Comparison of ML8-X10 (a prototype oil-in-water micro-emulsion based on a novel free fatty acid), taurolidine/citrate/heparin and vancomycin/heparin antimicrobial lock solutions in the eradication of biofilm-producing staphylococci from central venous catheters

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Objectives: Antimicrobial lock solutions are used for prevention and management of catheter-related bloodstream infections. ML8-X10 (a prototype oil-in-water micro-emulsion based on a novel free fatty acid), vancomycin/heparin and taurolidine/citrate/heparin (TaurolockTM-Hep500) lock solutions were tested against biofilm-forming Staphylococcus epidermidis and methicillin-susceptible Staphylococcus aureus.

Methods: MICs were tested in neutral broth (pH ≏ 7) and acidified broth (pH 5). In an established in vitro central venous catheter (CVC) lock model, solutions were introduced after 24 h of bacterial growth in a CVC incubated at 37°C. After an additional 8, 24 or 72 h of incubation, saline flush and cut catheter segments were processed for bacterial quantification. The cfu/mL at 0 h was subtracted from cfu/mL at the different timepoints.

Results: The activities of ML8-X10 and taurolidine solutions were enhanced at lower pH (P < 0.05). Against S. epidermidis, ML8-X10 solution demonstrated less activity than taurolidine at 8 h (P < 0.001), but was not significantly different from vancomycin. At 24 h, ML8-X10 solution demonstrated significantly less activity than taurolidine (P < 0.001), but was significantly more active than vancomycin (P < 0.001). Against S. aureus, ML8-X10 solution was less active than taurolidine at 8 and 24 h (P < 0.001 for both), but was similar to vancomycin. At 72 h, all lock solutions reduced colony counts to levels that approached or reached the limit of detection against both strains.

Conclusions: In our in vitro catheter lock model, the novel free fatty acid emulsion demonstrated activity against biofilm-forming staphylococci similar to or greater than that of vancomycin lock solution. Taurolidine was the most active lock solution at 8 and 24 h, with all lock solutions tested demonstrating high activity at 72 h.

Keywords: Staphylococcus aureus, Staphylococcus epidermidis, catheter lock solutions

Introduction

Staphylococcal catheter infections are often caused by biofilm-producing strains, which are difficult to eradicate. Vancomycin is commonly used in a catheter lock solution in managing such infections.1 Some free fatty acids have antithrombotic properties and antimicrobial activity against bacteria, yeast and viruses.2–4 ML8-X10 is a prototype, novel catheter lock solution under development. It is an aqueous-based emulsion of free fatty acid in lipid microspheres. We evaluated the activity of ML8-X10, vancomycin and taurolidine/citrate as catheter lock solutions against biofilm-forming staphylococci in an in vitro central venous catheter (CVC) model.

Methods

Test organisms
Known biofilm-producing strains of Staphylococcus epidermidis (ATCC 35984) and Staphylococcus aureus (ATCC 35556) were evaluated.

Media
Strains were grown overnight on tryptic soy agar (TSA). Mueller–Hinton broth (MHB) supplemented with 25 mg/L calcium and 12.5 mg/L magnesium was used for MIC analysis and modified as noted below. Tryptic soy broth (TSB) supplemented with 25 mg/L calcium, 1% glucose and 2% sodium chloride was used to optimize biofilm formation.
MIC
MICs and MBCs were determined in MHB. The MICs were also determined in MHB acidified with the addition of hydrochloric acid to pH 5.0 since ML8-X10 activity is greatest at low pH and is known to drop off quickly above pH 5.0.

