Antimicrobial Effects of Boric Acid against Periodontal Pathogens

Borik Asitin Periodontal Patojenler Üzerine Olan Antimikrobiyal Etkileri

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ABSTRACT

Purpose: Boron is a bioactive trace element found in humans and essential for the growth and maintenance of bone and also has reported anti-inflammatory and antimicrobial activity. Boric acid is a well-characterised boron-containing compound which reportedly can reduce periodontal inflammation. Thus, the aim of the current study was to evaluate the possible bactericidal and/or bacteriostatic effects of boric acid on the periodontal pathogens: Fusobacterium nucleatum and Porphyromonas gingivalis.

Materials and Methods: Minimum inhibitory concentration (MIC) of boric acid on F. nucleatum (ATCC 10953) and P. gingivalis (ATCC 33277) were determined by the broth microdilution method. Overnight cultures were diluted to the following starting concentrations: 5×10^5 cfu/ml for F. nucleatum and 5×10^6 cfu/ml for P. gingivalis. After incubation for 48h at 37°C in an anaerobic cabinet the absorbance of the cultures was measured. The minimum bactericidal concentration (MBC) was determined by plating an aliquot of the cell suspensions on agar plates, and bacteria were counted after incubation at 37°C for 48 h under anaerobic conditions.

Results: Boric acid was able to inhibit the growth of F. nucleatum at a concentration of 780 nM and P. gingivalis at a concentration of 1.56 µM. The MBC of boric acid was 19 mM for F. nucleatum however using tested concentrations (75 mM-1nm) were insufficient to provide an MBC for P. gingivalis.

Conclusion: Boric acid may be a possible candidate for providing local antimicrobial actions on periodontal pathogens and therefore may have potential as a therapeutic intervention in the management of periodontal disease.

Keywords: *Boric acid, Fusobacterium nucleatum, Porphyromonas gingivalis, periodontal disease, Antimicrobial Drug Resistance, boron*

ÖZ

Amaç: Bor, kemiğin gelişimi ve idamesi için gerekli olup antiinflamatuar ve antimikrobiyal özelliklere sahip biyoaktif eser bir elementtir. Borik asit sıklıkla kullanılan bir bor bileşiği olup periodontal inflamasyonu azaltabildiği gösterilmiştir. Bu çalışmanın amacı borik asitin periodontal patojenlerden Fusobacterium nucleatum and Porphyromonas gingivalis üzerine olabilecek mevcut bakterisidal ve/veya bakteriostatik etkilerini incelemektir.

Gereç ve yöntem: Borik asitin F. nucleatum (ATCC 10953) ve P. gingivalis (ATCC 33277) için minimum inhibitör konsantrasyonu (MİK) sıvı besiyeri mikrodülisyon metodu yardımıyla saptanmıştır. Gecelik kültürler F. nucleatum için 5×10^5 cfu/ml ve P. gingivalis için 5×10^6 cfu/ml konsantrasyonuna seyreltilmiştir. Aaerobik bir kabinde 37°C de 48 saat inkübasyon sonrasında kültürlerin absorbansı ölçülmüştür. Minimum bakterisidal konsantrasyon (MBK) bakteri süspansiyonundan alınan alikotların agarlar üzerinde 37°C de 48 saat boyunca anaerobik koşullarda bekletilmesinden sonra bakterilerin sayılması sonucu elde edilmiştir.

Bulgular: Borik asit F. nucleatum'un üremesini 780 nm da P. gingivalis'in üremesini ise 1.56 µM konsantrasyonda durdurabilmiştir. Borik asitin F. nucleatum için MBK değeri 18.75 nM olarak saptanmış fakat denenen konsantrasyonlar (75 mM-1nm) P.gingivalis için MBK değeri verememiştir.

Sonuç: Borik asit periodontal patojenlere karşı lokal antimikrobial etki gösteren bir aday olabileceğinden dolayı periodontal hastalığın tedavisinde terapötik potansiyeli olabilir.

