Original Article

In Vitro Evaluation of Flow and Working Time of AH Plus Alone and in Combination with Amoxicillin and Triple Antibiotic Paste

M. Mashhadi Esmaeili [1], M. Zare Jahromi [2], M. Ebrahimi Dastjerdi [2]

[1] Postgradute student, Department of Endodontics, School of Dentistry, Islamic Azad University of Isfahan (Khorasgan) Branch, Isfahan, Iran
[2] Assistant Professor, Department of Endodontics, School of Dentistry, Islamic Azad University of Isfahan (Khorasgan) Branch, Isfahan, Iran

Abstract

Background and Aim: Some researchers have attempted to enhance the antibacterial properties of sealers by addition of antibiotics while this may affect their physical properties. The purpose of this in vitro study was to compare the working time and flow of AH Plus sealer alone and in combination with amoxicillin and triple antibiotic paste (TAP).

Materials and Methods: This in vitro study assessed the flow and working time of AH Plus alone (control group) and in combination with amoxicillin and TAP in 1%,5%, 10% and 25% concentrations according to ANSI/ADA specification NO. 57. The data were analyzed by ANOVA and Tukey's test.

Results: Statistically significant differences were noted in the flow and working time of the control group with those of different concentrations of TAP in combination with sealer ($P<0.0001$). The working time decreased by an increase in the concentration of TAP. The AH Plus/1% TAP and AH Plus/5% TAP showed higher flow than the control group. The AH plus/1%amoxicillin, AH Plus/5% amoxicillin and AH Plus/10% TAP had a flow similar to that of the control group. Other groups showed lower flow than the control group.

Conclusion: All the alterations in the physical properties of AH Plus sealer in combination with amoxicillin and TAP were within the ANSI/ADA specification NO. 57 standard range.

Key Words: Flow, Working Time, AH Plus, Amoxicillin, Triple Antibiotic Paste

- **Cite this article as:** Mashhadi Esmaeili M, Zare Jahromi M, Ebrahimi Dastjerdi M. In Vitro Evaluation of Flow and Working Time of AH Plus Alone and in Combination with Amoxicillin and Triple Antibiotic Paste. J Islam Dent Assoc Iran. 2015; 27(3):126-131.

Corresponding author: M. Zare Jahromi, Assistant Professor, Department of Endodontics, School of Dentistry, Islamic Azad University of Isfahan (Khorasgan) Branch, Isfahan, Iran

m.zare@khuisf.ac.ir

Received: 24 Nov 2014
Accepted: 12 May 2015

Introduction

One important goal in endodontic treatment is to decrease the bacterial count in the dentinal tubules via biomechanical preparation of the root canal system [1,2]. Endodontic instruments, irrigating solutions and intracanal medicaments can significantly decrease microorganisms in the infected root canals [3-5]. Use of antibacterial medications during root canal filling may enable penetration of drugs into dentinal surfaces and irregularities of the root canal system and allow their passage through the apical foramen into the periapical tissues and thus, decrease bacterial count and enhance wound healing [6]. In chronic alveolar infections following pulp necrosis and destruction of periradicular tissues, antibiotics cannot access the infected site due to inadequate blood supply to the region. In such cases, intracanal medicaments and intracanal application of antibiotics are probably more effective than their systemic administration [7]. Side effects are less in this method and a higher concentration of antibiotics is obtained at the infected site [8]. In cases of pulp necrosis and apical periodontitis, selection of an optimal sealer with optimal antibacterial activity can inhibit or decrease the growth and proliferation

of residual microorganisms in the root canal system [9]. However, considering the complexity of root canal infections, use of only one type of antibiotic may not result in complete sterilization of root canal, and a combination of antibiotics may be required to efficiently eliminate microorganisms [10]. Use of multiple antibiotics in the form of a paste was first introduced by Grossman in 1951 for use in the root canal system [11]. It was a mixture of penicillin, bacteriocin, streptomycin and sodium caprylate. Penicillin was incorporated to eliminate Gram-positive microorganisms, bacteriocin was incorporated to eliminate penicillin-resistant bacteria and streptomycin was used to eliminate Gram-negative bacteria. Sodium caprylate was used to eliminate fungi [11]. Thus, due to the presence of a wide range of aerobic and anaerobic bacteria in the root canal system, a mixture of antibiotics referred to as TAP was introduced, which contained metronidazole, ciprofloxacin and minocycline [8,12].

