NVC-422 Prevents Urinary Catheter Blockage and Encrustation

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Abstract

Introduction: Untreated *Proteus mirabilis* infections can lead to pyelonephritis and septicemia. *P. mirabilis* forms a crystalline biofilm composed of struvite and hydroxyapatite that affects many patients with long-term indwelling urinary Foley catheters. These crystalline biofilms, if not treated, can result in the blockage of urine flow leading to urine leakage or painful distention of the patient's bladder. NovaBay is developing NVC-422 (*N,N*-dichloro-2,2-dimethyltaurine), a fast-acting, broad-spectrum antimicrobial instillation solution, with an excellent safety profile for the treatment of urinary catheter blockage and encrustation (UCBE). **Methods:** Experiments were performed in our laboratory models of a catheterized bladder fed artificial urine at 0.5 mL per minute. The artificial bladder chamber was inoculated daily with *P. mirabilis*. NVC-422 or control solutions were instilled through the catheter every other day. These experiments were conducted for 15 days or until the catheters blocked. The pH of the effluent, CFU counts and the time to catheter blockage were recorded. Blocked catheters were examined using Stereo Zoom and/or scanning electron microscopy. **Results:** In control samples the urinary pH increased from 6 to 9, high CFU counts were observed, resulting in blockage by 58 hours; a crystalline biofilm was clearly evident in the catheter eyeholes and lumen. Catheters treated with 0.2% NVC-422 formulated in acetate saline at pH 4 drained freely throughout the 15 day study, maintained a neutral pH, had lower CFU counts and showed only traces of crystalline material. **Conclusions:** NVC-422 instillations were effective in preventing crystalline biofilm formation caused by *P. mirabilis* in catheters in our *in vitro* UCBE model. The design of our clinical protocol is based on these results.

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 catheterized patients. Experiments were performed in laboratory models, where catheterized bladders were fed with artificial urine and inoculated with *P. mirabilis* Hauser ATCC 29245™. 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 chamber containing artificial urine was infected on a daily basis. Urinary pH and viable counts were measured during the inoculatation and irrigation regimen (Figure 3).

Artificial urine composition was prepared according to Minuth *et al.* (5) with minor modifications (0.49 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, 0.02g/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). Tryptic soy broth was added to the artificial urine at 10 g/L for daily inoculum cultures and at 1 g/L for the continuous cultures of biofilm experiment.

Following blockage of catheter or on day 15, the model was disassembled and the catheter was removed from the glass chamber. The pH of the effluent urine and the times to catheter blockage were recorded (Table 1). The catheter was sectioned and analyzed for viable cell counts. Biofilm formation was observed with Stereo Zoom (Figure 4) and Scanning Electron Microscopy (Figure 5).

Catheterized Bladder Model

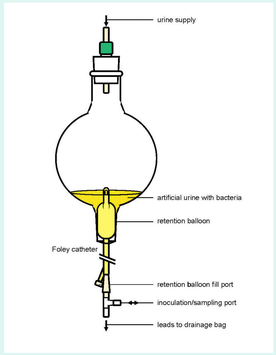

Fig. 1. Catheterized bladder model. Schematic of the *in vitro* catheterized bladder model. The model consists of a size 14 Foley catheter (all silicone) inserted into the bottom of a glass chamber simulating a human bladder. The catheter was held in place by inflating the retention balloon with 10 mL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

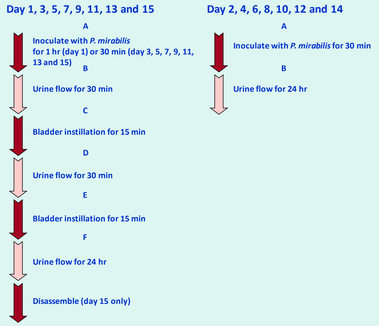

Fig. 2. Catheter bladder irrigation regimen for daily inoculation and every other day irrigation. On day 1, chambers were inoculated for 1 hr and received bladder irrigations twice. On days 2, 4, 6, 8, 10, 12 and 14, chambers were inoculated for 30 min and did not receive any irrigation. On days 3, 5, 7, 9, 11, 13 and 15, chambers were inoculated for 30 min and received irrigation solution twice. On day 15 (or after catheter blockage), chambers were taken apart and catheters were removed for analysis.