Anahtar kelimeler: *Borik asit, Fusobacterium nucleatum, Porphyromonas gingivalis, periodontal hastalık, Antimikrobiyal İlaç Direnci, bor.*

INTRODUCTION

Periodontal disease is a chronic inflammatory condition associated with multispecies biofilms causing destruction of periodontal tissues around teeth[1.] The periodontal pathogens *Porphyromonas gingivalis* (*P. gingivalis*), and *Fusobacterium nucleatum* (*F. nucleatum*) present in the dental plaque biofilm are associated with disease progression. *P. gingivalis is a non- motile, Gram negative, obligate anaerobic bacilli found in the subgingival sulcus of the oral cavity* and the numbers of this bacterium found in the periodontal pocket has been positively correlated with periodontitis while levels are lower or almost non-detectable in cases of subgingival health [2.] *F. nucleatum* is a Gram negative anaerobic bacterium that is considered as an intermediate colonizer bridging the attachment of commensals that colonize the tooth and epithelial surface with pathogenic species [3].

During the treatment of periodontal disease reduction of total periodontal pathogen load by subgingival and supragingival mechanical debridement is one of the key treatment steps. However some sub-gingival pathogens may reside in inaccessible areas and, mechanical therapies alone may fail to eliminate these bacteria present in the host tissues. Therefore, the use of antimicrobial therapy, such as application of topical antibiotics and topical antiseptics, adjunct with mechanical debridement has been proposed [4]. Notably the use of systemic antibiotics could lead to an increase in bacterial resistance and superinfections [5]. Re-infection from non-treated sites may also be a problem in the case of use of topical antibiotics [6]. Indeed the application of antimicrobials such as chlorhexidine (CHX) has been reported to have limited success because of its potential cytotoxicity [7] and limited antimicrobial activity within biofilms [8].

Boron is a trace element which is essential for the growth and maintenance of bone [9], and it has the reported ability to improve wound and alveolar bone healing [10]. Boron-containing compounds (BCCs) which possess unique and attractive biological properties including antibacterial, antifungal, antiparasitic, antiviral, and anti-inflammatory activities, have received increasing attention recently [11]. Boric acid is one of the most well-characterised BCCs reported in the literature and has been found to reduce alveolar bone loss [12, 13] and periodontal clinical parameters [14, 15]. A mouthwash form of boric acid was also found superior compared with CHX in chronic periodontitis patients in improving periodontal clinical parameters [16]. However to date no study has evaluated its possible bacteriostatic or bactericidal effects on periodontal pathogens *P. gingivalis* and *F. nucleatum*. Thus the aim of the current study was to investigate the minimum inhibitory concentration (MIC) that shows the inhibitor dose of bacterial growth and minimum bactericidal concentration (MBC) that reflects the non-viability of bacteria treated with boric acid on *P. gingivalis* and *F. nucleatum*.

MATERIALS AND METHODS

Preparation of Boric Acid

Boric Acid (Sigma-Aldrich, MO, USA) was dissolved in ddH_2O [17]. The solutions were sterilized by filter sterilisation using a 0.22 µm syringe-filter unit (Merck, Darmstadt, Germany). Concentrations were prepared ranging between 75 mM to 1 nm.

Bacterial Culture

F. nucleatum (ATCC 10953) was inoculated anaerobically (Don Whitley DG 250 anaerobic workstation, Don Whitley Scientific, West Yorkshire, England; atmosphere: 80% nitrogen, 10% carbon dioxide and 10% hydrogen) at 37 °C onto blood agar plates (Oxoid, Hampshire, UK) from a frozen stock for 48 hours [18].

P. gingivalis ATCC 33277 was also grown onto blood agar plates anaerobically (Don Whitley DG 250 anaerobic workstation, Don Whitley Scientific, West Yorkshire, England; atmosphere: 80% nitrogen, 10% carbon dioxide and 10% hydrogen) (Oxoid, Basingstoke, UK) at 37 °C for 72 hours [18].

A single representative colony from each bacteria was inoculated into 10 ml broth for each bacterial species and cultured for 24 hours. Subsequently, 1 ml of bacterial culture was taken from the overnight culture and read in the spectrophotometer at 600nm (Jenway 7315, Cole Parmer, Staffordshire, UK). The bacterial cell concentrations were determined with OD value according to the growth curve generated [18].

