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Research Article

Antimicrobial Effects of Triantibiotic Paste in Endodontic Treatment of Chronic Apical Periodontitis

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Abstract: The use of intracanal (ciprofloxacin,metronidazole, minocycline) dressing is essential to remove any remaining bacteria in the root canal treatment of chronic apical periodontitis (CAP). However, no single medicament commonly used has been found to be completely effective. This clinical study with pre and post test design was aimed to evaluate the antimicrobial effects of triantibiotic paste from the exudates of human root canals. Exudates samples were taken from 24 necrotic root canals after chemo-mechanical preparation and a 7-day inter appointment medication with calcium hydroxide paste. Samples were then allocated into two groups. Group 1 (G1) was locally delivered with triantibiotic paste in apical area. Group 2 (G2) was remedicated with $Ca(OH)_2$ paste. Exudates samples were taken after a 7-day medication. All samples were incubated in blood agar and nutrient agar and were evaluated for bacterial load (CFU) and presence of bacteria for a 14-day observation. All baseline samples were positive for bacteria. Following 7 days of $Ca(OH)_2$medication, median bacterial load of G1 was $3x10^3$, and $1.5x\ 10^3$ofG2. Following 7 days medication with triantibiotic paste, the median bacterial load for G1 was $1x10^3$ which was significantly decreased ($p=0.024$). For G2, the median bacterial load was $3x10^3$ and no significant difference was observed. ($p=0.441$). Disappearance of bacteria was found in 2 samples of each group before treatment. Following treatment with triantibiotic paste, disappearance of bacteriawas found in another 3 samples, while G2 showed positive for bacteria in 10 samples. We had concluded that triantibiotic paste showed additional antimicrobial effect over commonly used calcium hydroxide, and may prevent the growth of bacteria.
Keywords: triantibiotic paste, CFU, bacteria, endodontic treatment and CAP.

INTRODUCTION

It is well established that bacteria are essential for the development of pulpal and periapical diseases as demonstrated in animal models and human studies [1-3]. Eradication or controlled of bacteria through endodontic treatment may provide optimal treatment outcome[4]. However, elimination of bacteria from infected root canal systems is challenging. Numerous measures have been described to reduce the number of bacteria in the root canal system, including the use of various instrumentation techniques, irrigation regimens and intra-canal medicaments. There is no definitive evidence in the literature that all these measures result in a bacteria-free root canal system[5, 6]. Therefore additional methods such as the use of intra-canal medicaments are required in order to maximize disinfection of root canal system and kill as many bacteria as possible[5].

Calcium hydroxide is the most commonly intracanal dressing used between treatment sections, but studies have shown inconsistent results of its efficacy in significantly enhance disinfection. Additional intracanal dressing is sometimes needed to improve disinfection. Antibiotics have been proposed as intracanal medicament but any single antibiotic is unlikely effective to sterilize diverse flora in root canal infection [7-9].

Recently, triple antibiotic mixture has been reported by Japanese researchers for successfully disinfection of infected root dentine *in vitro*. This

mixture consist of ciprofloxacin, metronidazole and minocycline which consistently sterilize bacteria of infected dentine and infected pulps[10, 11]. In some case reports, triple antibiotic paste was also reported to provide successful management of a periapical lesion with sinus tract[12]. However, clinical evaluation of its antibacterial activityafter the root canal treatment of chronic apical periodontitis (CAP) is still scarce.

This clinical study with pre and post test design was aimed to evaluate the antimicrobial effects of inter-appointment medication with either triantibiotic paste or calcium hydroxide during treatment of primary infected root canals of teeth with chronic apical periodontitis.

MATERIAL and METHODS

Patient selection

Twenty four root patients consulting at the clinic of Endodontic Department, Oral and Dental Hospital, Hasanuddin University, were included if they had clinical diagnosis of Chronic Apical Periodontitis (CAP). The age of patients ranged from 16-38 years. Diagnostic criteria included the presence of apical lesion detected by periapical radiography in upper anterior teeth with clinical determination of non-vital pulp, and had indication of endodontic treatment. Exclusion criteria included marginal periodontal diseases, defined by the absence of clinical attachment loss (≥ 2 mm), increased probing depths (≥ 3 mm), systemic illness or previous antibiotics or non-steroid anti-inflammatory treatment during the 3-month period prior to the study. All the protocols and procedures were approved by the Ethics Commitee of Medical Faculty (0892/H4.8.4.5.31/PP36-KOMETIK/2014), Hasanuddin University, and informed consents were obtained from all individuals.