Although it seems that mixing the antibiotics with sealer may be effective for control of bacterial growth and proliferation, it may affect the physical properties of sealer. Durate et al, in 2010 evaluated the effect of addition of 5% and 10% concentrations of calcium hydroxide (CH) to AH Plus sealer on its physical and chemical properties such as flow, solubility, film thickness, dimensional stability after setting, setting time and working time and stated that physical and chemical properties of the combination of AH26 sealer and antibiotic were superior to those of AH26 sealer alone [13]. Kuga et al, in 2014 evaluated the effect of addition of iodoform to AH26 sealer on its physical properties such as setting time, flow, solubility and pH [14]. Two properties of solubility and setting time were significantly affected while the flow did not change [15].

This study aimed to assess the effect of addition of different percentages of amoxicillin and TAP to AH Plus sealer on its physical properties namely flow and working time.

Materials and Methods

In this in vitro, experimental study, flow and working time of AH Plus sealer alone as the control group, AH Plus in addition to 1% amoxicillin (group 1), AH Plus in addition to 5% amoxicillin (group 2), AH Plus in addition to 10% amoxicillin (group 3), AH Plus in addition to 25% amoxicillin (group 4), AH Plus in addition to 1% TAP (including metronidazole, minocycline and ciprofloxacin) (group 5), AH Plus in addition to 5% TAP (group 6), AH Plus in addition to 10% TAP (group 7) and AH Plus in addition to 25% TAP (group 8) were evaluated according to ANSI/ADA specification NO. 57 (in Fall 2008).

First, AH Plus sealer (Dentsply Maillefer, Tulsa, OK, USA) was mixed on a pad in 1:1 ratio according to the manufacturer's instructions to o btain a homogenous mixture. To prepare TAP, metronidazole (Kharazmi Pharmaceuticals, Tehran Iran), minocycline (STADA, Germany) and ciprofloxacin (Kharazmi Pharmaceuticals, Tehran Iran) were mixed in equal amounts to obtain a homogenous powder. To obtain 1%, 5%, 10% and 25% concentrations of amoxicillin (Farabi Pharmaceuticals, Tehran, Iran) and TAP for addition to AH Plus, weight percentage of each component was measured by a digital scale with 0.001g accuracy and they were then mixed. For the flow test, three samples were used in each group; 0.5cc of the samples was transferred (by an insulin syringe) to the center of a glass slab measuring 4×40×40mm with an approximate weight of 20g. After 180 seconds, another glass slab with the same dimensions and weight was placed on the sealer and a 100g weight was placed over it. After 10 minutes, 100g weight was removed and maximum and minimum diameters of sealer compressed between the two glass slabs were measured by a caliper (Inox, Belgium) and recorded. If the compressed discs were not round or minimum and maximum diameters were not within 1mm range, the test was repeated. The test was repeated three times for each sample and the mean value was recorded as the final data.

For working time testing, nine samples of each group (0.5cc each) were transferred to a polyethylene cylinder and stored at room temperature. Three hours after mixing of sealer, one sample of each percentage was placed on a glass slab and the procedures mentioned earlier for flow assessment were repeated. The minimum and maximum diameters of the compressed sealer disc were measured by a caliper. The same procedure was performed for all samples with 15-minute

intervals until the diameter of sealer discs was smaller than the recorded baseline value by 10%. The working time was measured from the beginning of testing to the time when the diameter was 10% smaller than the baseline. The data were analyzed using SPSS version 17, one way ANOVA and Tukey HSD test.

