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ORIGINAL RESEARCH

Determination of the minimum inhibitory concentration of four medicaments used as intracanal medication

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Abstract

The aim of this study was to determine the minimum inhibitory concentration (MIC) of iodoform, calcium hydroxide, IKI (iodine potassium iodine) and CFC (ciprofloxacin, Flagyl (metronidazole) and calcium hydroxide) required to kill *S. aureus, Pseudomonas aeruginosa, Enterococcus faecalis and B. fragilis*. In the experiment, medicaments were added to bacterial species into test tubes, in 10 different concentrations. The MIC was the lowest concentration of the drug at which bacterial growth could not be observed. In this investigation, CFC was the most effective medicament against all bacteria. All drugs were able to eliminate *E. faecalis* and *B. fragilis*, while IKI was not effective against *S. aureus*. IKI and calcium hydroxide were not able to eliminate *P. aeruginosa* as well.

Introduction

The presence of microorganisms and their by-products in the root canal system provokes a host response, which can be demonstrated clinically and radiographically as periapical alterations (1–4). The main goal of endodontic therapy is to decrease contamination allowing periapical repair (5).

Intracanal microorganisms are usually removed mechanically during root canal preparation. However, they are often found in areas that are not instrumentable (5–9). These bacteria, both inside the canal and in the adjacent periapical tissues, can organize themselves in such a way to form a biofilm (4,5,7,10). Periapical biofilm is usually found in teeth with pulp necrosis and radiographically visible periapical lesions (3). In these cases, conventional endodontic therapy tends to fail in a higher percentage of cases (6,8,9). Bacterial flora in this region is normally mixed, with the predominance of anaerobic and facultative species (4,6–8,11–14).

In these situations, the use of irrigating agents and an intracanal medication with an effective antibacterial action is recommended to achieve an adequate decontamination of this system (15–18). These medications must be able to kill bacteria, either by acting directly on them or by creating unsuitable conditions in which to survive (19–21).

The most widely used is calcium hydroxide (CH) (15,16,20–23). Its main mechanism of action is to raise the Ph sufficiently that few microorganisms are able to survive (20). However, there are some strains that are resistant to the use of this drug (22,23). To improve its antimicrobial activity, CH may be used in association with ciprofloxacin and CFC (ciprofloxacin, Flagyl (metronidazole) and calcium hydroxide) (14,17). Ciprofloxacin is a bactericidal drug, which acts by blocking bacterial DNA replication. Additionally, metronidazole has a specific selective toxicity for anaerobic bacteria as well as parasites (14,24).

Another drug used to improve the antibacterial activity is iodoform, which has been used successfully as a medicament and filling paste for many years (16,19,25). Iodoform seems to stimulate immunological response and to interact with bacterial contamination by promoting the growth of granulation tissue, and thus accelerating the healing process (19).

There are also specific antiseptics, such as iodine potassium iodide (IKI), an iodide compound which presents

excellent biocompatibility and good bactericidal action *in vitro*. However, clinical evaluations do not show the same antibacterial activity (15,16,23).

Considering the challenge of decontaminating the periapical tissues, the authors decided to evaluate the action of four medicaments prescribed in endodontics (iodoform, CH, IKI, and CFC) to kill four distinct bacteria *(Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis and Bacteroides fragilis)* by determining their minimum inhibitory concentration (MIC). These bacteria are characterised by great resistance to antibacterial drugs, either by their natural resistance or by the development of different defence mechanisms against the host response.

Materials and methods

Of the four selected bacterial species, the two aerobic bacteria (*P. aeruginosa* – ATCC 27853 and *S. aureus* – ATCC 6538) and the facultative one (*E. faecalis* – ATCC 29212) were in a solid culture medium of nutrient agar and stored in a test tube. *B. fragilis* (ATCC 25285), had to be reactivated by filling an ampoule of the lyophilised bacteria with Reinforced Clostridium Medium (RCM) and then inserted in anaerobic jars. All inoculums were transferred to a dry-heat oven at 37°C (98°F) where they remained for 24–48 h for initial growth, according to strain specifications.

At the end of the initial growth phase, the experimental groups to be studied were stratified, as follows: (i) according to the bacteria: *E. faecalis, S. aureus, P. aeruginosa and B. fragilis*; (ii) according to the medicament to be tested: iodoform, CH, CFC and IKI; and (iii) according to the drug concentration. The drugs were diluted in glycerin to reach the concentrations of 0.125 mg mL^{-1}, 0.25 mg mL^{-1}, 0.5 mg mL^{-1}, 1 mg mL^{-1}, 2 mg mL^{-1}, 4 mg mL^{-1}, 8 mg mL^{-1}, 16 mg mL^{-1}, 32 mg mL^{-1} and 64 mg mL^{-1}.

