

Antimicrobial Efficacy of Two Newer Intracanal Medicaments on *Enterococcus Faecalis*: An In Vitro Study

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ABSTRACT

Background: The purpose of this study is to evaluate the anti-microbial efficacy of two newer intracanal medicaments in the elimination of *Enterococcus faecalis* (e.faecalis) from the root canal system, a novel approach.

Materials and Method: Thirty intact single-rooted permanent human teeth were collected for the study. Then samples were cleaned, and access cavities were prepared and the canals were instrumented with the rotary file system. During preparation, all the samples were irrigated with saline, CHX, sodium hypochlorite and EDTA, simultaneously. The apex of all teeth was sealed with the composite filling material. Teeth were autoclaved to ensure the absence of bacteria before bacterial inoculation. The root canals were then inoculated with the bacterial mixture and closed with the sterile sponge and temporary restoration. All samples were then incubated at 37°C for 48 hours of the growth of *E.faecalis* and its penetration into the dentinal tubules. All the samples were then divided into three groups of 10 each-Tigecycline, L-arginine and negative control group, respectively. Orifices closed with the sterile sponge and temporary restoration and incubated for 7 days. After that, all samples were irrigated with saline and with the help of H-files dentinal scraping were inoculated in nutrient broth. *E.Faecalis* colonies on agar plates were evaluated. Statistical analysis was done with One way ANOVA test and Post Hoc test.

Result: Results showed almost negligible CFUs of e.faecalis in tigecycline compared to two other groups. There is a statistically significant difference between all three groups.

Conclusion: Tigecycline reduces bacterial growth significantly than two other groups.

Keywords: *E. faecalis*, Tigecycline, L- arginine.

INTRODUCTION

The main goal of endodontics is to prevent and treat diseases of the dental pulp and periapical tissue. Treatment protocols that are based on a thorough understanding of the aetiology of endodontic diseases (1). *E.faecalis* is recognized as a most predominant pathogen in the post-treatment endodontic infections, which is one of the most

notorious organism to tackle which was well adapted and tolerated in ecological conditions of root canals and hence results in failure of endodontic treatment.

The main rationale behind intracanal medicament is to kill the bacteria inside the root canal and to avert

Received: Dec. 19, 2019; Accepted: Feb. 25, 2020

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reinfection. In the absence of nutrients, the bacteria remain after obturation in root canal may die. If they survive, they may flourish. The bacteria which remain in dentinal tubules cannot be removed through chemomechanical preparation. If the root canal is not dressed properly with antiseptic medicaments between the visits, the residual bacteria may increase in the same number as it was in the starting stage of treatment. Thus the use of effective intracanal medication for disinfection of root canal is necessitated. Medicament with long effect and least irritated to periradicular tissue has to be introduced to infiltrate the dentinal tubule eliminating bacteria when the time does not allow to complete the treatment at one visit. According to McHugh et al. (7) conducted a study to explain why *E. faecalis* is able to survive intracanal treatment with calcium hydroxide: (a) *E. faecalis* passively maintain pH homeostasis. This occurs as a result of ions penetrating the cell membrane as well as the cytoplasm's buffering capacity. (b) *E. faecalis* has a proton pump that provides an additional means of maintaining pH homeostasis. This is accomplished by "pumping" protons into the cell to lower the internal pH. (c) At a pH of 12.5 or greater, *E. faecalis* is unable to survive. Triple Antibiotic Paste (TAP), Augmentin, and Tigecycline in a hydrogel form at 1 mg/mL of concentration reduced *e. faecalis* bacterial growth significantly with minimal colour change has been proved by T. AlSaeed et al. (4).

Ethan Kolderman et al. (5) proved that L-arginine destabilizes oral multi-species biofilm communities developed in human saliva. So, the aim of this study was to evaluate and compare the two newer intracanal medicaments, Tigecycline and L-arginine by counting colony-forming units of *e. faecalis*.

MATERIALS AND METHODS

30 extracted permanent single-rooted human teeth with a single canal were selected for the study. Teeth with closed apices were included in the study. Immature apex, internal resorption, carious teeth, cracks were excluded from the study. All the samples were decoronated and divided into three groups, 10 samples for each group. Access opening was done and bio-mechanical preparation was done by heroshaper rotary files till 25/04. The apical part of the root was etched, bonded and sealed with composite resin. Irrigation was carried out with 5% NaOCL, followed by normal saline and

chlorhexidine, simultaneously. Then all the samples were sealed in a sterilization pouch and autoclaved at 121-c, 15 lbs pressure for 15 mins.

For microbiological procedure

Enterococcus faecalis ATCC 29212 was used as challenging microorganism and which was obtained from the microbiology laboratory. *E. faecalis* suspension was made, one MacFarland unit and in each sample twenty microliter *e. faecalis* suspension was added and closed with a temporary restoration and incubated for 24 hours. All the samples were removed from incubation after 24 hours.

Group 1: Tigecycline

Group 2: L- Arginine

Group 3: Empty canal (negative control)

Ten samples, each were filled with tigecycline, L-arginine and 10 samples without any intracanal medicament, respectively and orifice were sealed with a temporary restoration. All the samples were incubated for seven days. After day seven, all the samples were irrigated with normal saline and with the help of 30 no. H-file dentinal scraping was collected into the nutrient broth. 100 microliter of nutrient broth was collected with a micropipette and incubate on an agar plate and spread with the help of L-spreader and again incubated for 24 hours. Colony Forming Units (CFUs) were calculated by placing agar plates into a digital colony counter.

