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Effect of boric acid and calcium ascorbatoborate esters against methicillin-resistant *Staphylococcus aureus* strain

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Abstract

Antibacterial studies of calcium ascorbatoborate esters (CABEs) – monoesters and diesters – and boric acid against methicillin-resistant Staphylococcus aureus (MRSA) highlighted a significant activity. Our study has confirmed and supported the effectiveness of calcium ascorbatoborate diesters (CABDs) and calcium ascorbatoborate monoesters (CABMs) as an antimicrobial coating due to their capacity to suppress the bacterial proliferation. The best in vitro response was found for the CABMs, which exhibited the highest antibacterial activity.

Keywords: *boric acid, calcium ascorbatoborate, monoesters, diesters, methicillin-resistant Staphylococcus aureus*

1. Introduction

The bacteriostatic or antiseptic effect of boric acid has been known for a long time. Boron compounds are essential micronutrients for many organisms and play important roles in the plant life. However, in large amounts, boron is also toxic to living cells. The gap between boron deficiency and toxicity is fairly small for all living organisms. Boron is involved in quorum sensing, an important mechanism in establishing antimicrobial activity (R.D. HOULSBY & al. [1]; S. WATANABE & al. [2]; O. REICHMAN & al. [3]). *Staphylococcus aureus* is a Gram-positive bacterium. The emergence of antibiotic-resistant forms of pathogenic *S. aureus*, *e.g.*, methicillin-resistant *S. aureus* (MRSA), is a worldwide problem in clinical medicine. *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of post-surgical wound infections. Each year, about 500,000 patients contract a staphylococcal infection in

the United States (U.S.) hospitals. *S. aureus* is extremely prevalent in persons with atopic dermatitis. It is mostly found in fertile, active places, including the armpits, hair, and scalp. Large pimples that appear in those areas may exacerbate the infection if lacerated (R. DANTES & al. [4]; G.M. ANSTEAD & al. [5]). MRSA is one of the many greatly feared strains of *S. aureus*, which have become resistant to most *β*-lactam antibiotics. A recent study by the Translational Genomics Research Institute showed that nearly half (47%) of the meat and poultry in the U.S. grocery stores were contaminated with *S. aureus*, with more than half (52%) of those bacteria resistant to antibiotics. The number of staphylococcal infections continues to increase – in parallel with the increased use of intravascular devices –, while the treatment of these infections becomes even more difficult because of the emergence of staphylococcal strains resistant to multiple antibiotics, including vancomycin. In U.S. and United Kingdom, 40% to 60% of nosocomial *S. aureus* strains are multidrug resistant. The mortality rate due to methicillin-resistant *versus* methicillin-sensitive *S. aureus* infections is almost threefold higher. This context explains the urgency in developing new antibacterial dietary supplements (A.E. WATERS & al. [6]).

Boric acid has been commonly used in small quantities in cosmetic formulations as an antiseptic agent. Boric acid has antiseptic and antiviral activity. Aqueous solutions of boric acid have been used as mouthwashes, eye drops, skin lotions and cosmetics. Various boron-containing materials have been synthesized and tested for antimicrobial activity (L. DINCA & R. SCOREI [7]). Boric acid reacts with polyhydroxy compounds as a Lewis acid to form complexes in aqueous solutions, producing tetrahedral anionic compounds. Besides some studies about the antimicrobial behavior of amine borate materials, to our knowledge, no biocompatibility and antimicrobial investigations about the ascorbatoborate esters have been reported to date (P.D. MEERS & C.K. CHOW [8]; D.A. KÖSE & B. ZÜMREOGLU-KARAN [9]; A.S. SEE & al. [10]; D.A. KÖSE & B. ZÜMREOGLU-KARAN [11]).

In this study, we highlighted the *in vitro* antibacterial properties for the calcium ascorbatoborate esters (CABEs) and boric acid against the MRSA strain. It was found that MRSA might have difficulty in acquiring resistance to boric acid.

2. Materials and methods

2.1. CABEs synthesis: The calcium ascorbatoborate complexes were isolated in salt form with Ca^{2+} ions by flash precipitation from aqueous solutions. The complexation reaction was based on the addition of the anionic ascorbate ligand to the Lewis acid boron center, followed by water elimination between the hydroxy groups of the organic ligand and boric acid through esterification (D.A. KÖSE & B. ZÜMREOGLU-KARAN [11]). The complexes were prepared in the form of mono-chelate (1:1) and *bis*-chelate (1:2) borate esters, which are readily soluble in water but slowly undergo hydrolytic dissociation to boric acid and ascorbate in aqueous solutions, as indicated by liquid nuclear magnetic resonance (NMR) studies (D.A. KÖSE & B. ZÜMREOGLU-KARAN [11]).

