Cerium, chitosan and hamamelitannin as novel biofilm inhibitors?

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Objectives: The colonization of indwelling medical devices and subsequent biofilm formation represents a global challenge since it promotes the persistence of infection and contributes to antimicrobial resistance. The aim of this study was to determine the antimicrobial activity of cerium, chitosan and hamamelitannin against usual microbial colonizers and to assess their efficacy regarding biofilm formation on polyurethane (PUR)-like catheters.

Methods: The antimicrobial and anti-biofilm effect of cerium nitrate, low molecular weight chitosan (LMWC) and hamamelitannin was tested against Staphylococcus epidermidis, Staphylococcus aureus, Acinetobacter baumannii and Candida albicans strains. Biofilm formation was assessed with PUR-like catheter segments and the metabolic activity was quantified by colorimetry with a tetrazolium reduction assay.

Results: Cerium nitrate and LMWC inhibited the microbial growth of all microbial strains tested; hamamelitannin showed no inhibition. Regarding biofilm formation on PUR-like catheters, with subinhibitory concentrations: cerium nitrate significantly inhibited the metabolic activity of C. albicans; LMWC reduced the metabolic activity of S. epidermidis and C. albicans; and hamamelitannin decreased the metabolic activity of all tested bacteria, but not of yeasts.

Conclusions: The microbicidal activity of cerium nitrate and LMWC was clearly demonstrated in this study, as was their fungistatic effect at lower concentrations. Hamamelitannin significantly reduced biofilm metabolic activity of all tested bacteria. These microbial inhibitors may play a promising role regarding different biomedical applications.

Keywords: catheter-related bloodstream infections, central venous catheters, S. epidermidis, S. aureus, A. baumannii, C. albicans, cerium nitrate, low molecular weight chitosan, hamamelitannin

Introduction

The colonization of medical indwelling devices through microbial adhesion and subsequent biofilm formation may precede bacteremia and sepsis in critically ill patients. Catheter-related bloodstream infections (CRBSIs) have been shown to result in longer hospital stays, increased costs and mortality.1 Staphylococcus epidermidis, Staphylococcus aureus, Acinetobacter baumannii and Candida albicans are among the most frequently isolated microorganisms from central venous catheters (CVCs).2 Preventive methods have been suggested to reduce the incidence of CRBSIs, such as the coating of catheters with antibiotics or antimicrobials. Nevertheless, more effective and less toxic alternatives would be highly desirable.

Cerium is a rare earth element belonging to the lanthanide group. Cerium nitrate has been in use for a long time in the management of burns. Its direct antimicrobial properties remain controversial,3 but the uptake of cerium into the cytoplasm with inhibition of cellular respiration, inhibition of glucose metabolism and disruption of the cell membrane have been the postulated mechanisms.

Chitosans are polyamino saccharides obtained by deacetylation of naturally occurring chitin, with biocompatibility. Chitosans are being investigated for their broad spectrum of antimicrobial activity, which has been explained by (i) cell wall leakage by ionic surface interaction or by teichoic acid binding and extraction of membrane lipids, (ii) mRNA and protein synthesis
inhibition and (iii) suppression of microbial growth through external barrier formation and metal chelation. Hamamelitannin is a naturally occurring polyphenol extracted from the bark of *Hamamelis virginiana* that belongs to the family of tannins. It is the ester of D-hamamelose (2-hydroxy-methyl-D-ribose) with two molecules of gallic acid. Hamamelitannin seems to prevent graft-associated infections caused by the staphylococci tested so far (including methicillin-resistant *S. aureus* and *S. epidermidis*) by inhibiting the quorum sensing system of such bacteria, thereby reducing their virulence.

The aim of this study was to determine the antimicrobial activity of cerium nitrate, low molecular weight chitosan (LMWC) and hamamelitannin against microbial strains usually involved in CRBSIs and to test their efficacy regarding biofilm formation on polyurethane (PUR)-like catheters.

## Materials and methods

### Chemicals

Cerium nitrate [cerium(III) nitrate hexahydrate; Sigma-Aldrich], LMWC (107 kDa, 75%–85% decyslated; Sigma-Aldrich; stock solution of 4.0×10³ mg/L prepared in 1% acetic acid, pH 4.0) and hamamelitannin [hamamelofuranose 2;5-digallate, 2-C-(hydroxyethyl)-D-ribofuranose 2;5-digallate; Sigma-Aldrich] were used in the experiments.

### Microbial strains

A type strain and a clinical isolate of each of four microbial species were used. The type strains from the ATCC were *S. aureus* ATCC 25923, *S. epidermidis* ATCC 155, *A. baumannii* ATCC 19606 and *C. albicans* ATCC 90028. The clinical strains of *S. aureus* (SA1), *S. epidermidis* (SE1), *A. baumannii* (AB1) and *C. albicans* (CA1) had been previously isolated from cultures of CVCs removed from critical care patients. All clinical isolates had been identified by the Vitek system (bioMérieux, Vercieux, France).

### Antimicrobial activity

The MICs of chemicals were determined according to the CLSI microdilution reference protocol M07-A87 for bacteria and protocol M27-A38 for yeasts. The type strains from the ATCC were identified by the Vitek system (bioMérieux, Vercieux, France).

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### Biofilm formation on PUR-like catheters

The strains were grown overnight and cells were harvested by centrifugation, washed with PBS and standardized to 1×10⁷ cells/mL in LB broth for bacteria and 1×10⁶ cells/mL in RPMI (Sigma-Aldrich) for yeasts. The antibiofilm effect of the different test compounds was evaluated in the presence of four concentrations of cerium nitrate (1.7×10³, 1.3×10³, 6.5×10² and 3.3×10² mg/L) and three of hamamelitannin (100, 80 and 50 mg/L). Regarding LMWC, concentrations tested were 625, 160 and 78 mg/L for bacteria and 1.0×10⁴, 5.0×10³ and 625 mg/mL for yeasts. One single fragment of PUR-like intravenous catheters (BD Vialon™, 16G, 1.7×45 mm), with 1 cm length, was placed in each well of 12-well microplates, containing 1 mL of the standardized microbial suspensions.