Statistical analysis

One way ANOVA test to compare all groups and Post Hoc test used to compare in between three groups ($p < 0.05$).

RESULTS

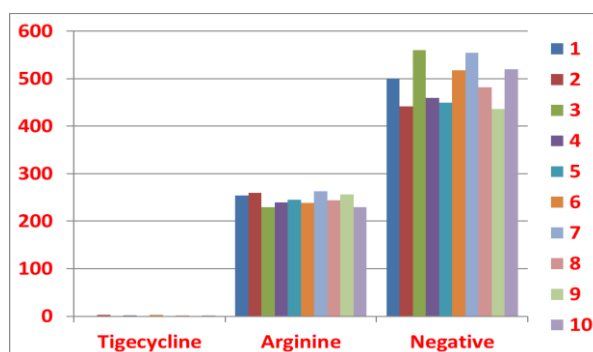


Fig 1: Chart showing CFU of *E. faecalis* in between tigecycline, L-arginine and negative control group.

Results showed almost negligible CFUs of *E. faecalis* in tigecycline compared to two other groups. There is a statistically significant difference between all three groups.

DISCUSSION

The main goal in endodontics is the prevention and treatment of diseases of the dental pulp and periapical tissues. Modern techniques and equipment have greatly contributed to increased clinical success rates and significantly shortened the time needed to complete the RCT, yet there are still limitations to the endodontic disinfection process; viable biofilm cells can persist in undertreated and untreated locations of the root canal system due to the inherent challenges associated with its complex anatomy (12,13,14). Undoubtedly, the major factors associated with endodontic failure are the persistence of microbial infection in the root canal system and the periradicular area. Endodontic diseases are biofilm-mediated infections, and the removal or reduction of persistent bacteria from the root canal system is challenging. Hence, it is important to develop and introduce new antimicrobial agents for effective root canal disinfection.

According to Kawashima et al., the intracanal medicament is a temporary placement of medicaments with good biocompatibility into root canals for the purpose of inhibiting coronal invasion of bacteria (24). Most commonly found bacteria is *E. faecalis*, which is gram-positive, non-spore forming, anaerobic bacteria, which was used in this study as it is one of the most predominant bacteria found in persistent endodontic infections. *E. faecalis* can survive very harsh environments including extreme alkaline PH and salt concentrations. According to Rocas et al., it can easily adhere, accumulate and form their organized colonies into root canals, which is difficult to remove with sodium hypochlorite, hydrogen peroxide, normal saline irrigation solution.

Widely used intracanal medicament is calcium hydroxide. Despite its good physical, biological and pharmaceutical properties, calcium hydroxide cannot completely eradicate resilient intracanal microorganisms as *E. faecalis* (19).

A triple antibiotic is used as an intracanal medicament in managing non-vital young permanent teeth as well as root canal pathogen like *E. faecalis*. but the major disadvantage it contains minocycline and thus it causes discolouration in young permanent teeth. According to Turkey AlSaeed et al. (4) evaluated in ex vivo study, the antibacterial efficacy and discolouration effect of triple antibiotic paste (TAP), Augmentin, and tigecycline as intracanal medicaments in regenerative endodontics and they concluded that TAP caused the greatest discolouration.

So, newer 3rd generation tetracycline, "Tigecycline" was used. Tigecycline is a broad-spectrum antibiotic, which is FDA and EMEA approved for skin and soft tissue infections, including those with vancomycin- susceptible *E. faecalis*. Tigecycline inhibits protein synthesis upon interaction with the bacterial 30S ribosomal subunit, and only one case of tigecycline-resistant *E. faecalis* has been documented, although the exact mechanism of resistance has not yet been elucidated (10).

Tigecycline belongs to a new group of glycylcycline antibiotics, which is and bacteriocidal in nature (9). Because of this nature, Tigecycline gave best results in necrotic canals cases (4). These were all reasons in favour that tigecycline gave excellent antimicrobial efficacy in this study and showed significant result than the other two groups.

L-arginine is an α -amino acid. It is the precursor for the biosynthesis of nitric oxide. L-arginine inhibits bacterial coaggregation, is involved in cell-cell signalling, and alters bacterial metabolism in a broad range of species present in the human oral cavity. So it is more effective against oral multi-species. Till now no any study conducted to check the role of L- arginine used as an intracanal medicament against *E. faecalis*.so because of this reason L-arginine was selected.

In our study, all the samples were closed at the apex level with composite resin to prevent apical leakage. Chlorhexidine was used as a final irrigant to reduce bacterial load effectively. After that samples were autoclaved before starting any procedures to sterilized the samples.

On the other hand, having a single-species does not represent the multimicrobial nature of the true

endodontic infection (11). Hence the antimicrobial efficacy of intracanal medicaments may differ when tested on a multimicrobial species. Thereby, future research efforts should focus on creating multimicrobial species studies that better reflects the complex composition of endodontic infections rather than single-species studies.

CONCLUSION

Within the limitation of the study,

- Tigecycline is more effective against *Enterococcus faecalis* than L-Arginine.
- Tigecycline can be considered as alternative intracanal medicament in persistent root canal infection.

But still in vivo studies were needed to confirm these findings.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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