Efficacy of Dilute Povidone-Iodine Preparations Against Multi-Drug Resistant Biofilms of Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Candida albicans

Introduction:
Recent work in otolaryngology has begun to appreciate the role of biofilm formation in the pathogenesis of chronic diseases and acute infectious conditions. Chronic rhinosinusitis (CRS) is a complicated inflammatory condition of the nose and paranasal sinuses often encountered by primary care providers, otolaryngology specialists and rhinology sub-specialists. Work in the last decade has shaped our understanding of the roles played by bacterial infection, fungal infection and the host immune response in the clinical CRS picture. Of particular recent interest is the identification of chronic biofilm formation as a potential etiologic agent and therapeutic target. Similarly, acute and chronic otitis externa have been shown to involve biofilm formation of both bacterial and fungal species. Though a variety of agents have shown promise in anti-biofilm studies, none have yet been developed that combine the antibacterial and antifungal properties likely needed to eradicate the causative agents of most sinus and external ear infections. A first approach to this problem is presented that employs the common antiseptic povidone-iodine at very low concentrations known to be safe for administration to patients. These novel dilute PVP-I formulations are effective anti-biofilm agents in vitro. Further evaluation in living models is warranted.

Materials and Methods:
Preparation of PVP-I Formulations. The test formulations consisted of a povidone-iodine at or below 2% (w/w) in solvent systems that can be tailored to form solutions or cellulosic gels incorporating dimethylsulfoxide as a co-solvent. These dilute povidone-iodine systems allow the preparation and room-temperature stabilization of povidone-iodine concentrations from 2% to as low as 0.15% in solutions and gel systems. For these experiments povidone-iodine solutions were prepared at 2% (w/w) and povidone-iodine gels were prepared at 0.25% (w/w). Subsequent dilutions were prepared of the solutions to achieve lower concentration solutions for evaluation.

ABSTRACT
Objective: To study the in vitro effect of novel low-dose povidone-iodine formulations against established biofilms of multi-drug resistant Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Candida albicans.
Methods: Biofilms of multi-drug resistant Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Candida albicans were developed on solid surfaces using the Calgary Biofilm Device (CBD) plate. Minimum biofilm eradication concentration (MBEC) was then determined for each test drug and for control samples of known antibiotics, ciprofloxacin and itraconazole.
Results: The low-dose povidone-iodine formulations completely eliminated all biofilms of bacterial and fungal species in the test systems. Ciprofloxacin was able to eradicate one bacterial biofilm only at concentrations greater than 0.25 µg/mL.
Conclusions: These novel dilute PVP-I formulations are effective anti-biofilm agents in vitro. Further evaluation in living models is warranted.

Table 1. Organism Details

<table>
<thead>
<tr>
<th>Organism</th>
<th>Isolate #</th>
<th>Phenotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>ATCC 3591</td>
<td>Multi-drug resistant</td>
<td>ATCC</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>ATCC BAA-2473</td>
<td>M</td>
<td>ATCC</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>1674623</td>
<td>Multi-drug resistant</td>
<td>Eurofins</td>
</tr>
<tr>
<td>C. albicans</td>
<td>3288194</td>
<td>Multi-drug resistant</td>
<td>Eurofins</td>
</tr>
</tbody>
</table>

Comparator antibiotics and controls:
Ciprofloxacin (Cat # 17850, Lot # 456829, Potency 98.0 µg/mL) and itraconazole (Cat # I6657, Lot # 0871322) were obtained from Sigma (St. Louis, MO, USA). Antibiotic stocks were prepared in appropriate solvents following the CLSI guidelines.

Minimum Biofilm Eradication Concentration:
Minimum biofilm eradication concentration (MBEC) values provide estimates on the concentration of an antimicrobial product required to kill/disrupt the bacterial biofilm. The Calgary Biofilm Device (CBD) plate allows for biofilm formation on a lid containing 96 pegs. The inoculum was diluted to 1 x 10^6 CFU/mL in Tryptic Soy Broth (TSB) for bacterial strains and RPMI media for C. albicans before inoculating the Calgary Biofilm Device (CBD) plate. The CBD plate was incubated with test microorganisms for 24 hours at 35°C on a shaker at 150 rpm. The MBEC assay was conducted as described in Ceri et al.
Biofilm eradication assay: Treatment with PVP-I

A treatment plate was made with the three test articles and comparator antibiotic. The 0.25% PVP-I gel test article was highly viscous, hence only one concentration (full strength at 100%) was tested. One percent (w/v) PVP-I solutions were serially diluted 2-fold and the resulting diluted test articles were tested down to 0.00018% (w/v). Ciprofloxacin and itraconazole were used as positive controls for bacterial strains and C. albicans, respectively. Ciprofloxacin was tested at the following concentrations (µg/mL): 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 µg/mL. Itraconazole was tested at the following concentrations (µg/mL): 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2 and 0.5 µg/mL.