2.2. Bacterial strain and culture media: The biological material consisted of a *S. aureus* wild-type strain isolated from the throat. *Staphylococcus* isolates were evaluated for methicillin resistance using the agar diffusion technique (J.L. WATTS & al. [12]). Oxacillin (30 μg) antibiotic discs (Oxoid Limited, Basingstoke, Hampshire, England) were used. The assay was conducted using the agar well diffusion assay (C. PEREZ & al. [13]). Studies of bacterial sensitivity towards the CABEs were carried out and the diameters of the inhibition zones of the bacteria using the ascorbatoborate esters and boric acid were measured. *S. aureus* culture was grown overnight on Müller–Hinton agar. Bacteria were selected using a sterile wooden inoculating stick and a bacterial suspension of approximately 1×10^8 colony-forming units

(CFU)/mL was prepared in 0.1% peptone water. The optical density measurement was used to approximate the bacterial concentration. The bacterial suspension was diluted to 10^7 CFU/mL in tryptic soy broth (TSB). Serial dilutions of the TSB suspension were made and plated on Müller–Hinton agar in order to determine the concentration of bacteria in the inoculum, CFU/spot, following recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) for sensitivity testing (M.B. COYLE & al. [14]). All measurements were performed in triplicate. The results were read after overnight incubation at 37^0C and the antimicrobial activity was quantified.

2.3. Statistical analysis: Student's *t*-test was applied for the analysis of experimental data. The differences were considered statistically significant for $p<0.05$.

3. Results and discussion

It was found that the calcium ascorbatoborate diester (CABD) has a bactericidal action stronger than boric acid. The calcium ascorbatoborate monoesters (CABMs) have the best antimicrobial activity. As it can be seen from the statistically significant experimental data (Tables 1 and 2; Figures 1 and 2), the diameter of inhibition zone of CABEs is higher than the boric acid.

Table 1. Diameter of inhibition zone [mm] determined for boric acid on MRSA strain (agar well diffusion assay)

	Diameter of inhibition zone (mean±SD) [mm]						
Boron concentration [mg/mL]	Control (0)	0.1	0.2	0.3	0.5	0.8	1.0
Boric acid	NI	NI	NI	6.1±0.1	6.1±0.1	6.2±0.1	6.4±0.1

MRSA: Methicillin-resistant *S. aureus*; SD: Standard deviation; NI: No inhibition.

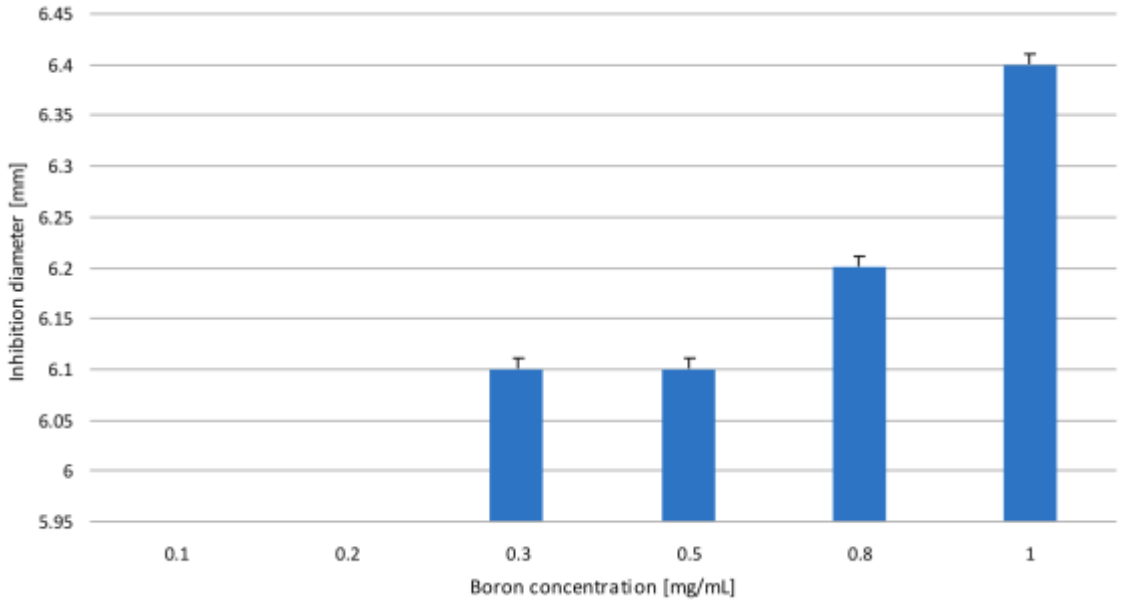

Fig. 1. Effect of boric acid on the growth of MRSA strain.

Table 2. Diameter of inhibition zone [mm] determined for CABEs on MRSA strain (agar well diffusion assay)

	Diameter of inhibition zone (mean±SD) [mm]						
Boron concentration [mg/mL]	Control (0)	0.01	0.02	0.05	0.08	0.1	0.2
CABMs	NI	NI	6.1±0.1	6.4±0.1	6.7±0.2	6.7±0.2	6.8±0.2

CABD	NI	NI	NI	NI	NI	6.2±0.1	6.2±0.1

CABEs: Calcium ascorbatoborate esters; MRSA: Methicillin-resistant *S. aureus*; SD: Standard deviation; CABMs: Calcium ascorbatoborate monoesters; CABD: Calcium ascorbatoborate diester; NI: No inhibition.

