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The effect of urease inhibitors on the encrustation of urethral catheters

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Abstract Encrustation and blockage of indwelling urethral catheters is primarily brought about by infection of the urinary tract by *Proteus mirabilis* or other urease-producing species. The bacteria colonise the catheter forming a biofilm community within a polysaccharide matrix. The activity of the urease drives up the urinary pH and causes the crystallisation of calcium and magnesium phosphates in the biofilm. We have used a simple physical model of the catheterised bladder to investigate the ability of urease inhibitors to control encrustation. It was observed that acetohydroxamic acid (1.0 mg/ml) and fluorofamide (1.0 μg/ml) restricted the increase in pH of *P. mirabilis*-infected urine from 9.1 to 7.6. Significant reductions in the deposition of calcium and magnesium salts were also recorded on the silicone catheters. Electron microscopy confirmed that encrustation and occlusion of the catheter lumen was minimal in the presence of the urease inhibitors. The data from this in vitro study suggests that urease inhibitors, particularly fluorofamide, could have clinical applications in the prevention of catheter encrustation and blockage.

Key words Urinary tract infections · Urinary catheterisation · Bacterial biofilms · Urease · *Proteus mirabilis*

Introduction

Long-term indwelling urethral catheters are commonly subject to encrustation [7]. Crystalline deposits can cover the balloon and obstruct the eye-hole and lumen of the catheter. They can cause trauma to the bladder mucosa and to the urethra on catheter withdrawal. Blockage of the catheter lumen can lead to retention of urine and painful distension of the bladder. As bacteriuria is inevitably associated with the encrustation and blockage, retention can facilitate ascending infection of the urinary tract, culminating in episodes of pyelonephritis, septicaemia and shock [13]. Unnoticed catheter blockage can thus be very dangerous, particularly for patients being cared for in the community where professional care is not immediately available. Surveys have reported that around 50% of patients undergoing this form of long-term bladder management will experience recurrent encrustation and blockage of catheters [12, 15].

The catheter encrustations resemble infection-induced stones in their crystalline structure, being composed of a mixture of struvite (magnesium ammonium phosphate hexahydrate) and the poorly crystalline form of calcium phosphate, hydroxyapatite [3, 11]. The early work of Griffith et al. [10] demonstrated the central role of urease-producing bacteria in the generation of the bladder and kidney stones. The enzymatic hydrolysis of the urea produces an alkaline environment in which calcium and magnesium phosphates crystallise out of solution. Examination of these infection-induced stones by scanning electron microscopy, confirmed the presence of microbial cells throughout their structure [22]. Similarly Cox et al. [4] showed the presence of large numbers of bacilli associated with catheter encrustations substantiating the view that they are generated by the processes responsible for the formation of infection-induced stones. Bacteriological analysis subsequently confirmed that the urease producer *P. mirabilis* was the predominant organism in the urine of patients prone to catheter obstruction [18] and in encrusted catheter biofilms [21]. The evidence now suggests that the steps in the encrustation process are (1) the infection of the urinary tract by *P. mirabilis*, (2) the adherence of the urease-producing bacteria to the catheter, (3) the development of a bacterial biofilm community within a matrix of bacterial exopolysaccharide, (4) the elevation of the pH of the urine and biofilm matrix by the action of the bacterial

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urease on urea, (5) the attraction of calcium and magnesium ions into the gel of the matrix, (6) the alkali-induced, gel-stabilised crystallisation of calcium and magnesium phosphates. It also seems that throughout the encrustation process, crystals formed in the alkaline urine of the bladder can attach to the surfaces of the catheter and the biofilm [2, 4, 5, 21, 24].

