Auriclosene Irrigation Solution Prevents Encrustation by Crystalline Biofilm Due to *Proteus mirabilis* in an *In Vitro* Urinary Catheter Patency Model

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Abstract

INTRODUCTION AND OBJECTIVES: Long-term indwelling urinary catheters are susceptible to blockage due to formation of crystalline biofilms by urease-producing microorganisms such as *Proteus mirabilis*. An *in vitro* catheter biofilm model (CBM) was developed to compare current methods for maintaining urinary catheter patency. We compared various bladder irrigation solutions or antimicrobial-coated urinary catheters, versus a novel anti-microbial catheter irrigation solution containing auriclosene (*N*,*N*-dichloro-2,2-dimethyltaurine; formerly designated NVC-422).
METHODS: CBM units were fed artificial urine at 0.5 mL per min. The artificial bladder chamber was inoculated with 10^8 colony forming units (CFU) of *P. mirabilis* and biofilm was allowed to establish for 48 hours before daily treatments commenced. A single treatment consisted of two sequential 50-mL irrigations. Each irrigation was retained in the catheter for 15 min and then drained, with a 30-min washout period between the two irrigations. Experiments were conducted for up to 10 days or until catheter blockage. The pH of the effluent, CFU counts in the bladder chamber and the time to catheter blockage were recorded. The area of catheter encrustation was measured using Stereo Zoom imaging.
RESULTS: Inoculation of the CBM reactor with 10^8 CFU of *P. mirabilis* resulted in blockage of the urinary catheters within 5 days. The use of silver-hydrogel or nitrofurazone-coated catheters did not extend the period of catheter patency. Catheters irrigated with 0.25% acetic acid, 10 mM acetate-buffered saline or isotonic saline blocked at the same rate as untreated catheters. Catheter irrigation with a citrate-buffered formulation of 0.2% auriclosene resulted in complete eradication of *P. mirabilis* biofilm within one treatment day. In contrast, daily irrigations of infected catheters with 0.2% auriclosene in 10 mM acetate-buffered saline (at pH 4) or Renacidin® Irrigation Solution had no effect on *P. mirabilis* colonization of the bladder chamber, even though catheter patency was maintained throughout 10-day studies.
CONCLUSIONS: Irrigation with the rapidly bactericidal antimicrobial auriclosene in a buffered acidic formulation – termed Auriclosene Irrigation Solution – significantly enhanced catheter patency *in vitro* versus other irrigation solutions and antimicrobial-coated urinary catheters. Clinical evaluation of Auriclosene Irrigation Solution is ongoing.

Introduction

Patients with long-term indwelling bladder catheters often face bacterial colonization causing encrustation and subsequent catheter blockage. Among the many urinary pathogens, urease-positive *Proteus mirabilis* is the most common culprit. These urease-producing bacilli catalyze the hydrolysis of urea to ammonia, thus creating alkaline conditions. As the urinary pH elevates, calcium and magnesium phosphates precipitate out of solution as struvite and hydroxyapatite crystals, respectively (1). The continued formation of crystalline biofilms blocks the normal flow of urine from the bladder, leading to incontinence (2) and increased risks of developing bacteriuria, pyelonephritis, bacteremia, and sepsis (3). Currently, there are no successful treatment protocols for controlling catheter encrustation and blockage (2).

In the present study, we utilized an *in vitro* catheter biofilm model that closely mimics the crystalline biofilm growth conditions in catheterized patients. Experiments were performed in laboratory models, where catheterized reactors were fed with artificial urine and inoculated with *P. mirabilis* Hauser ATCC 29245™. Auriclosene solutions and other control solutions were irrigated into the catheterized biofilm model following protocols to simulate clinical irrigation and drainage regimens.

Materials & Methods

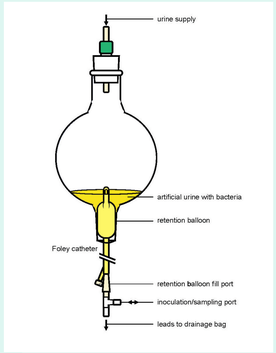

Fig. 1. *In vitro* catheter biofilm model

Schematic of *in vitro* catheter biofilm model. The model adapted from Stickler *et al.* (4) consists of a size 14 Foley catheter (all silicone) inserted into the bottom of a glass chamber simulating a human bladder. The catheter was held in place by inflating the retention balloon with 10 mL of sterile water. As artificial urine is pumped into the reactor, the urine drains through the eye-hole into a waste bag with minimal residual urine collecting in the glass chamber. Reactor was inoculated with *P. mirabilis* for 1 hour to allow cell attachment. Artificial urine flow was initiated for 48 hours to allow biofilm development prior to daily irrigation for 8 days. Following blockage of catheter or on Day 10, the reactor was disassembled.

