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Nitrofurantoin: Furious against bacteria? An in vitro study to test nitrofurantoin as an intracanal medicament against Enterococcus Faecalis

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***Abstract*---**In vitro evaluation of nitrofurantoin as an intracanal medicament against Enterococcus Faecalis. Materials and Methods: 150 single-rooted premolar teeth were chosen and instrumented using a Protaper gold rotary file system; the root canals of these teeth were inoculated for 21 days with an Enterococcus faecalis bacterial solution. The canals were inoculated with Nitrofurantoin, Calcium hydroxide, and MTAP after 21 days and left for 7 days. On the eighth day, the samples were examined to determine the degree of infection. The study's findings revealed that both Nitrofurantoin and MTAP were very successful, with no Colony Forming Units (CFU) production in their respective groups. The current investigation indicated that nitrofurantoin is effective in eradicating E. faecalis cells in pure culture as well as root canals.

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***Keywords*---**nitrofurantoin, MTAP, enterococcus faecalis, calcium hydroxide.

Introduction

Endodontic infections are mixed infections in which bacteria proliferate in the root canals as sessile biofilms. A number of microbial species can be identified in primary periapical infections, however in secondary infections, the most prevalent bacterium that can be isolated is Enterococcus faecalis (1). Enterococcus faecalis is a facultative anaerobic gramme positive bacteria. It is an opportunistic pathogen that lives naturally in the oral cavity (2-4). Enterococcus faecalis was found in around 24-70 percent of root canal failure patients (5). Due to its many special features, such as the ability to tolerate long periods of starvation, deep invasion of the dentinal tubules, antimicrobial resistance, and the ability to adapt to changing environments, E. fecalis is able to survive in the root canal even after complete disinfection of the canals (6).

Chemo-mechanical root canal preparation eliminates bacteria through mechanical shaping and alternating irrigation with different disinfection solutions. Despite proper disinfection and debridement, microorganisms such as E. Faecalis might survive inside the dentinal tubules or in inaccessible locations, resulting in re-infection and root canal treatment failure (7). As a result, the use of intracanal medicaments that can function throughout the root canal system will improve endodontic treatment outcomes. Local application of an antimicrobial agent in the form of intracanal medicament targets bacteria in every locus of the root canal that standard treatment methods such as dental instrumentation and irrigation cannot reach. (9) It is a more effective method than systemic antibiotics for preventing antibiotic-related side effects such as allergic responses or toxicities (8). However, due to the inappropriate use of antibiotics, this procedure may result in the development of bacterial resistance (10). To address this limitation, new chemical substances are constantly being researched for their appropriateness in endodontic antiseptic treatment.

Metronidazole, ciprofloxacin, and minocycline are the active ingredients of triple antibiotic paste (TAP). Because of its excellent antibacterial activity, it is employed as an intracanal medicament. However, several research have revealed that triple antibiotic paste has adverse effects such as bacterial resistance and crown discolouration due to the presence of minocycline (11). To address this issue, a modification of TAP known as Modified Triple Antibiotic Paste (MTAP) was created in which minocycline was replaced with clindamycin (12). Due to the drawbacks of TAP and its modification to MTAP, there was a need for the development of a new medicament that has less resistance potential, equivalent potency against E fecalis, is preferably a single drug rather than a combination, is also cost effective, and requires less time and effort to prepare.

Nitrofurantoin (Nit) is a widely used antibacterial drug as an oral antibiotic for urinary tract infections (UTIs). It is a nitrofuran molecule that has been synthesised by various processes. It is effective against the majority of gram-positive and gram-negative bacteria. It is also the first-line treatment for

infections caused by multidrug-resistant bacteria (13). A study conducted in 2001 by G. G. Zhanel et colleagues indicated that none of the 300 isolates tested displayed nitrofurantoin resistance. Several similar studies have been conducted, and the high efficiency of Nit against EF has been established (14).

Objectives

The purpose of this study is to compare the antibacterial activity of Nitrofurantoin against E. faecalis under controlled settings to Modified Triple Antibiotic paste (MTAP) in extracted teeth.

