Antimicrobial efficacy of Metapex (Calcium hydroxide with Iodoform formulation) at different concentrations against selected microorganisms-An in vitro study

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ABSTRACT

The objective of this study was to assess, in vitro, the effectiveness of several concentrations of Metapex (0.22gm/ml, 0.022gm/ml, 0.0022gm/ml) in the elimination of selected microorganisms. Different concentrations of Metapex were prepared by dissolving it in ethanol (99.9%) Pre-sterilized Whatman paper discs, 6 mm in diameter and soaked with the test solution, were prepared and placed onto the previously seeded agar Petri plates. Plates were incubated aerobically for *Enterococcus* faecalis (E. Faecalis) and Candida albicans (C. albicans), anaerobically for *Bacteroid fragilis* (B. fragilis) and Propionibacterium acne respectively. A zone of inhibition was recorded for each plate and the results were analysed statistically. There was significant reduction in the size of zone of inhibition against P. acne, as the concentration of metapex decreases. At lower concentrations of metapex there was no zone of inhibition observed against E. Faecalis, C. Albicans and B. Fragilis. The result of this study suggested that Metapex is a potent antimicrobial agent at higher concentration.

Keywords: B. fragilis, C. albicans, E. faecalis, metapex, P. acne.

INTRODUCTION

One of the most important objectives of root canal treatment is the elimination of microorganisms from the root canal system. Although chemo-mechanical preparation of root canals is able to reduce the number of bacteria, an intracanal medicament with antibacterial action is required to maximize the disinfection of the root canal system in infected cases.[1] The need for intracanal medication increases in those cases where bacteria are resistant to routine treatment, and where the therapy cannot be successfully completed due to the presence of pain or continuing exudates.[2]

Bacteria remaining in obturated root canals may proliferate and invade ramifications, apical deltas, isthmuses, and dentinal tubules.[3,4] In these locations, bacteria remain unaffected by chemomechanical preparation and may result in persistent endodontic infections.[5] Therefore, the use of intracanal medicaments is essential to eliminate bacteria that remain after mechanical debridement.[6]

Since its introduction in 1920 (Hermann 1920), calcium hydroxide has been widely used in endodontics. It is a strong alkaline substance, which has a pH of approximately 12.5. In an aqueous solution, calcium hydroxide dissociates into calcium and hydroxyl ions. Various biological properties have been attributed to this substance, such as antimicrobial activity,[7] high alkalinity,[8] inhibition of tooth resorption[9] and tissue-dissolving ability.[10] Because of such effects, calcium hydroxide has been recommended for use as intracanal medicaments and in several other clinical situations.

Several works[11] have studied the mixture of other substances to calcium hydroxide with the purpose of improving some of its properties. Among these additional substances are vehicles that can speed up or slow down ionic dissociation, substances that aid the filling of pulpal cavity by means of their consistency, substances used as antimicrobial medium and media that enhance radiopacity.[12]

Metapex, a silicone oil-based calcium hydroxide paste containing 38% iodoform is very popular.[13]

Bacteroid fragilis (B. fragilis) is a gram negative anaerobic bacilli and is the most common bacteria found in endodontic infections.[14,15] Previous studies have shown the presence of Propionibacterium acne (P. acne) in infected root canal.[16]

Enterococcus faecalis (E. faecalis) can occur in primary root canal infections, especially in teeth with coronal leakage, although typically in low numbers[17] and also is the most common organism cultured from failed root canal therapy, with 12–90% prevalence.[18] Candida albicans (C. albicans) is the most commonly isolated fungal species in the oral cavity (30–45%).[19]

Table1-1: Mean zone of inhibition of various concentration of metapex on selected microorganisms

Metapex (conc in gm/ml)	E. faecalis	C. albicans	B. fragilis	P. acne
0.22(Group1)	26.5±1.29	10.0±0.0	27.0±0.82	26.75±0.96
0.022(Group2)	0.0±0.0	0.0±0.0	0.0±0.0	12.0±0.80
0.0022(Group3)	0.0±0.0	0.0±0.0	0.0±0.0	4.25±0.96

MATERIALS AND METHODS

The microorganism employed in this study were , two obligate anaerobic bacteria (P. *acne* ATCC 6921 and B. fragilis ATCC 25285), one anaerobic facultative bacteria (Enterococcus *faecalis* ATCC 29212) , and one Candida *albicans* (ATCC 10231). All the bacterial strains were allowed to grow in respective media like Brain Heart Infusion broth (BHI) (Difco Co, Becton Dickinson, sparks, MD) supplemented with hemin (5 mg/l) and menadione (0.5 mg/l). C albicans grown in Sabouraud's dextrose agar broth.

