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Activity of sodium metabisulfite against planktonic and biofilm *Staphylococcus* species

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Abstract

Biofilm-forming staphylococci cause a majority of intravascular catheter-related infections. We evaluated the effect of sodium metabisulfite, a preservative commonly added to intravenously administered pharmaceuticals as an antioxidant and previously used as a catheter lock solution, on planktonic and biofilm staphylococci at clinically encountered concentrations. Sodium metabisulfite exhibited bactericidal activity against planktonic *Staphylococcus aureus*, *Staphylococcus lugdunensis*, and *Staphylococcus epidermidis* at concentrations of 512, 512, and 1024 μg/mL, respectively. A concentration of 720 μg/mL inhibited cell growth by all 3 species in a biofilm formation assay. However, established *S. aureus* and *S. lugdunensis* biofilms showed less than 1.5 $\log_{10}$ decreases in viable cell counts when treated with 720 μg/mL of sodium metabisulfite for 24 h. These in vitro results suggest that the use of sodium metabisulfite as a catheter lock may inhibit staphylococcal colonization of catheters, thereby preventing catheter-related infection.

Keywords: *Staphylococcus epidermidis*; *Staphylococcus aureus*; *Staphylococcus lugdunensis*; Catheter-related infection; Sodium metabisulfite

1. Introduction

Intravascular catheter-related infection accounts for a substantial number of nosocomial bloodstream infections each year, contributing to extended hospitalization and increased costs (Mermel et al., 2001; O'Grady et al., 2002). *Staphylococcus aureus* and coagulase-negative staphylococci are the microorganisms most frequently associated with intravascular catheter-related infection (O'Grady et al., 2002), due to their ability to attach to catheter surfaces and form biofilms (von Eiff et al., 2005).

As opposed to bacteria that are unattached to surfaces (i.e., planktonic bacteria), infections caused by bacteria growing in biofilms are characterized by production of a self-excreted extracellular matrix (Donlan and Costerton, 2002). Biofilm bacteria exhibit an altered physiologic state that promotes evasion of host immune defenses and decreases susceptibility to antimicrobial agents at conventional therapeutic levels, rendering biofilm-associated infections challenging and expensive to treat (Donlan and Costerton, 2002; von Eiff et al., 2005). Removal of infected catheters is warranted in many circumstances (Mermel et al., 2001). However, removal of surgically implanted catheters intended for prolonged usage in patients receiving parenteral nutrition, hemodialysis, or cancer chemotherapy constitutes a costly and complicated situation (Segarra-Newnham and Martin-Cooper, 2005; von Eiff et al., 2005).

Sodium metabisulfite ($Na_2S_2O_5$, SMBS) is an inorganic sulfite commonly used as a preservative in some wines and foods. SMBS also serves as an antioxidant in numerous cosmetic products and several pharmaceuticals. Despite the widespread use of SMBS in products intended for human use, few studies have examined its antimicrobial properties against human pathogens (Abalaka and Deibel, 1980;

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Freeman et al., 1982). To our knowledge, the susceptibility of pathogenic bacteria to SMBS under clinically relevant circumstances has not been previously reported.

We initially observed that a diluted inotrope preparation (dopamine hydrochloride injection, USP; American Regent, Shirley, NY) containing approximately 0.7 mg/mL of SMBS had an antimicrobial effect on staphylococci (unpublished). We hypothesized that SMBS may be active in the prevention or treatment of catheter-related staphylococcal biofilm infections. Therefore, we measured planktonic and biofilm susceptibilities to SMBS and determined the effect of SMBS on biofilm formation for 3 staphylococcal species.

2. Materials and methods

2.1. Bacterial strains and chemicals

Staphylococcus epidermidis ATCC 35984 (RP62A), *S. aureus* ATCC 35556 (SA113), and *Staphylococcus lugdunensis* IDRL-5258, a prosthetic knee infection isolate, were studied. All 3 strains are *icaA* positive (Cramton et al., 1999; Heilmann et al., 1996b, K.L. Frank and R. Patel, unpublished data), indicating that these strains are genetically capable of forming biofilm through at least 1 well-characterized mechanism of staphylococcal biofilm formation (Fitzpatrick et al., 2005). *S. aureus* ATCC 29213 was used as a reference strain for MICs and minimum bactericidal concentrations (MBCs). Isolates from freezer stocks stored at −70 °C were freshly streaked on tryptic soy agar before each experiment. All incubations were at 37 °C in ambient air.