Antimicrobial activity in a catheter model
ML8-X10 (Marvao Medical, Inc., Ireland), 5 mg/mL vancomycin (Hospira, Lake Forest, IL, USA) plus preservative-containing (0.45% benzyl alcohol) 5000 U/mL heparin (Hospira) and 1.35% (cyclo)taurodiloidine with 4% citrate and 500 U/mL heparin (Taurolock™-Hep500, TauroPharm GmbH, Germany)

Table 1. MIC and MBC results for vancomycin, ML8-X10 and taurolidine plus citrate and heparin against staphylococci in non-acidified MHB at pH ~7.5 and acidified broth (pH 5.0)

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Vancomycin</th>
<th>ML8-X10</th>
<th>Taurolidine plus citrate and heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/L)</td>
<td>MBC (mg/L)</td>
<td>MIC (%)</td>
</tr>
<tr>
<td>S. epidermidis ATCC 35984</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>&gt;12.5 (1.56)</td>
</tr>
<tr>
<td>S. aureus ATCC 35556</td>
<td>1 (0.5)</td>
<td>1 (1)</td>
<td>&gt;12.5 (3.13)</td>
</tr>
</tbody>
</table>

The MICs and MBCs of ML8-X10 and taurolidine are presented as percentages of the stock solution since they were only available as solutions. The MICs and MBCs in acidified broth are given in parentheses in the body of the table.

Figure 1. Activity of antimicrobial lock solutions against S. epidermidis after 8, 24 or 72 h of exposure. Activity was measured as change (average and SEM) in log_{10} cfu/mL from the 0 h timepoint for bacteria recovered from a 1 mL saline flush through the catheter segment ('flush', dark grey), from a cut catheter segment ('cut', light grey) and the combined total bacteria recovered ('total', white). ML8, ML8-X10; TLH, 1.35% taurolidine, 4% citrate and 500 U/mL heparin; V ANH, 5 mg/mL vancomycin with 5000 U/mL heparin.
were evaluated in a multi-lumen polyurethane CVC (Arrow-Howes CVC®
(MC-15703), Reading, PA, USA) model.6

Device inoculation and treatment
A 0.5 McFarland standard from overnight growth on TSA was diluted
(1:100) in TSB and added to the lumen of multi-lumen CVCs. Each lock
solution was introduced after 24 h of biofilm development, based on pre-
vious studies.7,8 Broth was drained after 24 h of incubation and catheter
lock solution sufficient to fill the catheter lumen was introduced. Since
the catheter lock solution completely filled the lumen of the CVC, the pH
of the experiment was that of the lock solution being tested.

Regimen simulations included ML8-X10 alone, 5 mg/mL vancomycin
plus 5000 U/mL heparin (plus 0.45% benzyl alcohol) or 1.35% taurolidine
plus 4% citrate plus 500 U/mL heparin. Catheters were clamped at the dis-
tal end as soon as the antimicrobial lock solution filled each lumen. Each
system was then incubated at 35°C for an additional 8, 24 or 72 h. Each
organism was tested against each agent in triplicate.

Recovery of test organisms
At the endpoint, catheter luminal fluid was obtained by opening the clamps
and draining the contents into a sterile test tube. A sterile needle was intro-
duced into the open lumen of each access port and 1 mL of normal saline
was flushed through the lumen and collected in a sterile test tube. To opti-
mize yield of viable bacteria, the flushed saline was then sonicated at 60 W
for 1 min, then vortexed for 15 s. Additionally, a 3 cm cut segment of each
catheter was sonicated at 60 W for 1 min. A 100 μL volume of the flushed,
sonicated saline and saline from the cut segment was serially diluted
10-fold and transferred to TSA plates, which were incubated overnight at
35°C. The limit of detection for the flushed, sonicated and vortexed cultures
from the access port and lumen and the sonicated saline of the catheter
segment is 2.0 log10 cfu/mL. Vacuum filtration was used to quantify bacte-
rial counts as necessary with a limit of detection of 1.0 log10 cfu/mL.

Data analysis
Statistical analyses were performed using SPSS software (release 20; SPSS,
Inc., Chicago, IL, USA). Activity at 8, 24 or 72 h was compared for the cath-
teter lock solutions using ANOVA followed by Tukey’s post hoc test for multiple
comparisons.9

Results and discussion
MIC
Catheter lock solutions were active at the concentrations used in
the catheter lock model (Table 1). Activities of ML8-X10 and

![Graphs showing activity of antimicrobial lock solutions against S. aureus after 8, 24 or 72 h of exposure.](image-url)
taurolidine plus citrate and heparin were enhanced at a lower pH (P<0.05). It is known that the activity of ML8-X10 is optimal at pH levels <5.0.

