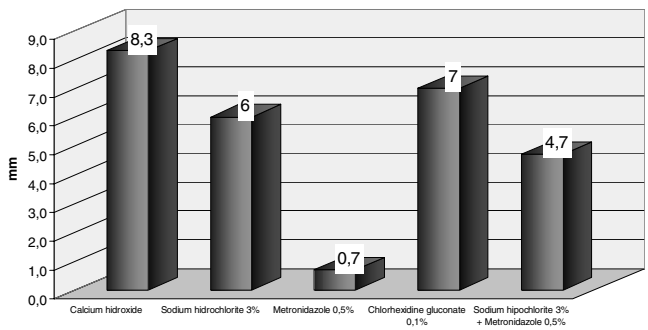

Diagram 2: Means of the diameters of the activity zones /in mm/ with Streptococcus mitis

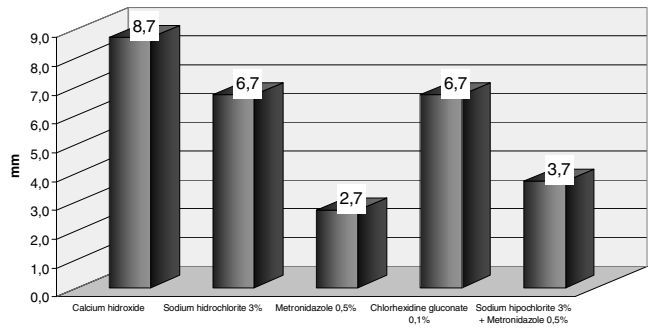

Diagram 3: Means of the diameters of the activity zones /in mm/ with Streptococcus bovis

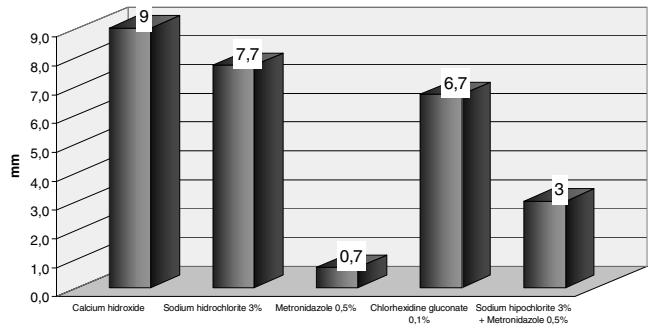

Diagram 4: Means of the diameters of the activity zones /in mm/ Streptococcus anginosus

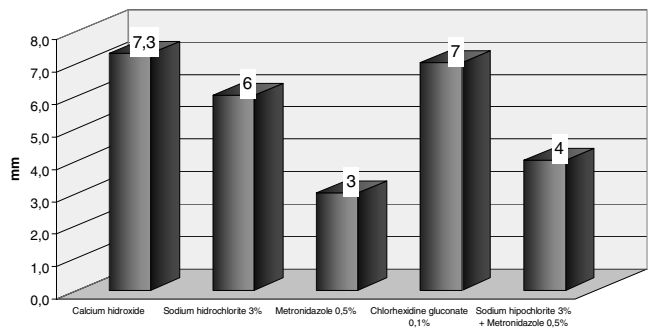

Diagram 5: Means of the diameters of the activity zones /in mm/ with Enterococcus faecalis

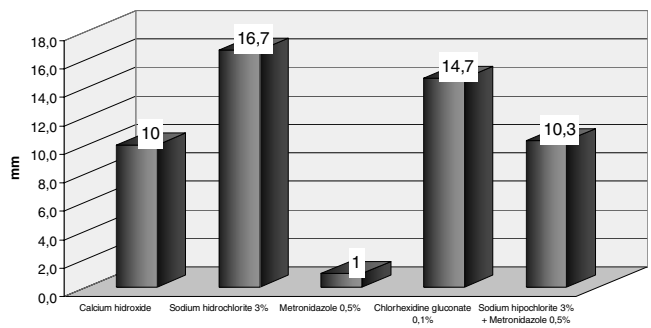

Diagram 6: Means of the diameters of the activity zones /in mm/ with Staphylococcus aureus

As Table 1 shows, Calcium hydroxide has the strongest influence against all microorganisms. This medicament has its largest activity zone in the case of Streptococcus pyogenes (14.33 mm), and its smallest zone in the case of Enterococcus faecalis (7.33 mm). Against Staphylococcus aureus the most effective medicament has been Sodium hypochlorite 3% (16.67 mm), while the leading medicament against other strains of microorganisms – Calcium hydroxide – is only in penultimate position.