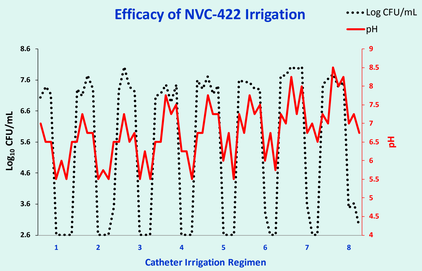

Fig. 3. Efficacy of NVC-422 irrigation. NVC-422 was effective in reducing bacterial counts to the limit of detection and lowering urinary pH immediately after each irrigation during the 15 day experimental period (excluding the last irrigation). Urinary effluent pH and viable cell counts were measured 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 15 days. Crystalline material was evident in catheter treated with control solutions (acetate saline).

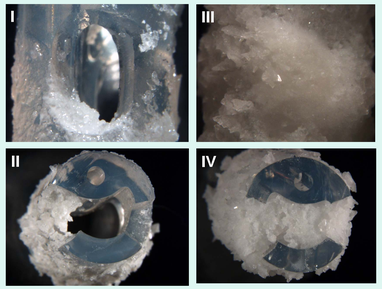

Fig. 4. Stereo Zoom images of *in vitro* NVC-422 treated and 10 mM acetate saline treated (control) catheters removed from *P. mirabilis* infected bladder models. (I) Eye-hole and (II) eye-hole cross section of an unblocked NVC-422 treated catheter draining freely at 15 days. (III) Eye-hole and (IV) eye-hole cross section of a control (acetate buffered saline) treated catheter after blockage on day 3.

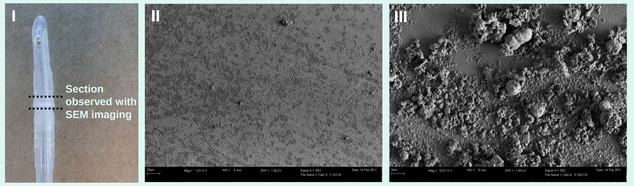

Fig. 5. Scanning electron microscopy images of NVC-422 treated and acetate saline treated (control) catheters colonized by *P. mirabilis* biofilm. (I) Proximal tip of catheter for SEM imaging. (II) Section analyzed after 15 day study irrigated every other day with NVC-422 formulated in acetate saline. (III) Section analyzed after control (acetate buffered saline) catheter blocked on day 3.

Catheter Blockage Time

Urinary Catheter Irrigation Solution	Time to Blockage (days)	pH[a]
0.2% NVC-422 in 10 mM acetate buffered saline (pH 4)	No blockage within 15 days	Initial pH 6.0 Final pH 7-7.5
10 mM acetate buffered saline (pH 4)	2.7	Initial pH 6.0 Final pH 9

[a] pH of the artificial urine in the bladder

Table 1. Catheter blockage time. Catheters irrigated with control solution (acetate buffered saline) blocked at average of 2.7 days and the pH of artificial urine shifted from 6 to 9. The catheters treated with NVC-422 remained patent throughout the 15 day experimental period. The pH of artificial urine remained between 7-7.5. Concentration of NVC-422 selected was based on safety and efficacy data.

Conclusions

- Treatment with 0.2% NVC-422 reduced *P. mirabilis* cell counts and urinary pH after each irrigation
- Treatment with 0.2% NVC-422 reduced crystalline encrustation in the biofilm model and maintained catheter patency for greater than 15 days
- NVC-422 irrigation solution has utility for the prevention and treatment of urinary catheter blockage and encrustation

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Acknowledgements

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