Minimal Inhibitory Concentration (MIC)

MICs of the compound were determined by broth microdilution method previously described [19, 20]. Serial dilutions of the boric acid were performed in Schaedler Anaerobe Broth [21] (at a volume of 100 µL per well in 96-well microtitre plates (Thermo-Fischer Scientific, MA, USA). Each well of a microwell plate was inoculated

with 10 μL of the diluted bacterial culture at a final concentration of 5×10^5 cfu/ml for *F. nucleatum* [19] and 5×10^6 cfu/ml for *P. gingivalis* [19]. After incubation for 24h and 48h at 37°C the absorbance of the plates was measured at 600 nm using a microplate reader (ELX800 absorbance microplate reader, Bio-Tek; USA). The MIC was defined as the lowest concentration of the compounds tested that completely inhibited growth or produced at least 90% reduction in absorbance compared with the negative (compound-free) control. The MIC value represented the average of at least 3 independent experiments [19].

Minimal Bactericidal Concentrations (MBC)

MBC was determined by plating an aliquot of cell cultures (10 μL) from four wells above the MIC on agar plates [22], and bacteria were counted after incubation at 37°C for 48 h. The MBC was defined as the lowest concentration of the compound at which more than 99.9% of the bacteria were killed compared with a non-treated control [19].

RESULTS

Boric acid concentrations of 75 mM to 1 nm were tested against *P. gingivalis* and *F. nucleatum* for MIC determination (Table 1). The MIC of Boric acid was determined as 780 nM for *F. nucleatum*. However, a higher dose 1.56 μM of Boric acid was found to be required for *P. gingivalis* (Table 1).

After determination of the MIC, aliquots were transferred onto agar plates for determination of the MBC.

The MBC value of Boric acid was determined to be considerable higher compared with the MIC value for *F. nucleatum* being 19mM. No MBC could be determined for *P. gingivalis,* concentrations between 75 mM to 1nm could not inhibit the bacteria. (Table 2)

DISCUSSION

Boric acid has previously been reported to have antibacterial effects on some oral bacteria including *Staphylococcus aureus* [23], *Streptococcus mutans* and *Enterococcus faecalis* [16]. However no study has evaluated the effects of Boric acid on *F. nucleatum* and *P. gingivalis*. Determination of MIC and MBC of a potential therapeutic agent for use in disease management is essential to determine the sensitivity and/or resistance of bacteria and, also to monitor their efficacy. In the present study, the MIC values of boric acid determined for *P. gingivalis* and *F. nucleatum* were determined to be in the μM and nM range respectively, but the MBC value of *F. nucleatum* was notably higher (mM range) and, finally no MBC could be determined for *P. gingivalis* within the tested concentration range.

Table 1: MIC of Boric Acid on *F. nucleatum* and *P. gingivalis*

Bacteria	Concentration												
	800 μM	400 μM	200 μM	100 μM	50 μM	25 μM	12.5 μM	6.25 μM	3.12 μM	1.56 μM	780 nM	390 nM	195 nM
F. nucleatum	S	S	S	S	S	S	S	S	S	S	S	R	R
P. gingivalis	S	S	S	S	S	S	S	S	S	S	R	R	R

S: Sensitive, R: Resistance

Table 2: MBC determination of Boric Acid on *F. nucleatum* and *P. gingivalis*

Bacteria	Concentration						
	75 mM	37.5 mM	18.75 mM	9.3 mM	4.68 mM	2.34 mM	1.17 mM
F. nucleatum	N-G	N-G	N-G	G	G	G	G
P. gingivalis	G	G	G	G	G	G	G