Currently there is no effective method available to block the formation of these encrustations [14]. The materials used in the manufacture of catheters provide particularly attractive surfaces for biofilm colonisation and a recent study demonstrated that none of 18 different types including silicone, silicone-coated latex, hydrogel-coated latex and silver-coated catheters were capable of resisting encrustation by *P. mirabilis* biofilms [19]. As the increase in urinary pH is the main factor inducing the precipitation of magnesium and calcium salts from urine, attempts have been made to acidify urine by dietary means or with bladder washes. The experiments of Bibby et al. [1], however, have shown the futility of this approach in urine infected with *P. mirabilis*. The addition of hydrogen ions to the urine simply causes more urea to be converted by the bacterial urease into ammonia. Alkaline conditions are thus quickly restored. In this study we have used a simple physical model of the catheterised bladder to investigate the effect of the urease inhibitors acetohydroxamic acid and fluorofamide, on the development of catheter encrustation.

Materials and methods

Growth media

The artificial urine used in the experimental work was based on that devised by Griffith et al. [10]. It contained calcium chloride 0.49 g/l, magnesium chloride hexahydrate 0.65 g/l, sodium chloride 4.6 g/l, disodium sulphate 2.3 g/l, trisodium citrate dihydrate 0.65 g/l, disodium oxalate 0.02 g/l, potassium dihydrogen phosphate 2.8 g/l, potassium chloride 1.6 g/l, ammonium chloride 1.0 g/l, urea 25 g/l, gelatine 5.0 g/l. The pH of the medium was adjusted to 6.1 and then sterilised by membrane filtration. Tryptone Soya Broth (Oxoid, Basingstoke, UK) was prepared separately, autoclaved and added to the sterile basal medium to a final concentration of 1.0 g/l.

The bladder model

The model of the catheterised bladder has been described previously [19]. In essence it consists of a glass fermentation flask maintained at 37°C by a water jacket. After sterilisation of the model by autoclaving, a size 14 all-silicone catheter (Bard Crawley, UK) was inserted aseptically into the flask through a section of silicone tubing (a "urethra") attached to a glass outlet at the base of the flask. The catheter balloon was then inflated in the usual way, securing the catheter in position and sealing the outlet from the "bladder". Sterile urine was then supplied to the bladder at 0.5 ml min^{-1}. In this way a residual volume of 30 ml collects in the bladder below the level of the catheter eyelet and then flows through the catheter and drainage tube to a collecting bag.

The experimental protocol

Sets of models were assembled and the bladders primed with urine with and without various concentrations of the urease inhibitors acetohydroxamic acid (Sigma, Poole, UK) or fluorofamide (kindly provided by SmithKline Beecham, Betchworth, UK). The test organism *Proteus mirabilis* NSM6 had been isolated from a patient's encrusted catheter 18 months prior to the start of this study. For experimental purposes cells were subcultured monthly from a stock suspension in 5% glycerol stored at −80°C. The bladders were inoculated with 10 ml of a 4-h urine culture of the test strain. After 1 h, to allow the organisms to establish themselves in the model, the supply of urine was switched on. The models were run for 24 h before the urine supply was switched off. The catheters were removed from the models and cut into 2-cm serial sections. The sections were placed into 4% nitric acid (100 ml) and the encrustations disrupted by sonication for 5 min (Transsonic Water Bath, Camlab, Cambridge, UK). The calcium and magnesium content of the resulting solutions were then determined by atomic absorption spectroscopy.

Visual assessment of the encrustation was made on sets of catheters using low vacuum scanning electron microscopy. Cross-sections were made of catheters just below the eye-holes, and viewed directly using the low vacuum setting of a JEOL 5200 LV SEM at 20 kv. The low vacuum facility allows the direct examination of specimens that have not been fixed, stained or treated in any way.

Viable cell counts on the cultures used to inoculate the models and on the urine in the "bladders" at the end of the experiment were performed on CLED Agar (Oxoid, Basingstake, UK). The pH of the urine was also monitored over the 24-h period.