Stock solutions of SMBS (9 mg/mL) (NF/FCC grade; Fisher Chemicals, Fair Lawn, NJ) were freshly prepared and filter sterilized immediately before use in susceptibility assays. A filter sterilized stock solution was stored at room temperature for microtiter plate assays.

2.2. MICs and MBCs

Standard- and high-inoculum MIC and MBC assays were performed 3 times in cation-adjusted Mueller–Hinton broth, according to the Clinical Laboratory Standards Institute guidelines for broth microdilution susceptibility testing (NCCLS, 1999, 2003). Doubling dilutions of sodium metabisulfite at concentrations between 0.125 and 1024 μg/mL were tested.

2.3. Microtiter plate biofilm formation assays

Biofilm formation was measured using a modification of the microtiter plate biofilm formation assay originally described by Christensen et al. (1985) (Deighton et al., 2001; Heilmann et al., 1996a). A single colony of each isolate was incubated for 22 h with shaking at 130 rpm in trypticase soy broth (BD BBL, Franklin Lakes, NJ) supplemented with 1% glucose ($TSB_{gluc1\%}$). Cultures were adjusted with $TSB_{gluc1\%}$ to a turbidity of 1.0 McFarland (~1–2 × 10^8 colony-forming units [CFU]/mL) and diluted 1:50 in $TSB_{gluc1\%}$ containing 0, 7.2, 72, or 720 μg/mL of SMBS. Two hundred-microliter aliquots of each dilution were placed into 4 wells of 96-well microtiter plates (Nuclon Delta, Nunc, Denmark), incubated for 24 h, and the OD_{600nm} was measured on a microplate reader (Multiskan; Thermo Electron, Waltham, MA). Plates were washed twice with deionized water, air dried overnight, then stained with 0.1% safranin for 1 min, rinsed under running tap water to remove excess stain, and air dried overnight. To ensure homogeneity among stained material in the wells, safranin-stained biofilms were resuspended in 200 μL of 30% glacial acetic acid by pipetting (Shanks et al., 2005). The OD_{492nm} of safranin-resuspended wells was measured on a microplate reader. Wells containing uninoculated $TSB_{gluc1\%}$ served as a negative control. Each SMBS concentration was assayed 3 times.

2.4. Silicone elastomer disk biofilm antimicrobial susceptibility assays

Ten-millimeter diameter disks were cut from 0.020-in. thick nonreinforced medical grade silicone elastomer sheeting (Bentec Medical, Woodland, CA) with a skin biopsy punch and autoclaved. Overnight cultures were adjusted to a turbidity of 1.0 McFarland and diluted 1:50 in $TSB_{gluc1\%}$ as described for the microtiter plate assay. One-milliliter aliquots were inoculated into disk-containing wells of a 24-well plate (Falcon; BD Biosciences Franklin Lakes, NJ). Plates were incubated for 24 h for biofilm formation; disks were then removed from wells with sterile forceps and rinsed on each side with 1 mL of sterile phosphate-buffered saline (PBS) to remove planktonic bacteria. Disks were incubated for 24 h in wells containing $TSB_{gluc1\%}$ or $TSB_{gluc1\%}$ + 720 μg/mL of SMBS, rinsed with sterile PBS, transferred to tubes containing 1 mL of sterile PBS, and vortexed for 10 s at high speed. Biofilm was removed by probe sonication at approximately 40 W (80% output setting, VirSonic 50; Virtis, Gardiner, NY) for 10 s. Vortexing and sonication were repeated once. Cells were quantitated by serial dilution and reported as the CFU per disk. Two disks were assayed for each condition in 2 independent experiments.

2.5. Statistical analysis

Data were analyzed with the Student's *t* test using JMP 5.1.2 software (SAS Institute, Cary, NC).

3. Results

S. aureus, *S. epidermidis*, and *S. lugdunensis* are 3 clinically important species that are capable of colonizing and infecting catheters (O'Grady et al., 2002). In this study, we tested the activity of the frequently used preservative SMBS as an antimicrobial compound against planktonic and biofilm forms of these species.

Standard- and high-inoculum MICs and MBCs of SMBS against *S. aureus* ATCC 29213, *S. aureus* SA113, *S.*