Methods and Materials

The study included 150 extracted single-rooted premolars. A diamond saw installed on a micromotor handpiece was used to decoronate the coronal region of the teeth. The remaining roots were then grouped into three categories. The canals of the teeth were instrumented with engine driven protaper gold files up to size F2 utilising the crown down procedure. Side vent needles were utilised to irrigate with 5.25 percent sodium hypochlorite. After completing root canal treatment on all roots, they were dried with sterile paper points and autoclaved for 15 minutes at 121 degrees Celsius.

Bacterial Inoculation

A bacterial sample of E. Faecalis was received from the microbiology lab, and the bacterial solution was put into each root canal. The root canals were completely filled, sealed, and incubated in a closed container at 37 degrees Celsius for 21 days. The canals were reinoculated with fresh bacterial solution every third day. After a few days, samples were removed from the canals using sterile paper points and cultured under aerobic conditions to test cell survival and culture purity.

Intracanal medication administration

The roots were taken from the incubators after 21 days and the canal contents were aspirated. The canals were cleaned with saline solution, and separate groups received intracanal medications.

Group I- Nitrofurantoin paste 25 mg/ml (Nitrofurantoin solution was prepared by mixing 25 mg nitrofurantoin tablets with distilled water).

Group II- MTAP paste at a concentration of 25mg/mL (MTAP paste was prepared by mixing equal proportions 25 mg metronidazole, 25 mg ciprofloxacin and 25 mg clindamycin with 1 ml of distilled water.)

Group III- Calcium hydroxide paste 25 mg/ml

The root canal openings were sealed and incubated for 7 days at 37 degrees Celsius. After 7 days, the roots were evaluated to check for infection of the radicular dentin.

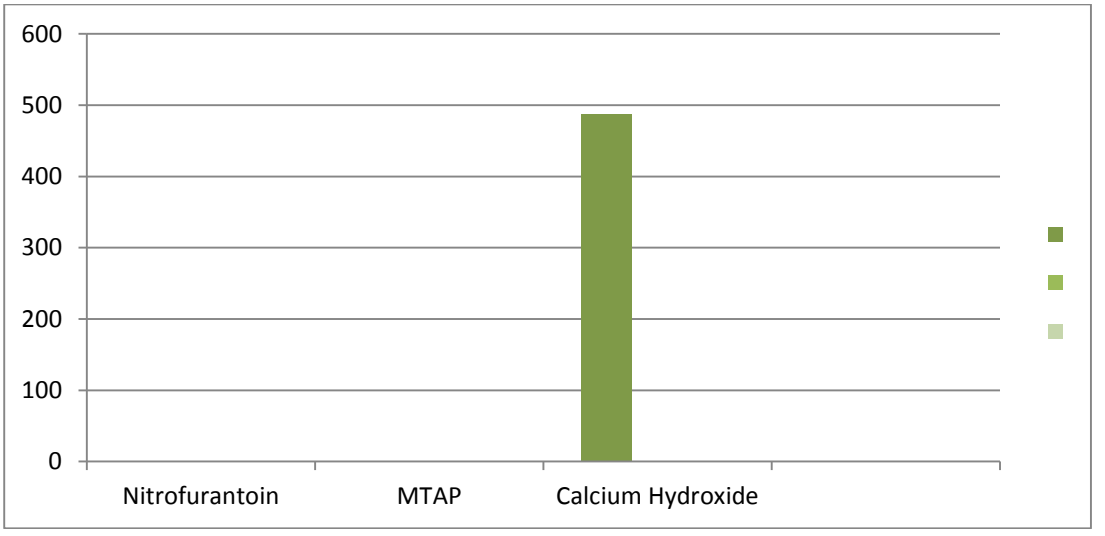
Results

The number of colony forming units was used to evaluate the efficacy of various intracanal medicaments. The samples from Group I (Nitrofurantoin paste) and Group II (MTAP paste) showed zero colony forming units, indicating that E. Faecalis had been completely eradicated from their respective samples, but the samples from Group III (Calcium hydroxide group) showed the presence of colony forming units, indicating that calcium hydroxide was not very effective in eliminating E. Faecalis with 488 CFU (105/ml). When the data was statistically analysed using the Students Unpaired 't' test, the p value discovered was (0.001), which is considered very significant, as is p value (0.05). When compared to the calcium hydroxide group, both the nitrofurantoin and MTAP groups exhibited a significant difference in eliminating E. faecalis.