Results

The mean and standard deviation of flow of sealers are shown in Table 1. Based on the results, AH Plus/1% TAP had the highest flow and AH Plus/amoxicillin had the lowest flow rate. Since the data had normal distribution and considering the homogeneity of variances, one-way ANOVA was applied to analyze flow data. A significant difference was noted among the groups in this respect (P=0.001). Thus, Tukey's test was applied for pairwise comparisons of the groups (Diagram 1). The results showed that addition of 25% amoxicillin to sealer significantly decreased its flow compared to the control group (P=0.01). On the other hand, addition of 1% and 5% TAP to sealer significantly increased its flow compared to sealer alone (P=00.001). Regarding working time (Table 2), addition of amoxicillin or TAP at all concentrations significantly decreased the working time compared to the control group (P<0.001). Overall, the higher the concentration of antibiotic, the greater the reduction in working time (Diagram 2).

Discussion

Elimination of microorganisms from the root canal system is among the main goals of endodontic treatment, which is achieved by mechanical cleaning and shaping, use of irrigating solutions, adequate filling of root canal system, and use of intracranial medicaments if necessary in between treatment sessions. However, pathogens may colonize in the dentinal tubules and cause reinfection of the root canal system and periapical tissues [4]. It has been shown that filling of prepared root canals with antimicrobial agents allows for their penetration into dentin and root canal irregularities and also into the periapical tissues and may consequently decrease microbial count and enhance periapical tissue healing [16,17]. Thus, attempts have been made to increase the antimicrobial properties of sealers and their substantivity.

Considering the importance of increasing the antibacterial activity of endodontic sealers by addition of antibiotics and since incorporation of antibiotics may affect physical properties of sealers, this study aimed to assess the effect of addition of different percentages of amoxicillin and TAP on flow and working time of AH Plus sealer. AH plus sealer was used in this study because it is commonly used for this purpose and its antimicrobial properties have been assessed in many previous studies; it has been shown that freshly mixed AH Plus sealer has antimicrobial properties [18,19]. Antimicrobial activity of AH Plus sealer is attributed to the release of formaldehyde during its polymerization and presence of Bisphenol A diglycidyl ether in its composition, which is considered mutagenic [18]. Mild antimicrobial activity of AH Plus against Enterococcus faecalis may be due to the slow release of formaldehyde over time [20].

Selection of TAP for this study was because of its common use in open apex teeth and its favorable clinical efficacy. Different concentrations of amoxicillin were also used in our study due to its effects on E. Faecalis and its common use in endodontic treatments [1].

To assess the physical properties of sealer in our study, ANSI/ADA specification NO. 57 was used [21]. Working time has been defined as the time period from mixing the sealer to its setting (working with it without adversely affecting its properties). Working time depends on the composition of sealer, size of particles, environmental temperature and relative humidity [22]. In the current study, working time decreased following the addition of antibiotics to sealer, which is probably explained by the fact that when added to sealer, antibiotics serve as fillers and decrease the resin/filler ratio and eventually decrease the working time [22]. It should be noted that decreased working time following addition of all concentrations of antibiotics was within the ADA standard range and thus, would have no adverse effect on the clinical efficacy of sealer. On the other hand, decreased working time is somehow favorable because in case of leakage through the apex, set sealer would cause less

Table 1. Working time results (minutes)

Test group	Mean	Standard deviation
AH Plus+1% amoxicillin	341.66	10.40
AH Plus+5% amoxicillin	310.00	8.66
AH Plus+10% amoxicillin	270.000	5.00
AH Plus+25% amoxicillin	221.66	2.88
AH Plus+1% TAP	298.33	12.58
AH Plus+5% TAP	280.00	5.00
AH Plus+10% TAP	246.66	5.77
AH Plus+25% TAP	196.66	7.63
AH Plus alone (control group)	376.66	7.63