Iodoform and CH were directly diluted in glycerin up to the desired dilution. The three CFC compounds, (CH, ciprofloxacin and metronidazole) were mixed in the commonly used proportion (2:1:1 respectively), and then this mixture was diluted in glycerin. Regarding IKI, this is the association of 2% iodine and 4% potassium iodide. In this study, IKI was prepared in the proportion of 1:2 of iodine and potassium iodide, respectively, and diluted in glycerin up to the desired concentration.

Initially, the experimental groups were prepared in test tubes containing pre-sterilised medium. One millilitre was removed from each of these tubes and incubated to be used as negative control. The experiment itself consisted of removing 1-mL aliquots from tubes with bacterial growth, and repeatedly inoculating them into tubes containing sterile medium until the desired turbidity pattern was reached (approximately 10^4–10^5 bacteria mL^{-1}). One millilitre of this combination (bacteria + medium with no medication) was transferred to an empty tube as positive control.

Treated groups consisted of test tubes containing 0.9 mL of this initial bacteria + medium combination, and 0.1 mL of the chosen medicament in every concentration. Using the above protocol, the experiment was repeated in 20 tubes for each of the 10 medicament concentrations.

For *S. aureus* and *P. aeruginosa*, and *E. faecalis*, the medium used was brain heart infusion during the entire experiment, and they were incubated at 37°C (98°F) for 24 h. For *B. fragilis*, the test tubes containing the bacteria and medicament combinations in RCM were stored in anaerobiosis jars, which were kept sealed up for 48 h. At the end of the incubation period, the bacterial growth in the test tubes was checked.

The MICs of the drugs for each bacterial species were determined. The MIC was the lowest concentration of the drug at which bacterial growth could not be observed. For the aerobe or facultative strains, the results were confirmed by transferring samples, by means of an inoculating loop, from tubes with no bacterial growth into sterile nutrient agar plates. For *B. fragilis*, tubes where no growth could be observed after 48 h had a sample of 0.1 mL placed in test tubes containing sterile RCM. All these new samples were once again incubated and the presence of bacteria checked.

Data were compiled into tables and individual values and mean values were statistically analysed, with a level of significance of 5%. Comparisons were made among the different drugs in terms of MIC required to kill the same microorganism. These comparisons were then submitted to statistical analysis using the ANOVA test.

Results

MICs of the drugs required to kill the four specific bacterial species are shown in Table 1 and Figure 1. The evaluation showed that IKI was not active against *S. aureus* and *P. aeruginosa*, even in the highest concentration used in this study (64.0 mg mL^{-1}), and CH was not active against *P. aeruginosa*.

At the concentrations used, all drugs were found to be able to eliminate *E. faecalis* and *B. fragilis*. For *E. faecalis*, the MICs were 32.0 mg mL^{-1} for iodoform, 16.0 mg mL^{-1} for CH, 0.125 mg mL^{-1} for CFC and 2.0 mg mL^{-1} for IKI. For *B. fragilis*, MIC values were 0.25 mg mL^{-1} for iodoform and CFC, 4.0 mg mL^{-1} for IKI and 16.0 mg mL^{-1} for CH.

MIC values for *S. aureus* were 0.5 mg mL^{-1} for CFC, 2.0 mg mL^{-1} for iodoform and 16.0 mg mL^{-1} for CH,

Table 1 Minimum inhibitory concentration (MIC) of iodoform, calcium hydroxide, CFC (ciprofloxacin, Flagyl (metronidazole) and calcium hydroxide) and iodine potassium iodine (IKI) required to kill *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa and Bacteroides fragilis*

	Bacteria			
Drug	*E. faecalis*†	*S. aureus*†	*P. aeruginosa*†	*B. fragilis*†
Iodoform	32.0 mg mL^{-1}	2.0 mg mL^{-1}	64.0 mg mL^{-1}	0.125 mg mL^{-1}
Calcium hydroxide	16.0 mg mL^{-1}	16.0 mg mL^{-1}	–	16.0 mg mL^{-1}
CFC	0.125 mg mL^{-1}	0.5 mg mL^{-1}	0.125 mg mL^{-1}	0.125 mg mL^{-1}
IKI	2.0 mg mL^{-1}	–	–	4.0 mg mL^{-1}

†Statistically significant difference ($P < 0.05$) between the MIC of the drugs.