N-G: Non-growth, G: Growth

Previously boric acid has been reportedly used for the treatment of periodontal disease in human and animal studies [12-16]. Kanoriya *et al.*[15] evaluated the efficiency of 0.75% subgingivally delivered boric acid in addition to conventional mechanical therapy and found that it significant improved periodontal disease parameters including probing depth and clinical attachment level and reduced the defect depth radiologically compared with the placebo group over a 6 month treatment period. Singhal *et al.*[14] also investigated the same concentration of Boric acid gel in addition to conventional periodontal treatment in the treatment of class II furcation defects at 6 months and reported greater probing depth reduction and clinical attachment gain and also bone formation in the boric acid gel treatment group compared with the placebo group at 6 months. Saglam *et al.*[16] also compared the effects of a 0.75% boric acid mouthwash with chlorhexidine on clinical parameters and bacterial counts for several periodontal pathogens including *P. gingivalis*, *Tannerella forsythia*, and *Treponema denticola* at 3 months in chronic periodontitis patients. They reported a significant reduction in bleeding on probing, probing pocket depth and clinical attachment level in moderate periodontal pockets (probing depth ≥5 and <7) at the 1 month treatment period however no differences were found for bacterial counts in any time period up to 3 months. In the current study the maximum tested concentration was (75mM=0.50%) lower than all of the three clinical studies reported above. The ability of Boric acid to reduce periodontal pathogen infection may be due to the bacteriostatic effects of the compound on *P. gingivalis* that was shown in the current study. However, the compound should also be tested on biofilms as it is known that this growth mode results in bacteria being more resistant to antimicrobial agents [24]. The previously mentioned studies also provided data on the toxicity of these boron containing compounds on gingival fibroblasts and periodontal ligament cells, reporting that 0.75% boric acid did not exhibit any adverse effects. However no data was reported regarding the cytotoxicity of the compounds on osteoblasts or keratinocytes and these are major cell types present in the oral cavity and relevant to periodontitis pathogenesis. Further studies should also be conducted to confirm the safe dose for this compound on these cell types as this would have clinical relevance.

In the literature *in vivo* studies have also evaluated the effects of boric acid as a dietary supplement for the treatment of experimental periodontitis in rats. Saglam *et al.*[12] applied boric acid at 3mg/kg daily for 11 days in rats with experimental periodontitis and they concluded that administration of boric acid may reduce alveolar bone loss by affecting the Receptor activator of nuclear factorkappa B ligand (RANKL)/Osteoprotegerin (OPG) ratio which is a good indicator of bone turnover in experimental periodontitis. Demirer *et al.*[13] also evaluated the effects of the same dose of Boric acid over the same time period (3mg/kg administered daily for 11 days) on periodontal inflammation and osteoblast/osteoclast number in rats with experimental periodontitis. They detected significant improvements for periodontal inflammation and bone formation in rats supplemented with boric acid. However the two studies [12, 13] did not report on any data for bacterial counts preventing any discussion about the compound's antimicrobial effects in experimental periodontitis.

In the literature the effects of boron compounds on microorganisms was also evaluated and Boron containing compounds were found to impair protein synthesis and the activity of certain enzymes including serine-protease, β-lactamase [25]. In the current study although we detected some inhibitory effects of boric acid on *P. gingivalis* and *F. nucleatum* the exact mechanism of action of the compound on the bacteria has not been elucidated and is the focus of future studies.

This is the first study to show the antimicrobial effects of boric acid *on P. gingivalis* and *F. nucleatum*. Therefore, boric acid may have potential to use as an antimicrobial agent including mouthwash and gel in the management of periodontal disease. In future, bone protective and anti-inflammatory effects of boric acid will also be assessed *in vitro* that may also extend the use of boric acid in the field of periodontology.

CONCLUSION

Our findings established MICs of 780 nM and 1.56 μM respectively of boric acid for both *F. nucleatum* and *P. gingivalis*. Therefore, boric acid has antimicrobial effects towards periodontal pathogens, that can make it a desirable antimicrobial agent in mouthwash or subgingival gel for periodontal disease management. Further work is required to evaluate the possible anti-inflammatory potential and bone protective effects of boric acid on periodontal inflammation *in vitro*, which may widen the application of boric acid in periodontology.

REFERENCES

1. Kinane DF, Lappin DF. Immune processes in periodontal disease: a review. *Ann Periodontol* 2002;7:62-71.

2. Schmidt J, Jentsch H, Stingu CS, Sack U. General immune status and oral microbiology in patients with different forms of periodontitis and healthy control subjects. *PLoS One* 2014;9:e109187.

3. Kolenbrander PE. Oral microbial communities: biofilms, interactions, and genetic systems. *Annu Rev Microbiol* 2000;54:413-437.

4. Slots J. Selection of antimicrobial agents in periodontal therapy. *J Periodontal Res* 2002;37:389-398.

5. Heta S, Robo I. The Side Effects of the Most Commonly Used Group of Antibiotics in Periodontal Treatments. *Med Sci (Basel)* 2018;6.