Clinical procedures

The teeth were isolated with a rubber dam, disinfection of their external surfaces and the surrounding structure field was carried out by using 30% hydrogen peroxide, followed by 2.5% NaOCl. The solutions were inactivated with 5% sodium thiosulfate to avoid interference with bacteriologic sampling. The sterility of the external surfaces of the crown was checked by taking a swab sample from the crown surface and streaking it on blood agar plates. The access preparation was performed without the use of water spray but under manual irrigation with sterile saline and high-speed diamond bur. Before entering the pulp chamber, the access cavity was disinfected again following the protocol described above.

The root canal was prepared by using hand K-files with back-and-forth alternated rotation motion (size 15/40 and 45/80; FKG Dentaire, Switzerland). The working length was established with an apex locator and confirmed by radiographs. Master apical files ranged from 30-40, depending on the root anatomy and initial diameter of the root canal. Chemomechanical preparation was completed at the same appointment in all cases. The irrigant was 6% sodium hypochlorite (local medical supplies). A 27-gauge needle was used to deliver 3 mL of sodium hypochlorite after each instrument size. Each canal was then flushed with 2 mL of sterile aquadest solution to inactivate any residual sodium hypochlorite. Smear layer was removed by rinsing the canals with 3 mL of 17% EDTA and leaving the canal filled with this solution for 3 minutes. The canal was then flushed with 2 mL sterile aquadest. the canal was dried with sterile paper points. A sterile papper point (size 20; Dochem, China) was introduced into the full length of the canal, and retained in position for 60 seconds for sampling. Afterwards, the paper point was placed in a screw-cap container containing Stuart-transport medium and was directly sent to the microbiology laboratory for microbial cultivation.

The canal was medicated with calcium hydroxide paste (Ultradent, Utah, USA) which was placed by means of lentulo spiral fillers and packed with a cotton pellet at the level of canal entrance. A radiograph was taken to ensure proper placement of the calcium hydroxide paste in the canal. Access cavity was filled with a temporary filling, Cavit-G (3M, ESPE,USA).

Seven days later, the tooth was isolated and the operative field was cleaned and disinfected as described above. A sterile control sample of the operative field was obtained. The temporary filling was removed and the paste was carried out of the canal by using sterile file. Medication sample was taken as above. The canals were then irrigated with the same procedure outlined above and dried with sterile paper points. Triantibiotic paste was prepared by mixing 500 µg of each antibiotic (ciprofloxacin, Metronidazole (IPHARS, Pharmaceutical Company, Solo), Minocycline (Sigma Aldrich, St Louis, USA), mixed with propylene glycol to obtain paste form. The paste was packed into the apical area by using guttapercha into the Group 1 and the access was sealed with temporary restoration, Cavit-G (3M, ESPE, USA). For Group 2, the canal was remedicated with calcium hydroxide paste (Ultradent, Utah, USA). Patients were recalled following one week, and a post medication sample was taken in both groups as decribed above. The canals were then obturated with gutta-percha and AH plus sealer (Dentsply-Maillefer, Switzerland),by lateral compaction technique, and the teeth was finalized with permanent restoration.

Isolating and detection of species

The paper point was taken out from the container and inserted into the other container filled with enhancement medium of Brain-Heart Infusion Broth (BHIB), vortexed for 60 seconds to remove all bacteria and spread on BHIB. One mL of BHIB was added with 9 mL NaCl 0.9%, diluted and repeated in 3 series until it reached 10^3 CFU bacteria. One mL was swabbed on a sterile petri dish contained Blood Agar (BA) and

incubated anaerobically for 24 h at 37°C in gas vac. Onother 1 mL was swabbed on a sterile petri dish contained Nutrient Agar (NA) to detect facultative anaerob bacteria. Appeared colonies were counted visually.

RESULTS

This study was held in the Endodontic Department Dental Hospital of Hasanuddin University in the period of May to September 2014. A total of 24 subjects, consists of 7 males and 17 females, with age ranged from 16 to 38 years old. The subjects were divided into two groups of twelve, triantibiotic paste group and control group (Table 1).

Bacterial load difference of each treatment group before and after treatment shown on Table 2.

Median of bacterial load of triantibiotic paste showed a significant decrease after treatment with triantibiotic paste ($p<0.05$) as shown in Table 2. Meanwhile, bacterial load of control group showed a tendency to increase after treatment, although it is not statistically significant.