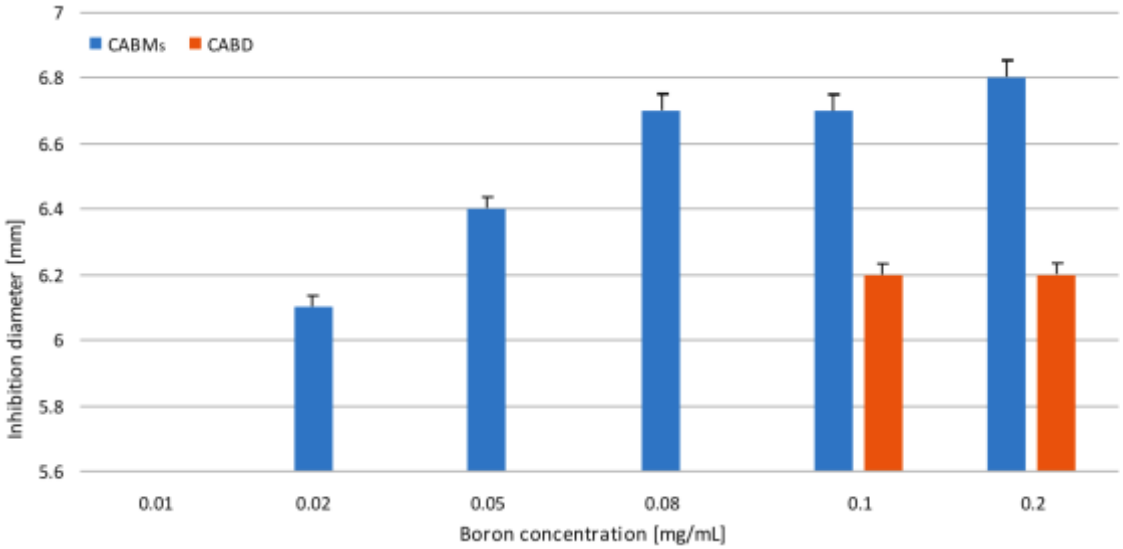

Fig. 2. Effect of CABEs on the growth of MRSA strain. CABEs: Calcium ascorbatoborate esters; CABMs: Calcium ascorbatoborate monoesters; CABD: Calcium ascorbatoborate diester.

The growth of methicillin-resistant *S. aureus* wild-type strain was completely inhibited by 0.3 mg/mL boron from the boric acid added to the culture media. The minimum inhibitory concentrations (MICs) were distributed between 0.2 and 0.3 mg/mL. Compared with about 5–25 years ago, the antibacterial effect of boric acid against *S. aureus* has not changed. Other scientific papers highlighted similar results for boric acid (S. WATANABE & al. [2]; C. KATSUKAWA & al. [15]; F. HAESEBROUCK & al. [16]; YILMAZ [17]). This fact shows that MRSA might have difficulty in acquiring resistance to boric acid. The effect of boric acid suggests that this formerly used reagent might be able to be used to treat MRSA infections. Biological properties of CABEs are crucial because of the growing interest in using them in biomedical applications. Many potential applications of ascorbatoborate esters are based on their boron transport capacity. This specific property makes ascorbatoborates suitable for a variety of high technological uses, including biomedical and industrial applications. The results show that two mechanisms may be responsible for the antimicrobial properties of borate esters: boron delivery and the interaction of the synthesized boron compounds with the cellular membranes. The anionic nature of ascorbatoborate esters leads to the interaction with the electropositive bacterial cell surface and the disruption of the barrier properties of the outer membrane of Gram-positive bacteria. However, the exact mode of action of the CABEs on the tested microorganism needs more investigation and is actually under study in our laboratories.

4. Conclusions

Our results have shown that complexation of ascorbic acid with boron provides an interesting way to produce antimicrobial coatings. The synthesized CABEs exhibited lower cytotoxicity, suggesting that the macromolecule is a biocompatible material. Preliminary studies of the antimicrobial activity suggest that CABEs may be useful to prevent the bacterial contamination and may act effectively against *S. aureus.* Additionally, the results show that

the inhibition increased by increasing the concentration of CABEs, which may indicate a strong interaction with the cell membrane of the microorganism. The boron delivery or the interaction of the polyanionic borate esters might be another factor responsible for the antimicrobial properties. The best *in vitro* response was found for CABEs with high monoester *vs.* diester ratio. The results show that the whole ascorbatoborate molecule is more active than the boric acid molecule. If we consider the boron level, 0.01–0.02 B mg/mL in CABEs predominantly monoesters *vs.* 0.2–0.3 mg/mL B in boric acid, *i.e.*, ascorbatoborate monoesters are about 30 times more active than boric acid.

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