Table 2. The flow results (mm)

Test group	Mean	Standard deviation
AH Plus+1% amoxicillin	1.23	0.110
AH Plus+5% amoxicillin	1.28	0.041
AH Plus+10% amoxicillin	1.07	0.112
AH Plus+25% amoxicillin	0.95	0.086
AH Plus+1% TAP	1.70	0.068
AH Plus+5% TAP	1.60	0.070
AH Plus+10% TAP	1.22	0.065
AH Plus+25% TAP	1.03	0.075
AH Plus alone (control group)	1.22	0.058

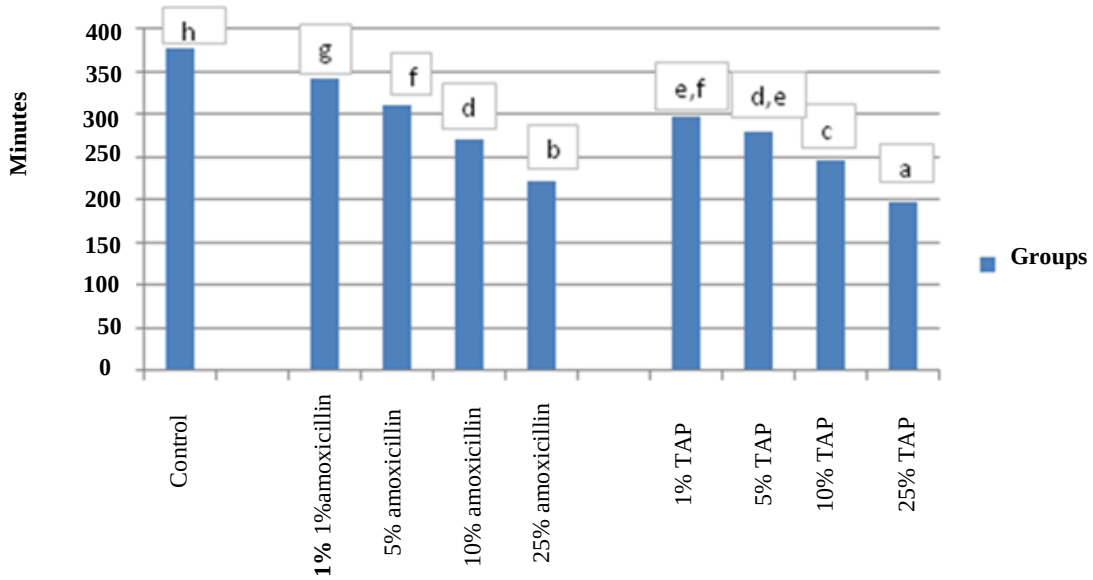

Diagram 1. Results of working time (minutes) in the study groups

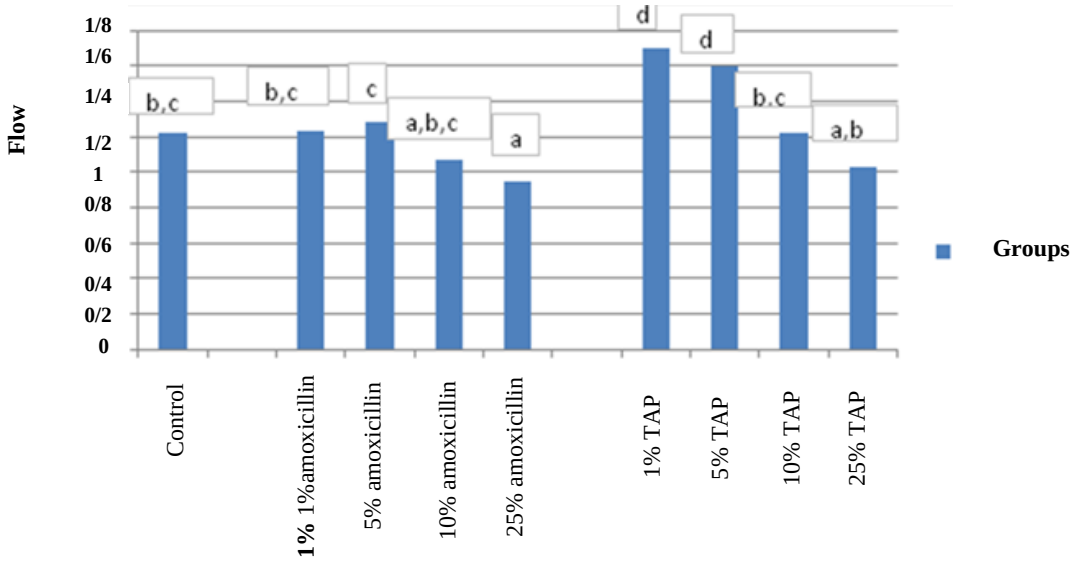

Diagram 2. Results of flow rate (mm) in the study groups

inflammation in the periapical tissue [23].

Flow is another important property of endodontic sealers, which is responsible for penetration of sealer into dentin irregularities and accessory canals of the root canal system. In case of low flow of sealer, it cannot fill the gaps between dentin and root canal filling material. In contrast, if the flow of sealer is too much, it is more likely to pass through the apex into the periapical tissue and since AH Plus sealer is cytotoxic and has poor biocompatibility in its primary stages of setting, it can damage the periapical tissue [24,25]. In the current study, addition of antibiotic to sealer decreased its flow, which may be due to change in its viscosity and can decrease the toxic effects of sealer on periapical tissues (by lowering the likelihood of its passage through the apex).

However, it should be noted that reduction in sealer flow following the use of different concentrations of antibiotic was within the ADA standard range.