Metronidazole 0.5% has the weakest impact on all microorganisms. If combined with Sodium hypochlorite 3% its impact increases slightly.

With almost all microorganisms Sodium hypochlorite 3% and Chlorhexidine gluconate 0.1% have an intermediate impact. These medicaments have the largest activity zone only in the case of Staphylococcus aureus (see Table 1).

Table 1: Arithmetic means of the diameters of the zones of activity (in mm) of the studied medicaments with each individual microorganism

Microorganism → Medicament ↓	**Streptococcus pyogenes**	**Streptococcus mitis**	**Streptococcus bovis**	**Streptococcus anginosus**	**Enterococcus faecalis**	**Staphylococcus aureus**
Calcium hydroxide	14,33^{a}	8,33^{a}	8,67^{ac}	9,00^{a}	7,33^{a}	10,00^{a}
Sodium hypochlorite 3%	7,00^{b}	6,00^{ac}	6,67^{a}	7,67^{a}	6,00^{ac}	16,67^{bd}
Metronidazole 0.5%	1,00^{c}	0,67^{b}	2,67^{bc}	0,67^{b}	3,00^{bc}	1,00^{ce}
Chlorhexidine gluconate 0.1%	9,67^{b}	7,00^{ac}	6,67^{ac}	6,67^{ac}	7,00^{a}	14,67^{ade}
Sodium hypochlorite 3% + Metronidazole 0.5%	2,67^{c}	4,67^{c}	3,67^{bc}	3,00^{bc}	4,00^{ac}	10,33^{a}

* - Identical letters show the lack of significant difference, while different letters show that there is a difference ($p < 0,05$).

DISCUSSION

Sodium hypochlorite has a wide-ranging antimicrobial activity. It is effective against vegetative bacteria, sporangial bacteria, fungi, protozoans and viruses. By using different concentrations of NaOCl a substantial decrease in the bacterial count is achieved (5, 7, 11, 14). Despite being highly effective in extinguishing the bacteria, it does not penetrate well into the difficult-to-access segments of micro-canal system of the dentine. In addition, it remains in the canal for a short period of time. Still, the use of larger quantities of the irrigant may be assumed to lead to extinguishing larger quantities of cells of difficultly influenced microorganisms, such as E. faecalis. In the present study when applied to Staphylococcus aureus, this endodontic irrigant produced the largest area of activity (16,67 mm), followed by Chlorhexidine gluconate 0,1%.

Chlorhexidine belongs to the group of the bi-guanidine antiseptics. These are antibacterial substances that act on the surface; they are easily adsorbed by the tissues and of negligible toxicity. Chlorhexidine has a wide range of activity. It is important because of its long-term disinfecting action, resulting from adhesion to the dentine and duration of the emission for about seven days (1, 7, 12).

Our results show that the medicaments Sodium hypochlorite 3% and Chlorhexidine gluconate 0,1% have an impact of medium power on almost all microorganisms.

Other authors (1, 11, 12) have studied the effect of Chlorhexidine as endodontic irrigant and intracanal medicament and have established that it has an antibacterial activity comparable to that of NaOCl, and that it is effective against strains resilient to Calcium hydroxide. It has a large spectrum of antibacterial activity and low toxicity. It is absorbed by the hydroxylapatite in the dentine by forming depots and has the quality of being released in time.

$Ca(OH)_2$ owes its effectiveness to its antimicrobial, anti-inflammatory and osteogenic potential. Due to its high alkalinity it has a high antibacterial activity against most bacteria found in an endodontic infection. Its high pH - 12.5 - has destructive effect over bacteria's cellular membrane and protein structure (3, 4, 9, 12, 13). The strongest effect of this medicament is found with Streptococcus pyogenes- 14,33 mm, and the weakest - with Enterococcus faecalis (7,3 mm).

Another substance used in the present study - Met-

ronidazole 0,5%, is also used as an endodontic irrigant and intracanal medicament for treating infected root canals. This medicament has demonstrated the weakest impact against the tested microorganisms. The combination of Sodium hypochlorite 3% and Metronidazole 0,5% shows greater effectiveness, probably due to the expressed antibacterial action of Sodium hypochlorite. This topic requires further research. There are publications reporting resistibility of some anaerobic organisms against Metronidazole. Such organisms are the gram-positive coccal Peptostreptococcus micros and the gram-positive rodlike Propionibacterium acnes.

Regardless of the intracanal irrigants and medicaments used, bacteria can never be completely eliminated from the root canal. In order to eradicate the bacterial cells from the root canal, the medicament has to penetrate and reach them. Many segments of the canal system of the root have the ability to retain bacteria, which makes them inaccessible for the effects of the used intracanal medicaments.

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