The cation-adjusted Mueller Hinton Broth (CAMHB)/RPMI (for C. albicans) was used as negative control. The CBD plate with the pegs containing a robust biofilm was first rinsed in PBS and then transferred to the treatment plate containing the test articles and control. The plate was incubated for 24 hours at 35°C and then read for determination of MIC values. After incubation, the pegs were rinsed in PBS twice and transferred to a recovery plate containing fresh culture media. The pegs were sonicated in a water bath sonicator for 30 minutes to detach any remaining adherent biofilm. The plate was incubated overnight at 35°C to evaluate growth and the MBE values were determined.

**RESULTS:**

MBEC assay was carried to determine if the low-dose PVP-I test articles can disrupt a pre-existing robust biofilm of the microorganisms that were grown on the pegs for 24 hours. PVP-I solutions at concentration as low as 0.25% (w/v) and PVP-I gel at 0.25% (w/v) completely eradicated the biofilms of all the test microorganisms (Table 2). Comparator antibiotics were ineffective in eradicating biofilms of test microorganism except ciprofloxacin which had a MBE value of 0.25 µg/mL against S. aureus ATCC 35951 (Table 2).

**Table 2: MBEC value for test articles and comparator antibiotics.**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Isolate ID</th>
<th>Phenotype</th>
<th>MBEC (% PVP-I and µg/mL)</th>
<th>1% PVP-I Solution</th>
<th>0.25% PVP-I Gel</th>
<th>Ciprofloxacin</th>
<th>Itraconazole</th>
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</thead>
<tbody>
<tr>
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<td>ATCC 3591</td>
<td>MDR</td>
<td>25</td>
<td>100</td>
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<td>MDR</td>
<td>25</td>
<td>100</td>
<td>&gt;128</td>
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<td>NA</td>
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<tr>
<td>P. aeruginosa</td>
<td>1674623</td>
<td>MDR</td>
<td>25</td>
<td>100</td>
<td>&gt;128</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>C. albicans</td>
<td>3288198</td>
<td>NA</td>
<td>25</td>
<td>100</td>
<td>&gt;128</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Discussion:**

Povidone-iodine, well known to almost every medical specialty, is a complex of elemental iodine and the organic polymer povidone that improves the tolerability, stability and solubility of iodine in water. Free iodine (I₂) is delivered from the PVP-I complex when dissolved in aqueous solvent systems. The delivery of this free molecular iodine provides for the rapid antibacterial, antifungal, antiviral and antiprotozoal activity of PVP-I-based disinfectants. Though most medical PVP-I products are supplied as 5% (w/v) or 10% (w/v) solutions, in vitro analysis paradoxically suggests that solutions with a lower concentration may be more effective than those with higher concentrations. This behavior enables the use of lower concentrations of PVP-I at 0.25-1.0% (w/v) as tested in the current study, which are perfectly tolerable to the ciliated epithelium in the sinuses and the nose. These concentrations are also non-toxic to the external, middle and inner ear and safe for use on mucosal surfaces.

While a variety of PVP-I formulations have gained recent interest beyond simple disinfection as a therapy for acute infections in the eye, ear and skin, this is the first formulation that has been designed as a low-concentration solution or gel specifically for the irrigation treatment of CRS or the single-dose gel treatment of otitis externa. Though there is no known antibiotic resistance to PVP-I and no known species of yeast or fungus that can be eliminated with PVP-I, there is less information describing the anti-biofilm activity of solutions that are tolerable for acute irrigation into the sinuses and the middle ear. The present study clearly shows the anti-biofilm potential of this novel dilute PVP-I system. As the role of biofilm-forming organisms becomes more clearly linked to the development of disease in the sinuses and the ear, the development for non-toxic anti-biofilm agents becomes imperative. Though traditional antibiotics and antifungals may evolve to play some role, the use of potent iodine-based antiseptics, could lead to the development of a new class of topicaly administered therapeutics that are able to eradicate persistent infection via non-specific chemical mechanisms (i.e. non-specific oxidation) may come to define the standard of care. A formulation of PVP-I identical to that tested in this investigation has recently been shown in a small human study to provide significant symptomatic relief in a series of chronic CRS cases and has been employed in our practice (BT, BW) for the treatment of CRS and sinusitis externa. Further in vivo work is being planned and undertaken to further characterize the utility of these PVP-I containing agents.

**Conclusion:**

Established biofilms of multi-drug resistant S. aureus, P. aeruginosa, K. pneumoniae and C. albicans can be successfully disrupted in vitro by treatment with a dilute PVP-I solution or gel. As search for potent, non-toxic anti-biofilm agents continues to evolve, further investigation of dilute PVP-I-containing systems in vitro and in vivo could yield a new class of therapeutic agents with important efficacy in sinus disease and otitis externa.

**References:**

10. Tessenba M et al. Treatment of Recalcitrant Chronic Sinusitis with Topical Irrigation of A Povidone-Iodine / Budesonide Suspension. (In preparation)