Razmi et al. assessed the physical properties of AH 26 sealer in combination with amoxicillin and doxycycline and reported that its combination with 1% amoxicillin and 1% doxycycline decreased its flow within the ADA standard range [14]. Durate et al. evaluated the effect of CH on physical properties of AH Plus sealer and showed that addition of 5% and 10% CH to sealer caused no significant change in its setting time or radiopacity. Also, 10% CH (compared to 5%) combined with sealer decreased the flow of sealer. They showed that addition of 5% and 10% CH to AH Plus sealer increased its solubility and its film thickness [13]. Kuga et al, in 2014 evaluated the effect of addition of iodoform to AH26 sealer powder on its physical properties such as setting time, flow, solubility and pH. They showed that solubility and setting time were significantly affected while flow did not change [15].

Conclusion

The results showed that by an increase in the concentration of antibiotics added to sealer, the flow and working time further decreased; however, these changes were within the ADA standard range; thus, if the results of this study are confirmed by further microbiological and cell culture assessments, addition of antibiotics to AH Plus sealer may be suggested as an alternative for use in the clinical setting.

References

1. Baer J, Maki JS. In vitro evaluation of the antimicrobial effect of three endodontic sealers mixed with amoxicillin. J Endod. 2010 Jul; 36(7): 1170-3.

2. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008 Nov; 34(11): 1291-1301.

