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Efficacy of NVC-422, a Novel Derivative of *N*-Chlorotaurine, in Controlling Crystalline *Proteus mirabilis* Biofilm Formation on Urinary Catheters

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Abstract

Background: Infection by *Proteus mirabilis* can complicate patient care with long-term indwelling bladder catheters. These urease-producing bacilli elevate urinary pH, colonize catheters and produce crystalline biofilms that can block the flow of urine from the bladder. NovaBay has developed a non-antibiotic, anti-infective compound (NVC-422; *N,N*-dichloro-2,2-dimethyltaurine), a stable analog of the natural antimicrobial *N*-chlorotaurine that exhibits potent broad spectrum antimicrobial activity with an excellent safety profile. Here we investigated the potential use of NVC-422 in an instillation solution for the control of catheter blockage by *P. mirabilis* biofilm. **Methods:** Experiments were performed in laboratory models of the catheterized bladder fed artificial urine and inoculated with *P. mirabilis*. NVC-422 or saline was instilled daily into the catheterized bladder chambers following protocols to simulate clinical bladder washout regimens. The experiments were run for 144 h or until catheters blocked. The pH of the effluent urine and the times to catheter blockage were measured. Biofilm formation was observed with scanning electron microscopy. **Results:** In control models (saline) catheters blocked at 46 h. Electron microscopy confirmed that catheter blockage was due to the accumulation of crystalline biofilm in the lumen and around the eyeholes. In these control models, the pH of urine increased from 6 to 9. The catheters treated with 0.2% NVC-422 in saline formulated at pH 4 drained freely for the 144 h experimental period, the urinary pH remained at 6, and no biofilm was visible. **Conclusions:** Instillations of NVC-422 were effective in preventing crystalline *P. mirabilis* biofilms on catheters in an *in vitro* test system. These results suggest that a bladder washout regime using solutions of NVC-422 could be used to manage catheter encrustation that complicates the care of patients undergoing long-term bladder catheterization.

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* catheterized bladder model that closely mimics the crystalline biofilm growth conditions in the catheterized bladders. Experiments were performed in laboratory models, where catheterized bladders were fed with artificial urine and inoculated with *P. mirabilis*. NVC-422 or placebo (control) was instilled into the catheterized bladder model following protocols to simulate clinical bladder instillation and drainage regimens.

Materials & Methods

The catheterized bladder biofilm model was used to simulate crystalline biofilms in patients with long-term indwelling catheters (Figure 1). The model was adapted from Stickler *et al.* (4) with the following modifications. Irrigation solutions were instilled through the catheter into the bladder as per schematic in Figure 2. The vessel containing artificial urine was infected either in a single inoculation or on a daily basis (see Table 1). Artificial urine composition (0.65 g/L calcium chloride, 0.65 g/L magnesium chloride hexahydrate, 4.6 g/L sodium chloride, 2.3 g/L sodium sulfate, 0.65 g/L sodium citrate dihydrate, 0.2g/L sodium oxalate, 2.8 g/L potassium dihydrogen phosphate,1.6 g/L potassium chloride, 2 g/L ammonium chloride, 12 g/L urea, 1.1 g/L creatinine).

Following blockage of catheter or on day 7, the assembly was dismantled and the catheter was removed from the glass vessel. The pH of the effluent urine and the times to catheter blockage were recorded. The catheter was sectioned and analyzed for viable cell counts. Biofilm formation was observed with Scanning Electron Microscopy (SEM) and Stereo Zoom imaging.

Catheterized Bladder Model

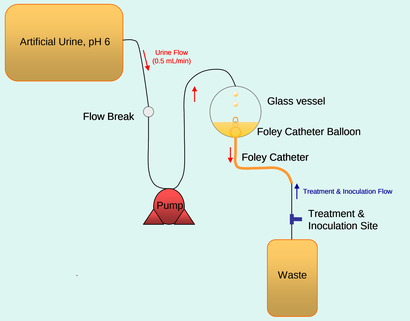

Fig. 1. Catheterized bladder model. Schematic of the *in vitro* catheterized bladder model. The model consists of a size 14 Foley catheter (all silicone) inserted into the bottom of a glass vessel simulating a human bladder. The catheter was held in place by inflating the retention balloon with 10 mL of sterile water. As artificial urine is pumped into the catheterized bladder, the urine drains through the eye-hole into a waste bag with minimal residual urine collecting in the glass vessel.