6. Mombelli A, Samaranayake LP. Topical and systemic antibiotics in the management of periodontal diseases. *Int Dent J* 2004;54:3-14.

7. Mariotti AJ, Rumpf DA. Chlorhexidine-induced changes to human gingival fibroblast collagen and non-collagen protein production. *J Periodontol* 1999;70:1443-1448.

8. Walker CB, Karpinia K, Baehni P. Chemotherapeutics: antibiotics and other antimicrobials. *Periodontol 2000* 2004;36:146-165.

9. Benderdour M, Bui-Van T, Dicko A, Belleville F. In vivo and in vitro effects of boron and boronated compounds. *J Trace Elem Med Biol* 1998;12:2-7.

10. Gorustovich AA, Steimetz T, Nielsen FH, Guglielmotti MB. Histomorphometric study of alveolar bone healing in rats fed a boron-deficient diet. *Anat Rec (Hoboken)* 2008;291:441-447.

11. Yang F, Zhu M, Zhang J, Zhou H. Synthesis of biologically active boron-containing compounds. *Medchemcomm* 2018;9:201-211.

12. Saglam M, Hatipoglu M, Koseoglu S, Esen HH, Kelebek S. Boric acid inhibits alveolar bone loss in rats by affecting RANKL and osteoprotegerin expression. *J Periodontal Res* 2014;49:472-479.

13. Demirer S, Kara MI, Erciyas K, Ozdemir H, Ozer H, Ay S. Effects of boric acid on experimental periodontitis and alveolar bone loss in rats. *Arch Oral Biol* 2012;57:60-65.

14. Singhal S, Pradeep AR, Kanoriya D, Garg S, Garg V. Boric acid gel as local drug delivery in the treatment of class II furcation defects in chronic periodontitis: a randomized, controlled clinical trial. *J Investig Clin Dent* 2018;9.

15. Kanoriya D, Singhal S, Garg V, Pradeep AR, Garg S, Kumar A. Clinical efficacy of subgingivally-delivered 0.75% boric acid gel as an adjunct to mechanotherapy in chronic periodontitis: A randomized, controlled clinical trial. *J Investig Clin Dent* 2018;9.

16. Saglam M, Arslan U, Buket Bozkurt S, Hakki SS. Boric acid irrigation as an adjunct to mechanical periodontal therapy in patients with chronic periodontitis: a randomized clinical trial. *J Periodontol* 2013;84:1297-1308.

17. Tepedelen BE, Soya E, Korkmaz M. Boric Acid Reduces the Formation of DNA Double Strand Breaks and Accelerates Wound Healing Process. *Biol Trace Elem Res* 2016;174:309-318.

18. Hirschfeld J, White PC, Milward MR, Cooper PR, Chapple ILC. Modulation of Neutrophil Extracellular Trap and Reactive Oxygen Species Release by Periodontal Bacteria. *Infect Immun* 2017;85.

19. Wei GX, Campagna AN, Bobek LA. Effect of MUC7 peptides on the growth of bacteria and on Streptococcus mutans biofilm. *J Antimicrob Chemother* 2006;57:1100-1109.

20. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clinical Microbiology and Infection* 2003;9:1-7.

21. Rotilie CA, Fass RJ, Prior RB, Perkins RL. Microdilution technique for antimicrobial susceptibility testing of anaerobic bacteria. *Antimicrob Agents Chemother* 1975;7:311-315.

22. Leandro LF, Moraes Tda S, de Oliveira PF, et al. Assessment of the antibacterial, cytotoxic and mutagenic potential of the phenolic-rich hydroalcoholic extract from Copaifera trapezifolia Hayne leaves. *J Med Microbiol* 2016;65:937-950.

23. Sayin Z, Ucan US, Sakmanoglu A. Antibacterial and Antibiofilm Effects of Boron on Different Bacteria. *Biol Trace Elem Res* 2016;173:241-246.

24. Adriztina I, Adenin LI, Lubis YM. Efficacy of Boric Acid as a Treatment of Choice for Chronic Suppurative Otitis Media and Its Ototoxicity. *Korean J Fam Med* 2018;39:2-9.

25. Yilmaz MT. Minimum inhibitory and minimum bactericidal concentrations of boron compounds against several bacterial strains. *Turk J Med Sci* 2012;42:1423-1429.