Results

Sets of four models were primed with urine containing various concentrations of either acetohydroxamic acid (0.0 mg/ml, 0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml) or fluorofamide (0.0 μg/ml, 0.1 μg/ml, 0.5 μg/ml and 1.0 μg/ml). The results from triplicate experiments in which the extent of encrustation produced on the catheters after 24 h growth of the test organism in the presence and absence of the urease inhibitors are shown in Figs. 1 and 3. The effects of the urease inhibitors on the pH of the bladder urine throughout the test period are presented in

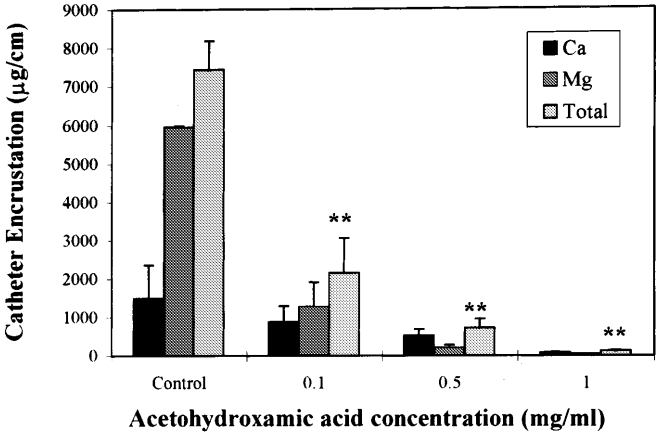

Fig. 1 Effect of acetohydroxamic acid on the encrustation of silicone catheters by *Proteus mirabilis* biofilms. Each value is the mean calculated from three replicated experiments. ** Indicates a significant difference ($P < 0.01$) from the control values (analysis of variance). The mean values for the log of the number of viable cells/ml of urine at 24 h were 8.02 (control), 8.16 (0.1 mg/ml), 8.20 (0.5 mg/ml) and 8.09 (1.0 mg/ml)

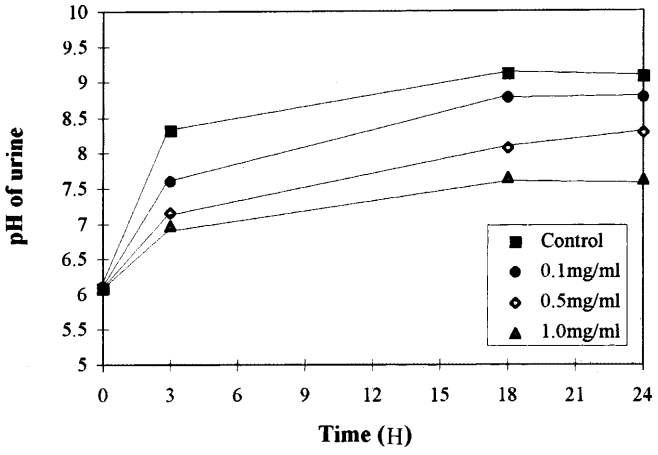

Fig. 2 Effect of acetohydroxamic acid on urinary pH. Each data point is a mean value calculated from three replicated experiments

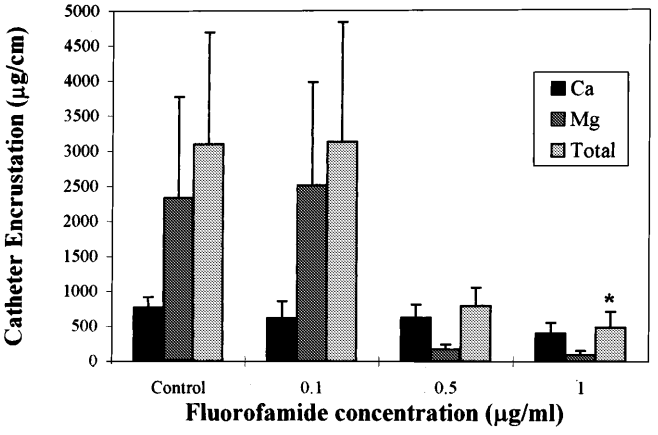

Fig. 3 Effect of fluorofamide on the encrustation of silicone catheters by *Proteus mirabilis* biofilms. Each value is the mean calculated from three replicated experiments. *Indicates a significant difference ($P < 0.05$) from the control values (analysis of variance). The mean values for the log of the number of viable cells/ml of urine at 24 h were 7.98 (control), 8.01 (0.1 μg/ml), 8.20 (0.5 μg/ml) and 8.16 (1.0 μg/ml)