Antibiotic discs preparation: Antibiotic discs of 6 millimetre diameter were prepared from Whatman paper No.1 which were sterilized by hot air oven.

Antibiotic Preparation: Antibiotic solution of Metapex (calcium hydroxide with iodoform) that obtained from Meta Biomed Co. Ltd, was prepared in three different concentration from 0.22gm/ml to 0.0022gm/ml by dissolving it in ethanol (99.9%) , these solution directly poured in in-house made disc . The control discs used are normal saline disc and ethanol discs.

After revival of the organisms in about 48 hours, these organisms were adjusted to 0.5 ml McFarland scale (1.5 $X10^8$ CFU/ml) and streak on Muller – Hilton – Agar plate (MHA plate) for Enterococcus *faecalis*, Wilkinson Charles green agar plate for obligate anaerobes and Sabouraud's dextrose agar plate for Candida *albicans* . After streaking the organisms were applied to antibiotics discs and on control.

Anaerobes were incubated in sealed anaerobic jar with mixture of gas (N2, H2 and CO_2). Enterococcus *faecalis* and Candida *albicans* were incubated in CO_2 incubator. All organisms were incubated for 48 hours after which microbial zone of inhibition were observed for each group and measured in millimetres (Fig. 1 and 2).

Table-2: Comparison of effect of Metapex on E. Faecalis [0.22gm/ml =1, 0.022gm/ml=2, 0.0022gm/ml=3]

Inter group comparision	P value	Level of Significance
Group1 v Group 2	0	Significant
Group1 v Group 3	0	Significant
Group2 v Group 3	1	Non significant

Table-3: Comparison of effect of Metapex on C. albicans

Inter group comparision	P value	Level of Significance
Group1 v Group 2	0	Significant
Group1 v Group 3	0	Significant
Group2 v Group 3	1	Non significant

RESULTS

There was significant reduction in the size of zone of inhibition against P. acne, as the concentration of metapex decreases. At lower concentrations of metapex there was no zone of inhibition observed against E. Faecalis, C. Albicans and B. Fragilis. Effects of different concentration of Metapex on the zone of inhibition of bacterial population have been subjected to statistical analysis and comparison between different groups with level of significance have been detailed in Table- 1 to 5.

DISCUSSION

Metapex contains radiopaque component barium sulfate which can help to control the deposition of material when seen radiographically. Iodoform is incorporated to improve the antibacterial properties of the material. Silicone oil acts as a vehicle.

The mechanism of action of calcium hydroxide on microorganisms can be explained by the influence of pH on growth, metabolism and bacterial cell division. It is believed that the hydroxyl ions from calcium hydroxide develop their mechanism of action in the cytoplasmic membrane, because enzymatic sites are located in the cytoplasmic membrane. This membrane is responsible for essential functions such as metabolism, cellular division and growth and it takes part in the final stages of cellular wall formation, biosynthesis of lipids, transport of electrons and oxidative phosphorylation.[20,21] The pH gradient of the cytoplasmic membrane is altered by the high concentration of hydroxyl ions of calcium hydroxide acting on the proteins of the membrane (proteic denaturation).[22] The effect of the high pH of calcium hydroxide alters the integrity of the cytoplasmic membrane by means of chemical injury to organic components and transport of nutrients, or by means of the destruction of phospholipids or unsaturated fatty acids of the cytoplasmic membrane, observed in the peroxidation process, which is a saponification reaction.[20]

Table-4: Comparison of effect of Metapex on B. fragilis

Inter group comparision	P value	Level of Significance
Group1 v Group 2	0	Significant
Group1 v Group3	0	Significant
Group2 v Group 3	1	Non significant

Table-5: Comparison of effect of Metapex on P. acne

Inter group comparision	P value	Level of significance
Group1 v Group 2	0	Significant
Group1 v Group 3	0	Significant
Group2 v Group 3	0.029	Significant

During this study it is observed that as the concentration of calcium hydroxide decreases antimicrobial action of Metapex decreases or disappears, this might be due to low concentration of hydroxyl ions which further leads to decline of pH.