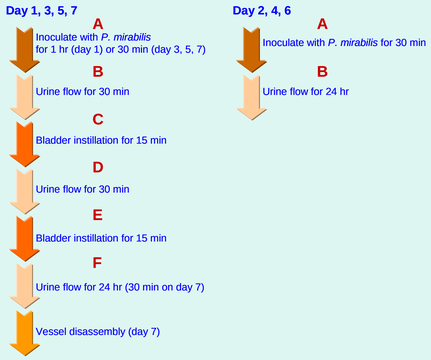

Fig. 2. Catheter bladder irrigation regimen for daily inoculation and every other day irrigation. On day 1, vessels were inoculated for 1 hr and received bladder irrigations twice. On day 2, 4, and 6, vessels were inoculated for 30 min and did not receive any irrigation. On day 3, 5, and 7, vessels were inoculated for 30 min and received bladder irrigations twice. On day 7 (or after catheter blockage), vessels were taken apart and catheters were removed for analysis.

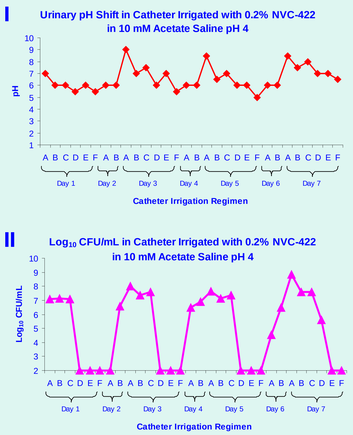

Fig. 3. Efficacy of NVC-422 irrigation. NVC-422 was effective in reducing bacterial counts to the limit of detection and lowering urinary pH immediately after each irrigation during the 7 day experimental period. (I) Urinary effluent pH and (II) viable cell counts measured during the inoculation and irrigation regimen.

Stereo Zoom and SEM Imaging

Treatment with 0.2% NVC-422 maintained catheter patency in the catheterized bladder model within 7 days. Crystalline material was evident in catheter treated with control solutions (saline).

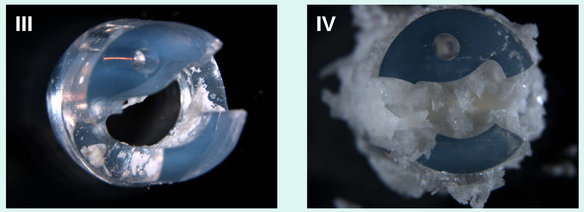

Fig. 4. Stereo Zoom images of NVC-422 treated and 10 mM acetate saline treated (control) catheters removed from *P. mirabilis* infected bladder models. (III) Cross section of the eyehole lumen of an unblocked NVC-422 treated catheter draining freely at 7 days. (IV) Cross section of the eyehole of a control treated catheter after blockage on day 3.

Fig. 5. Scanning electron microscopy images of NVC-422 treated and saline treated (control) catheters colonized by *P. mirabilis* biofilm. (V) Section analyzed after daily treatment with NVC-422 in saline. (VI) Sectioned analyzed after control (saline) catheter blocked on day 3. Photo shows a crystal embedded in *P. mirabilis* biofilm.

Catheter Blockage Time

Inoculation	Treatment	Irrigation Solution	Time to block (day)
Single	Daily (excluding day 7)	Saline pH 4 (n=3)	2
Single	Daily (excluding day 7)	0.2% NVC-422 in Saline pH 4 (n=4)	>7
Daily	Every other day	10 mM Acetate Saline pH 4 (n=4)	3
Daily	Every other day	20 mM Acetate Saline pH 4 (n=1)	4
Daily	Every other day	0.2% NVC-422 in 10 mM Acetate Saline pH 4 (n=4)	>7
Daily	Every other day	0.2% NVC-422 in 20 mM Acetate Saline pH 4 (n=1)	>7
Daily	Every other day	Renacidin® Irrigation (n=2)	3

*0.2% NVC-422 was selected based on our previous *in vitro* and *in vivo* studies

Table 1. Catheter blockage time. Catheters irrigated with control solutions (saline and acetate saline) blocked between 2-4 days and the pH of artificial urine shifted from 6 to 9. Increase of urinary pH indicates continued formation of crystalline biofilm. The catheters treated with NVC-422 remained patent throughout the 7 day experimental period. The pH of artificial urine remained between 6-7.

Catheters treated with Renacidin® (6.6% citric acid, 0.2% glucono delta-lactone, and 3.4% magnesium carbonate) blocked on day 3. Crystalline biofilm partially dissolved immediately after irrigation with Renacidin®, however, biofilm encrustation developed the next day.

Conclusions

- Treatment with 0.2% NVC-422 maintained catheter patency for greater than 7 days
- Treatment with 0.2% NVC-422 greatly reduced *P. mirabilis* cell counts after each irrigation
- Treatment with 0.2% NVC-422 reduced crystalline encrustation in the biofilm model
- Urinary pH remained between 6-7 in NVC-422 treated catheters

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