3. Sundqvist G, Figdor D, Persson S, Sjögren U.

Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998 Jan; 85(1): 86-93.
4. Slutzky-Goldberg I, Slutzky H, Solomonov M, Moshonov J, Weiss EI, Matalon S. Antibacterial properties of four endodontic sealers. J Endod. 2008 Jun; 34(6):735-8.
5. Hoelscher AA, Bahcall JK, Maki JS. In vitro evaluation of the antimicrobial effects of a root canal sealer-antibiotic combination against Enterococcus faecalis. J Endod. 2006 Feb; 32(2): 145-7.
6. Abbott PV. Selective and intelligent use of antibiotics in endodontics. Aust Endod J. 2000 Apr; 26(1):30-9.
7. Mata E, Koren LZ, Morse DR, Sinai IH. Prophylactic use of penicillin V in teeth with necrotic pulps and asymptomatic periapical radiolucencies. Oral Surg Oral Med Oral Pathol. 1985 Aug;60(2):201-7.
8. Mohammadi Z, Abbott PV. On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology. Int Endod J. 2009 Jul;42(7):555-67.
9. Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. Endod Dent Traumatol. 1996 Aug; 12(4):179-84.
10. Hoshino E, Ando N, Sato M, Kota K. Bacterial invasion of non-exposed dental pulp. Int Endod J. 1992 Jan; 25(1):2-5.
11. Grossman LI. Polyantibiotic Treatment of Pulpless Teeth. J Am Dent Assoc. 1951 Sept; 43 (3):265-278.
12. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J. 1996 Mar; 29(2): 125-30.
13. Duarte MA, Ordinola-Zapata R, Bernardes RA, Bramante CM, Bernardineli N, Garcia RB, et al. Influence of Calcium hydroxide association on. The physical properties of AH Plus. J Endod. 2010 June; 36(6):1048-51.
14. Razmi H, Parvizi S, Khorshidian A. Comparison of AH26 Physicochemical Properties with Two AH26/ Antibiotic Combinations. Iran Endod J. 2010 Winter; 5(1):6-10.
15. Kuga MC, Faria G, Só MV, Keine KC, Santos AD dos , Duarte MAH, et al. The impact of the addition of iodoform on the physicochemical properties of an epoxy-based endodontic sealer. J Appl Oral Sci. 2014 Mar-Apr; 22(2):125-30.
16. Hoelscher AA, Bahcall JK, Maki JS. In vitro evaluation of the antimicrobial effects of a root canal sealer-antibiotic combination against Enterococcus faecalis. J Endod. 2006 Feb; 32(2): 145-7.
17. Kayaoglu G, Erten H, Alaçam T, Ørstavik D. Short-term antibacterial activity of root canal sealers towards Enterococcus faecalis. Int Endod J. 2005 Jul; 38(7):483-8.
18. Fang DY, Lee WC, Lai CH. Antimicrobial activity of eight root canal sealers before and after setting. Rest Dent Endod. 2002 Mar; 27(2):207-211.
19. Mickel AK, Nguyen TH, Chogle S. Antimicrobial activity of endodontic sealers on Enterococcus faecalis. J Endod. 2003 Apr; 29(4): 257-8.
20. Leonardo MR, Bezerra da Silva LA, Filho MT, Santana da Silva R. Release of formaldehyde by 4 endodontic sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999 Aug;88(2):221-5.
21. ANSI/ADA specification no. 57 for endodontic filling materials: J Am Dent Assoc. 1984 Jan; 108 (1);88
22. Vitti RP, Prati C, Silva EJ, Sinhoreti MA, Zanchi CH, de Souza e Silva MG, et al. Physical properties of MTA Fillapex sealer. J Endod. 2013 Jul; 39(7):915-8.
23. Almeida JF, Gomes BP, Ferraz CC, Souza-Filho FJ, Zaia AA. Filling of artificial lateral canals and microleakage and flow of five endodontic sealers. Int Endod J. 2007 Sept; 40 (9): 692-9.
24. Brackett MG, Marshall A, Lockwood PE, Lewis JB, Mosser RL, Bouillagvet S, et al. Cytotoxicity of endodontic materials over 6-weeks ex vivo. Int Endod J. 2008 Dec;41(12):1072-8.
25. Scarparo RK, Grecca FS, Fachin EV. Analysis of tissue reactions to methacrylate resin-based, epoxy resin-based, and zinc oxide-eugenol endodontic sealers. J Endod. 2009 Feb;35(2):229-32.