**Catheter lock model**

Against *S. epidermidis*, total ML8-X10 activity was not significantly different from that of vancomycin, but it was less than that of taurolidine at 8 h (P<0.001) (Figure 1). For the catheter lumen flush at 24 h, ML8-X10 was as active as taurolidine and vancomycin, but less active than taurolidine against the bacteria found in the cut catheter segment culture (P<0.001). For the combined total bacterial counts of the flush and cut models, taurolidine was more active than ML8-X10 (P<0.001), which was more active than vancomycin (P=0.001). At 72 h, activity measured as colony counts of bacteria recovered from the catheter lumen flush and cut catheter segments was similar for all the lock solutions, approaching or meeting the limit of detection.

Against *S. aureus*, for the catheter lumen flush, ML8-X10 activity was not significantly different from taurolidine activity after 8 h; both were greater than vancomycin activity at 8 h (P<0.001) (Figure 2). For bacteria recovered from the cut catheter segments, ML8-X10 activity was not significantly different from vancomycin activity, but lower than taurolidine activity at 8 h (P<0.001). At 24 h, all lock solutions demonstrated similar activity against the bacteria recovered from catheter lumen flush. Bacterial counts recovered from the cut catheter segments and the overall counts recovered were similar. Taurolidine solution was the most active (P<0.001) and activities of ML8-X10 and vancomycin were not significantly different. At 72 h, all lock solutions reduced colony counts to levels that approached or reached the limit of detection; however, taurolidine and vancomycin solutions were more active than ML8-X10 against the catheter lumen flush organisms (P<0.004). The same was true for the total bacteria recovered, but no significant differences were found in activity of any of the lock solutions against the bacteria from the cut catheter segments.

We tested ML8-X10, a prototype oil-in-water emulsion, for potential use as an antimicrobial catheter lock solution. Antimicrobial activity of free fatty acids involves disruption of the electron transport chain and oxidative phosphorylation. Naturally occurring free fatty acids have cis-oriented double bonds, which have greater antimicrobial activity. One example is cis-2-decenoic acid, which inhibits *S. aureus* growth and biofilm formation in addition to dispersing biofilm. Taurolidine (1.35%) plus sodium citrate (4%) and heparin (500 U/mL) is a commercially available catheter lock solution in Europe, but not in the USA. Taurolidine-containing lock solutions are effective in the prevention and treatment of catheter-related bloodstream infections.

There are some limitations to our study. While the concentrations and dwell times of heparin and the taurolidine lock solution with citrate and heparin have been used in haemodialysis patients with similar antithrombotic activity, the antithrombotic activity of ML8-X10 was not evaluated in our study. In addition, this study focused on antimicrobial activity in a pre-established CVC biofilm and we did not assess the potential utility of ML8-X10 as a prophylactic antimicrobial lock solution.

**Conclusions**

In conclusion, ML8-X10, taurolidine plus citrate and heparin, and vancomycin plus heparin were active against biofilm-forming staphylococci at the concentrations used in our CVC model. Taurolidine was more active than the other two lock solutions at 8 h. Activity was greatest at 72 h, where all lock solutions were highly active and reduced colony counts to levels that approached or reached the limit of detection. Our findings suggest that the antimicrobial activity of the taurolidine lock solution was more rapidly acting than the prototype ML8-X10 solution and the vancomycin lock solution, particularly against the adherent bacteria from the cut catheter segment. ML8-X10 demonstrated similar or greater activity than a vancomycin plus heparin lock solution and, based on our data, free fatty acid emulsions show promise as a new class of antimicrobial lock solution worthy of additional development and study.

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**Transparency declarations**


All data collection, extraction and analyses were carried out by the Department of Veterans Affairs study team.

**Disclaimer**

The views expressed are those of the authors and do not necessarily reflect the position or policy of the United States Department of Veterans Affairs.

**References**