As a result, both the intracanal antibiotics Nitrofurantoin and MTAP are equally effective against E. faecalis. Thus, this proves that both the intracanal medicaments Nitrofurantoin and MTAP are equally effective against the E. faecalis.

S.no	Group	Number of samples	Mean Colony forming units	Standard deviation	p Value
1.	Nitrofurantoin	50	0	0	0.001
2.	MTAP (Modified Triple Antibiotic Paste)	50	0	0	0.001
3.	Calcium hydroxide	50	488	7.76	-

Comparison of all the Groups in Relation to CFU of Enterococcus Faecalis



Discussion

Enterococcus faecalis is an anaerobic, facultative, gram-positive bacteria that is thought to be the predominant causal microorganism in root canal infections that result in root canal treatment failure (15, 16). Enterococci can penetrate dentinal tubules even in adverse environmental conditions, making it resistant to chemomechanical instrumentation and leading to re infection (17). E. faecalis' principal resistance mechanism is its ability to thrive in alkaline environments up to pH 11.1. This feature is attributed to the presence of a proton pump, which resists the alkaline effects of calcium hydroxide (18). The pH required to kill E. faecalis is 11.5, which cannot be obtained by using Calcium hydroxide, which has a pH of 10.3, as reported in several in vitro experiments. Dentin is also thought to have an influence because its buffering reduces the pH gradient from the inner to the peripheral root potion of dentin (19, 20).

Nitrofurantoin was chosen for this investigation because it has a broad spectrum of antibacterial activity and is both bactericidal as well as bacteriostatic. It is the treatment of choice against E faecalis since numerous studies have demonstrated that it is effective in urinary tract infections as well as chronic and recurring infections caused by E faecalis. It features a one-of-a-kind structure composed of a hydantoin ring and a nitro-substituted furanyl side chain. It has a distinct mechanism of action in that it denatures bacterial ribosomal proteins after they have been reduced by bacterial flavoproteins, resulting in the suppression of many essential processes within the bacteria such as aerobic energy metabolism, cell wall synthesis, DNA synthesis, protein synthesis, and RNA synthesis. Resistance to nitrofurantoin is extremely unlikely due to its broad spectrum of activity and inhibition of all basic bacterial activities. As a result, bacterial resistance to Nit has been extremely rare since its introduction and FDA clearance was obtained in 1953. (21, 22).

MTAP (a multidrug paste containing three antibiotics: ciprofloxacin, clindamycin, and metronidazole) was used to test a new antibiotic agent, nitrofurantoin, as an experimental intracanal medicament and to analyse the efficacy of a single agent vs MTAP. Algarni et al. discovered that MTAP had the same efficacy as TAP against E faecalis strains (23). Several studies conducted by Mozayeni et al. (24), Ravi (25), and Sabarathinam et al (26) demonstrated that TAP outperformed nonantibiotic-based intracanal medicaments such as chlorohexidine gel and calcium hydroxide in terms of antibacterial effectiveness against E faecalis. However, MTAP was unable to completely eliminate E faecalis since the bacteria have demonstrated the potential to revert to their natural state when the environmental factors are favourable (27). This conclusion of several investigations undertaken by different authors necessitates the quest for an intracanal medicament capable of completely eliminating E faecalis.

Conclusion

When used as an intracanal medication, nitrofurntoin at a concentration of 25 mg/mL is entirely successful in eradicating E faecalis. Nitrofurantoin and its efficacy in eliminating E. faecalis could be a game changer in the field of endodontics due to its low drug resistance development. It also has the advantage

of being a one-drug therapy. This study does not support the use of Nitrofurantoin in everyday clinical practise since more research is needed to examine its efficiency against other microorganisms because endodontic infections are polymicrobial in nature.

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