Results

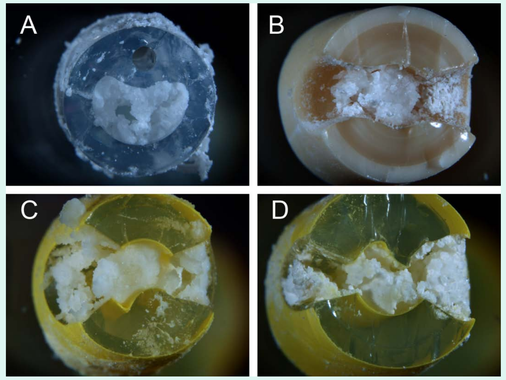

Fig. 2. Stereo Zoom images of untreated catheters. (A) All-silicone (B) Silver-alloy (C) Nitrofurazone Strata-NF (D) Nitrofurazone Release-NF

In vitro catheter biofilm studies were performed, in which catheters were left untreated until blockage. Antimicrobial-coated catheters blocked at the same rate as all-silicone catheters.

Results (continued)

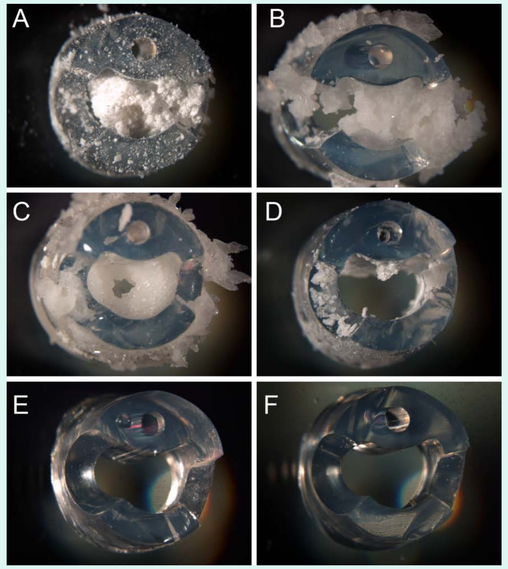

Fig. 3. Stereo Zoom images of all-silicone catheters irrigated daily. (A) Saline pH 7 (B) 10 mM acetate-buffered saline, pH 4 (C) 0.25% acetic acid (D) Renacidin® (E) 0.2% auriclosene in 10 mM acetate saline (F) 0.2% auriclosene in 6.6% citrate buffer

Test Solution	Initial urinary pH	Final urinary pH in reactor	Time to block (days)	CFU/mL in reactor	Avg $\log_{10}$ CFU/cm^2	n
0.2% auriclosene in 6.6% citrate buffer	6.5	5.7	No blockage*	<400	<3.3	3
0.2% auriclosene in 10 mM acetate saline	6.5	6.5	No blockage in 10 days	<400	<3.3	3
Renacidin®	6.5	8.7	No blockage in 10 days	3.5E+07	6.3	3
10 mM acetate saline	6.5	8.3	6.0	1.6E+07	6.7	3
0.25% Acetic acid	6.5	8.5	5.1	4.4E+07	7.0	3
Isotonic saline pH 7	6.5	9	4.2	6.9E+07	8.3	3

Table 1. Catheter blockage time Catheters irrigated with 10 mM acetate saline, 0.25% acetic acid, and isotonic saline blocked between Days 4 to 6. Catheters irrigated with 0.2% auriclosene in 10 mM acetate saline and Renacidin® remained patent during the experimental study, but the irrigation had no effect on *P. mirabilis* colonization. Catheters irrigated with 0.2% auriclosene in 6.6% citrate buffer resulted in complete eradication of *P. mirabilis* within one treatment day.

Results (continued)

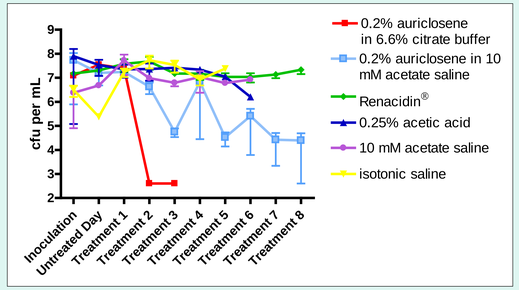

Fig. 4. Daily measurement of the colony forming units of *P. mirabilis* per mL before irrigation. 0.2% auriclosene in 6.6% citrate buffer eradicated *P. mirabilis* to the limit of detection by one treatment day. Irrigation with 0.2% auriclosene in 10 mM acetate saline reduced *P. mirabilis* counts. All other irrigation solutions (Renacidin®, 0.25% acetic acid, 10 mM acetate saline, and isotonic saline) did not reduce *P. mirabilis* counts. The limit of detection was 400 CFU/mL.

Conclusions

- Irrigation with 0.2% auriclosene in 6.6% citrate buffer resulted in complete eradication of *P. mirabilis* biofilm within one treatment day
- Irrigation with isotonic saline, 0.25% acetic acid and 10 mM acetate saline resulted in catheter blockage within 4 to 6 days
- Catheters irrigated with Renacidin® or 0.2% auriclosene in 10 mM acetate saline maintained patency throughout the study but *P. mirabilis* colonization was not reduced
- Clinical evaluation of 0.2% auriclosene solution is on-going

References

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