Table-1: Charasteric of patients

		Treatment Group	
		Triantibiotic Paste (A)	Control (B)
Gender	Male	5	2
	Female	7	10
Mean age		24,25 ± 6,45	20,67 ± 4,35

Tabel -2: Difference of bacterial load of each treatment group

Group	Median Bacterial Count			p-value
	Before	After	Difference	
Triantibiotic(n=12)	$3,0 \times 10^3$	$1,0 \times 10^3$	$1,50 \times 10^3$	0,024*
Control (n=12)	$1,5 \times 10^3$	$3,0 \times 10^3$	$0,50 \times 10^3$	0,441

*Wilcoxon test, $p<0.05$ = significant

Table-3: Difference of bacterial appearance in each group before and after treatment

Group	Before	N	After		RR (95% CI)
			None	Exist	
Triantibiotic	None	2	2	0	3,3 (1,29 - 8,59)
(12)	Exist	10	3	7	
	Total	12	5	7	
	None	2	0	2	
Control	Exist	10	2	8	1,3 (0,92 – 1,70)
(12)	Total	12	2	10	

Bacterial appearance in triantibiotic paste group showed 2 out of 12 samples disappeared before treatment and increased to 5 out of 12 after treatment with triantibiotic paste. Three out of ten samples showed bacterial dissapearance and no new appeared bacteria was detected after treatment. The relative risk ratio was 3.3, and was statistically significant. In the control group, two out of ten samples showed bacterial dissapearance before treatment, however new bacterial appearance was detected in 2 out of 10 samples. Relative risk was 1.3 and was not statistically significant. This result showed that triantibiotic paste reduced bacterial appearance and prevented the growth of new bacteria..

In triantibotic paste group, there was a possibilty of 3.3 times (RR=3.3) of no new bacterial appearance after triantibiotic paste treatment, however in the control group there was a possibility of 1.3 times of no new bacterial apperance after calcium hydroxide remedication.

DISCUSSION

The success of endodontic treatment is based on appropriate cleaning, shaping, asepsis and filling the root canal. Based on epidemiological data, the success rate of root canal treatment performed by general practitioner is 15% lower than trained specialist. Failure treatment is associated with the presence of bacteria in the apical part of root canal system that have endured or evaded antimicrobial treatment, survived in the filled canal and are capable of inflaming the periapical tissue[17]. In order to sterilize the infected root dentine, antibacterial medicaments are useful. Calcium hydroxide is widely recommended medicament for sterilizing the root canal system. Its high alkalinity is toxic for bacterial cells, and clinical studies have reported high success rate in one-month dressing with calcium hydroxide. On the other hand, some clinical

studies showed no progression of healing in large periradicular lesion following multiple visits of calcium hydroxide used as the dressing material[18]. Therefore in this study, calcium hydroxide is used in the first appointment of both groups to evaluate the bacterial load and the next visit with triantibiotic paste.

The results showed that bacteria could still be observed in both groups following one-week dressing with calcium hydroxide (Table 2). The bacterial load changed significantly follow one-week dressing with triantibiotic paste placed in apical part the root canal (Table 2). Triantibiotic paste consists a combination of ciprofloxacin, metronidazole and minocycline which are bactericidal for diverse of bacteria. Propylene glycol used as the carrier in the paste has been considered to increase its efficacy through diffusion of active components to the deeper layers of infected dentine. This may explained the result obtained in this study. However, in the control group, bacterial load tended to increase although it was nort significantly different (Table 2). This result is in accordance with other study [19] which reported the increase of bacterial load in 7 out of 15 root canals (46.67%) following calcium hydroxide dressing[19]. This may be due to a small number of bacteria resided in dentinal tubules and anastomoses of the root dentine. In addition, calcium hydroxide is capable to increase adhesion of bacteria to collagen and add the invasion to the dentinal tubules which cause resistence to infection. This confirmed the meta-analysis study that calcium hydroxide has limited effectivenss in eliminating bacteria from human root canals[13, 14].

Dressing with local application of triantibiotic paste (TAP) has been reported to be successful in treating non-vital pulp with persisting sinus tract. The result of this study also showed that triantibiotic paste not only kill the bacteria but also prevent them from growing during inter-appointment of root canal treatment (Table 3). Three out of 10 samples showed disappearance of bacteria while no new appeared bacteria was detected. The relative risk of bacterial appeared was 3.3 and was statistically significant. In control group, there was a decrease of bacterial appeared in 8 out of 10 samples, however new bacterial appeared was also observed. The relative risk was 1.3 and was not statistically significant. This might be due incomplete disinfecting of the remaining bacteria in the root canal, especiallyin apical part of the root canal which is challenging to control the bacteria [15-16].

In this study culture method was used to assess the bacterial load. Although it is simple, cultur emethod is still reliable to evaluate the effectiveness of medication during root canal treatment.

CONCLUSION

Under the limitation of this study, can be concluded that triantibiotic paste offers additional antimicrobial advantage over calcium hydroxide, and prevent the growing bacteria therefore it could be used as medicament of non-vital pulp in endodontic therapy.

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