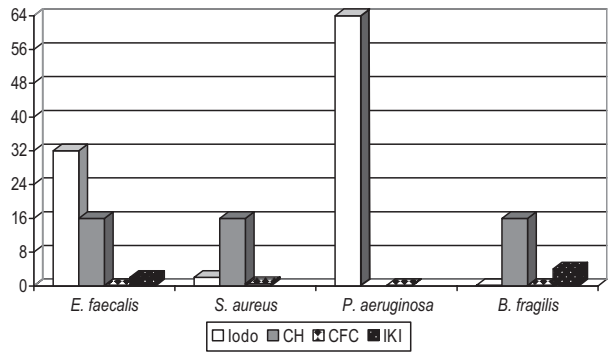

Figure 1 Minimum inhibitory concentration of iodoform, calcium hydroxide, CFC (ciprofloxacin, Flagyl (metronidazole) and calcium hydroxide) and iodine potassium iodine (IKI) required to kill *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa and Bacteroides fragilis*. CH, calcium hydroxide.

whereas, for *P. aeruginosa*, MICs were 0.125 mg mL^{-1} for CFC and 64.0 mg mL^{-1} for iodoform.

Discussion

Incomplete decontamination of infected root canals may lead to failure of the endodontic therapy and the development of periapical lesions (1–3). Clinically, the presence of anatomic variations as well as the high number and great variety of microorganisms make it difficult to completely eliminate microorganisms from the canal (5,6,8,9). Also, microorganisms can organise themselves in a biofilm, and conventional endodontic therapy tends to fail in a higher percentage of these cases (3,4,6,7,9,17). In order to eliminate as many remaining bacteria as possible following debridement, intracanal medication is highly recommended (14–16,18,19,21,24).

The determination of the MICs is used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the *in vitro* activity of new antimicrobials (26). This study was carried out to determine the MIC of iodoform, CH, IKI, and an association of CH, metronidazole and ciprofloxacin, the CFC.

The bacteria evaluated in this study are representative of the different groups normally found in cases of therapy-resistant lesions in root canals and have significant resistance characteristics (2,4,6,8–10,12,13). *E. faecalis* is usually associated with refractory lesions (8,9,17,18,22,24), owing to its capacity to survive for long periods without nutrients (15,22,23). *B. fragilis* is a gram-negative anaerobic bacilli and is the most common bacteria found in endodontic infections (2,4,9–11). Owing to its great capacity for adaptation and resistance, *S. aureus* can be found in both pulp and periapical infections (12). In endodontics, *P. aeruginosa* is found in teeth with periapical lesions exposed to the oral cavity and this bacterium is usually related to monoinfections (2,13).

CFC was shown to be the most effective drug in this investigation. CFC demonstrated the ability to eliminate all the bacterial strains at the lowest concentration used, that is, 0.125 mg mL^{-1}, except for *S. aureus*. The great antibacterial action of CFC is due to the presence of two specific antibiotics in its composition: ciprofloxacin (14), which is an antibiotic specific for enterobcateria, such as *E. faecalis* and *P. aeruginosa*; and metronidazole, which is able to eliminate anaerobic bacteria, such as *B. fragilis* (14).

The evaluation of the action of IKI showed that this medication was not able to kill *S. aureus* and *P. aeruginosa*, even at the highest concentration used. However, IKI was effective against *E. faecalis* in a low concentration, which is consistent with other studies (15,16). IKI was able to kill *B. fragilis* as well.

CH is the drug most commonly used as intracanal medication. The MIC of CH for *B. fragilis* and *S. aureus* was 16.0 mg mL^{-1}, the highest value found for these bacteria. The MIC of CH against *E. faecalis* was 16.0 mg mL^{-1} as well; however, the MIC of iodoform was higher, although without statistical difference. This drug was not able to eliminate *P. aeruginosa* in this investigation. Therefore, CH showed the higher MIC values. The direct antibacterial action of CH on *E. faecalis* (15,16,18,19), *B. fragilis* and *S. aureus* (2,12) observed in this study was expected based on other investigations.

Furthermore, iodoform presented antibacterial activity against all microorganisms tested. The MIC for *B. fragilis* was the same found with CFC. Iodoform was expected to be effective in anaerobic conditions owing to the higher release of iodine (19). Iodoform was also effective against *S. aureus* in a lower concentration than CH and IKI. According to other investigations, this drug is able to eliminate *E. faecalis* (16,19), but the MIC observed was higher than those of the other drugs used. Similarly, iodoform was effective against *P. aeruginosa*.

Conclusion

CFC showed the best results against the four bacterial species evaluated. The antibacterial action of CFC was similar to iodoform against *S. aureus* and *B. fragilis*, and similar to IKI against *E. faecalis*. CH showed a MIC value for *E. faecalis* that was close to the one of iodoform and presented the higher MIC values for all bacterial species, except for *S. aureus*, against which IKI was ineffective.

The results were achieved *in vitro* and cannot be directly related to the clinical situation. This is especially so when drug mixtures (such as CFC) interact with mixed bacterial flora such as that infecting most root canals (27).

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