After incubation (24 h for bacteria and 24 and 48 h for yeasts), at 37°C, catheter fragments were removed, gently washed with PBS and placed in new microplates to assess the biofilm metabolic activity with XTT, as previously described. All the assays were performed in triplicate.

### Data analysis

Biofilm metabolic activity in the presence of cerium nitrate, LMWC and hamamelitannin was expressed as the percentage in relation to the control. Values were divided by 1000 and arc sine square root transformed to achieve a normal distribution and homoscedasticity. For each compound, each concentration and each bioorganism, biofilm formation was compared by one-way ANOVA, followed by a Dunnett’s test to identify significant effects; a *P* value <0.05 was considered significant.

### Results

### Antimicrobial activity

Cerium nitrate MICs ranged between 3.3×10² and 1.3×10³ mg/L for bacterial strains. For yeasts, concentrations up to 2.6×10² mg/L were needed. Globally, MLCs corresponded to ≥2× the MICs for all microbial species (Table 1).

For LMWC, MICs ranged between 1.6×10² and 3.1×10² mg/L for all bacteria. A higher concentration (1.0×10⁴ mg/L) was needed to inhibit *C. albicans* growth. The MLCs were nearly ≥2× the MICs for all bacterial species. The MLC and MIC were the same for *C. albicans* (Table 1).

Hamamelitannin resulted in no inhibition of microbial growth, at concentrations ranging from 10 to 100 mg/L.

### Biofilm formation on PUR-like catheters

Cerium nitrate, at a concentration of 1.7×10³ mg/L, reduced the mean biofilm metabolic activity of *C. albicans* (24 h) by 58.9% (+7.4%) and of *C. albicans* (48 h) by 59.14% (+13.0%). No
Figure 1. Effect of cerium nitrate (a, b and c), LMWC (d, e and f) and hamamelitannin (g, h and i) on biofilm formation (as a percentage of the control) by Gram-positive cocci, Gram-negative bacteria and yeasts (at 24 and 48 h). Differences between control and antimicrobial groups were statistically significant (P < 0.01).
relevant biofilm inhibition was found for bacterial strains with cerium nitrate at concentrations lower than the MLC (Figure 1a, b and c).

LMWC, at a concentration of 78 mg/L, reduced the biofilm metabolic activity of S. epidermidis by 80.46% (±0.0%) (Figure 1d). At a concentration of 5.0×10^3 mg/L, LMWC reduced C. albicans biofilm metabolic activity at 24 h by 87.5% (±0.0%) and at 48 h by 90.06% (±0.0%) (Figure 1f). However, inconsistent results were found with A. baumannii and S. aureus (Figure 1d and e).

Hamamelitannin, at a concentration of 100 mg/L, decreased significantly the mean biofilm metabolic activity of S. aureus by 23.0% (±10.7%), of S. epidermidis by 42.2% (±3.78%) and of A. baumannii by 31.8% (±0.88%) (Figure 1g and h). Inconclusive results were found with C. albicans strains at 24 and 48 h (Figure 1i).

Discussion

A review conducted by Garner and Heppell,4 gathered information on the bacteriostatic effect of cerium nitrate against a panel of bacteria. Our study not only adds to this effect, but also documents unequivocally the microbicidal effect of cerium nitrate against bacteria and yeasts. This might be quite useful in medical applications because toxicity is rare with lanthanides.

Concerning cerium nitrate microbial growth inhibition, our results indicate MICs for S. aureus similar to previous descriptions,6 while determining for the first time MICs for S. epidermidis, A. baumannii and C. albicans. Furthermore, the so far unclear cerium nitrate microbicidal effect was documented and MLCs were established. At lower concentrations, cerium nitrate was only effective against C. albicans biofilm.

The broad spectrum of antimicrobial activity of chitosans depends on several factors, including the molecular weight.10 However, inconclusive data regarding the actual efficacy of LMWC is available.

Although there is a tendency in the literature to characterize chitosans as bacteriostatic and fungstatic, as stated by Goy et al.5 in 2009, our results suggest unequivocally that LMWC 107 kDa exhibits a microbicidal effect (Table 1). At subinhibitory concentrations, a significant reduction of the biofilm metabolic activity was only found for S. epidermidis and C. albicans.

Hamamelitannin produced no inhibition of microbial growth, as expected. However, regarding the experiments on PUR-like catheter segments, inhibition of the biofilm metabolic activity was found at a concentration of 100 mg/L for all tested bacteria. The inhibition of the quorum sensing system of Gram-positive bacteria, such as S. aureus and S. epidermidis, has already been documented by Kiran et al.6 in 2008. The biofilm inhibitory effect upon a Gram-negative bacterium was originally described by our study. A. baumannii is a conspicuous pathogen usually related to multiresistance to antimicrobials in intensive care patients.

Nowadays, medical device colonization constitutes a global threat that may contribute to antimicrobial resistance. The microbicidal effect of cerium nitrate and LMWC upon usual CVC colonizers was clearly demonstrated. Moreover, at lower concentrations, both were found to be fungistatic. Hamamelitannin inhibited the biofilm metabolic activity of all tested bacteria: for A. baumannii, this effect was an original description. Further studies are being conducted in order to clarify the mechanism of biofilm inhibition and the endovascular biocompatibility of these inhibitors, which may play a promising role in future biomedical applications.

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Transparency declarations

None to declare.

References