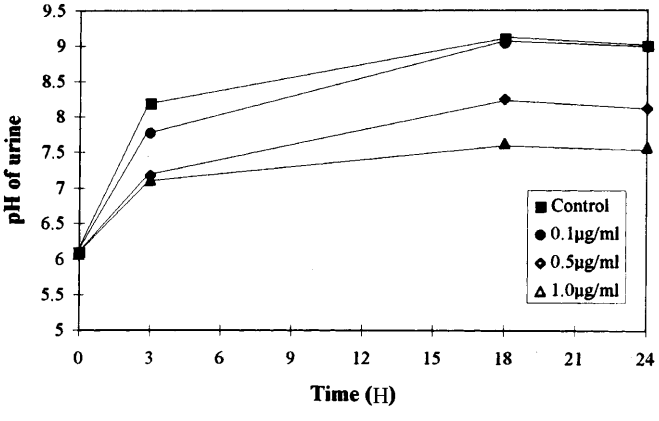

Fig. 4 Effect of acetohydroxamic acid on urinary pH. Each data point is a mean value calculated from three replicated experiments

Figs. 2 and 4. The scanning electron micrographs of catheter cross-sections (Fig. 5) confirm the observations that the inclusion of the urease inhibitors in the urine significantly reduced the extent of catheter encrustation and occlusion of the lumen.

Discussion

The driving force promoting the deposition of the calcium and magnesium phosphates on indwelling catheters is the urease of *Proteus mirabilis*. The results presented in Figs. 1–4 show that it is possible to inhibit

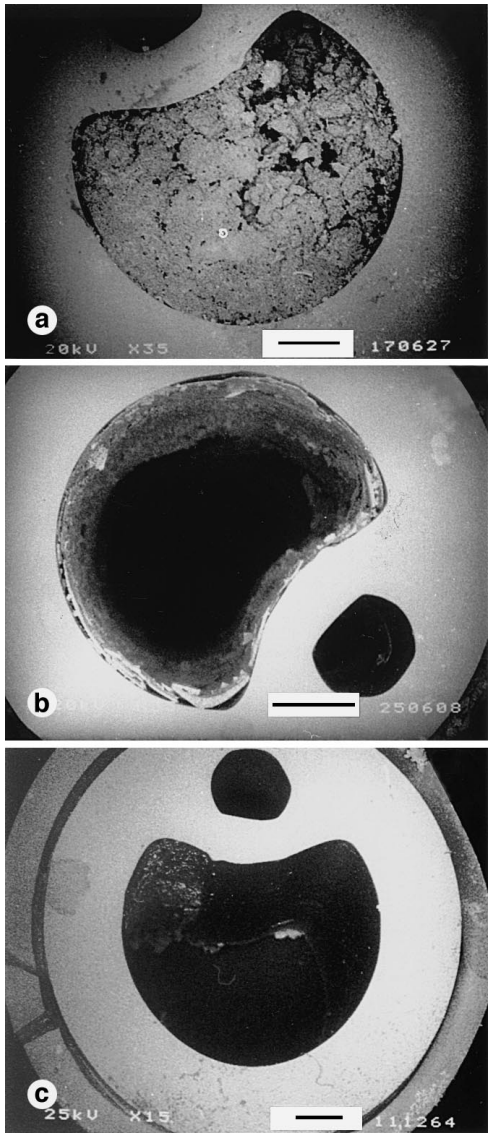

Fig. 5A–C Scanning electron micrographs of cross-sections of catheters taken 1 cm below the eye-holes. The catheters had been indwelling for 24 h in bladder models infected with *Proteus mirabilis*. Catheter (**a**) had been exposed to control urine, (**b**) to urine containing fluorofamide (1.0 μg/ml) and (**c**) to urine containing acetohydroxamic acid (1.0 mg/ml). In each case the scale bar represents 500 μm