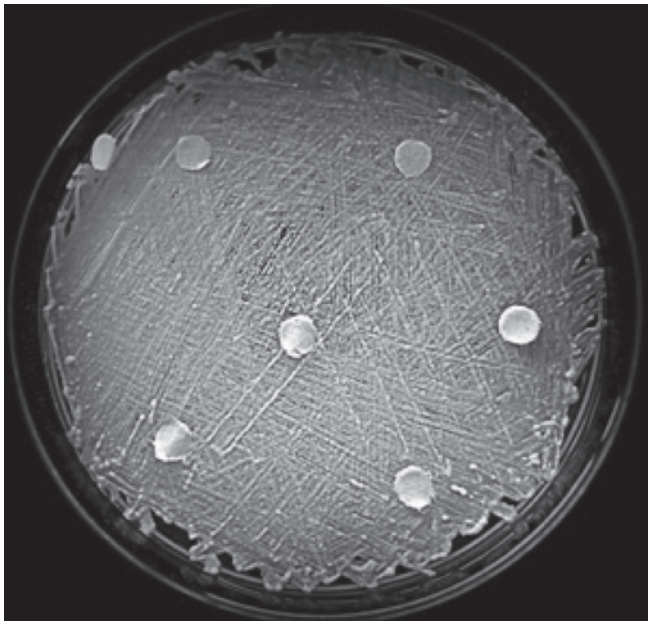

Fig.1: Zone of inhibition against C. albicans

Calcium hydroxide usually increases pH and allowed an unfavourable microenvironment to the growth of B. fragilis strains.[23] Past literatures have shown bactericidal effect of iodoform against B. Fragilis,which is also observed in our study. Same might be the reason of effect on P. acne, at lower concentrations metapex is effective against P. acne which might be due to enzymatic activity difference.

Metapex contains silicone oil as its vehicle and has a pH below that which is effective to kill E. faecalis. The superior antimicrobial effects of Metapex may be due to the combination with iodoform and to the viscous and oily vehicle, which may prolong the action of the medicament. Accordingly, Gomes *et al* showed that oily vehicles increase the antimicrobial effects of calcium hydroxide against E. faecalis and other bacteria.[24] The strong bactericidal properties of iodoform paste have been demonstrated in previous studies.[25]

Iodine is bactericidal as well as fungicidal.[26] The antimicrobial action of calcium hydroxide with iodoform

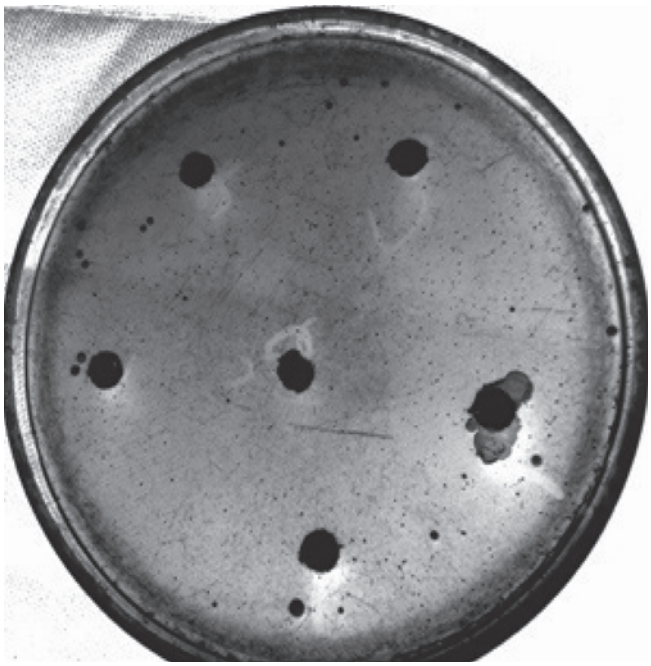

Fig.2: Zone of inhibition against E. faecalis

against *C. albicans* might be due to the role of the calcium ions in the regulation of *C. albicans* morphogenesis. It inhibited the mycelial growth of *C.albicans.*[27] The antimicrobial effect of calcium hydroxide, due to the release of hydroxyl ions, might be enhanced due to the inhibition of *C. albicans* growth by calcium ions.

Metapex (0.22gm/ml) has potent antimicrobial ability, a decreased concentrations of metapex (0.022gm/ml and 0.22gm/ml) resulted in significantly decreased antimicrobial effects. Further scientific investigation is required to elucidate its antimicrobial effectiveness and mechanisms inside the root canal system, as well as the need to associate it with other medicaments.

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