the activity of this enzyme produced by dense cultures of *P. mirabilis* (10^8 cfu/ml) in urine, with acetohydroxamic acid (1 mg/ml) or fluorofamide (1.0 μg/ml). Under these conditions the urinary pH was maintained at < 7.6, and the extent of catheter encrustation was significantly reduced over the 24-h test period. The numbers of viable cells present in the urine at 24 h, (Figs. 1 and 3) show that the urease inhibitors had no bactericidal activity against the test organism, populations of 10^8 cfu/ml being maintained in the bladders throughout the experiments. The scanning electron micrographs (Fig. 5) show that the lumen-occluding crystalline biofilm usually associated with *P. mirabilis* was much reduced in the presence of the inhibitors.

Acetohydroxamic acid has been shown to lower urinary pH in patients infected with *P. mirabilis* [8]. It is readily absorbed from the gastrointestinal tract and peak concentrations appear in the bloodstream approximately 1 h after oral administration. It is partially metabolised to carbon dioxide and acetamide and about 20%–48% is excreted unchanged in the urine [20]. Clinical trials with this urease inhibitor have demonstrated that it is effective in inhibiting the growth of infection-associated renal struvite stones [9, 23]. The effective doses of 250 mg of acetohydroxamic acid every 8 h produced urinary concentrations of 0.15–0.3 mg/ml. The data presented in Fig. 1 suggest that this concentration would also have a significant effect in inhibiting the formation of catheter encrustation. Unfortunately, the significant toxicity of acetohydroxamic acid has limited its use in treating urolithiasis. The major side effects include haemolytic anaemia, deep venous thrombosis, gastrointestinal and neurological symptoms [9, 23].

Fluorofamide (*N*-[diaminophosphinyl]-4-fluorobenzamide) is a significantly more potent inhibitor of *P. mirabilis* urease than acetohydroxamic acid [17]. The results presented in Figs. 1 and 3 also show that fluorofamide is approximately 1000 × more active in inhibiting catheter encrustation. Experiments in rats suggest that 30%–50% of orally administered fluorofamide is excreted in the urine and that doses of 15 mg/kg per 24 h were effective in inhibiting the development of urinary stones [17].

Little information is available on the toxicity of fluorofamide, though Millner et al. [17] reported that it was not mutagenic in the Ames test and that the acute LD50 for mice was high (2125 mg/kg). It has also been used to eliminate ureaplasma infections in mice and marmosets without any apparent toxic effects [6, 16].

An effective method of controlling or preventing the deposition of struvite on catheters would be an advance in the care of the many patients undergoing long-term urethral catheterisation. The results presented in Fig. 3 suggest that fluorofamide at 1.0 μg/l in urine in the bladder model will inhibit catheter encrustation by *P. mirabilis*. It is therefore possible that low doses of oral fluorafamide could control catheter encrustation in patients.

In view of the toxicity of acetohydroxamic acid, any clinical studies on the efficacy of urease inhibitors in controlling catheter encrustation should of course be conducted with care. It might be feasible, however, to introduce these agents into the bladder urine or catheter biofilm in ways which avoid any systemic toxicity.

These results have confirmed that inhibition of urease activity is a feasible approach to the control of catheter encrustation. It is possible that certain natural components of human urine might have inhibitory effects on urease-induced crystallisation. The identification of any individuals whose catheters remain free of encrustation even when they have urinary infections with *P. mirabilis* could help in the isolation and characterisation of such inhibitors.

Conclusions

Acetohydroxamic acid (1.0 mg/ml) and fluorofamide (1.0 μg/ml) restricted the increase in pH of *P. mirabilis*-infected urine from pH 9.1 to 7.6. These compounds also significantly reduced the deposition of calcium and magnesium salts on silicone catheters exposed to *P. mirabilis*-infected urine. The results from this in vitro study suggest that urease inhibitors particularly fluorofamide, could have clinical applications in the control of catheter blockage and encrustation by mineralised *P. mirabilis* biofilms.

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