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ORIGINAL PAPER

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Factors affecting crystal precipitation from urine in individuals with long-term urinary catheters colonized with urease-positive bacterial species

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Abstract Weekly urinalysis was conducted for 12 weeks on a group of 21 long-term catheter users with confirmed catheter encrustation and urinary tract colonization with urease-positive bacteria, in order to explore the cause of considerable variation in the severity of encrustation between sufferers. The rapidity of catheter blockage correlated significantly with the pH above which crystals precipitated from urine (the nucleation pH) but not the pH of the voided urine itself. Linear regression showed the nucleation pH to be significantly predicted by a combination of urinary calcium and magnesium concentrations, with calcium being the more influential variable. Reducing the rate of catheter encrustation could be achieved by lowering the urinary concentration of calcium and magnesium, which may only require catheter users to increase their fluid intake.

Keywords Catheter encrustation · *Proteus mirabilis* · Urinary pH · Bacterial biofilms

Introduction

The most common of the many complications experienced by patients undergoing long-term indwelling bladder catheterization is caused by encrustation of the catheter by a crystalline bacterial biofilm [1]. In almost half of these patients, crystalline deposits accumulate in the catheter lumen and cause frequent and unpredictable obstruction of urine drainage from the bladder. Managing the resulting incontinence or painful retention of urine increases the workload of health care providers and results in significant distress for the catheter user [2]. Typically, the crystalline component of the blocking material is composed of struvite (magnesium ammonium phosphate) and carbonate apatite (a form of calcium phosphate) [3]. The main cause of catheter encrustation is colonization of the urinary tract by urease-producing bacteria, particularly *Proteus mirabilis* [4]. These organisms colonize the catheter, forming biofilm communities consisting of layers of bacteria embedded in an extensive extracellular polysaccharide matrix. The urease catalyses the hydrolysis of urinary urea to ammonia, leading to the increased formation of various ionic species as the pH rises, and these then precipitate as struvite and carbonate apatite crystals. These crystals become incorporated into the extracellular matrix produced by the organisms to form the crystalline biofilm which blocks the catheter [5].

It is apparent to the clinician that even among those with *Proteus* colonization, alkaline urine and visible encrustation on their catheters, there is considerable variation in how long the catheters of different individuals take to block. The factors responsible for controlling the rate of crystalline biofilm formation on catheters are poorly understood. Several previous studies on risk factors for the complication have examined the differences between patients who suffer catheter blockage and those who do not [6–9]. The groups have usually been divided on the basis of catheter lifespan without reference to the organisms present in the urine, with patients whose catheters last for less than 2–6 weeks being designated 'blockers'. Many patients designated as 'non-blockers' in these comparative studies will not have been colonized by urease producers and would therefore not produce encrustation irrespective of the other variables under investigation. The study of these urease-free

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'non-blockers' is thus largely unhelpful in identifying factors that modulate encrustation. Further, as it is difficult to eliminate some of the most troublesome urease-positive organisms, such as *P. mirabilis* and related species, from the catheterized urinary tract, such studies are unlikely to identify factors which might lead to better treatment for sufferers of encrustation. In not identifying patients who are colonized by urease producers and yet encrust more slowly than the time allowed for the definition of a 'blocker', these studies have failed to focus on just the group that could provide valuable insights into the problem.

In a recent prospective study, we showed that in patients colonized with urease-positive *Proteus* species, catheter lifespan varied enormously between individuals. Some catheters blocked after 2 days while others drained for up to 98 days [10]. It was also shown that the pH of the voided urine (pH_v) was not significantly different between groups of catheter users designated as rapid and slow catheter encrusters. However, the pH above which crystals precipitate from the urine, the nucleation pH (pH_n), was significantly higher in the group of slow encrusters. The aim of the current study was to further examine the correlation between pH_n and catheter lifespan and to investigate the relationship between pH_n and the calcium and magnesium contents of urine in these patients who are colonized by urease-producing organisms.

Patients and methods

A prospective study was conducted on a group of 21 long-term catheter users known to have urease-positive urinary tract colonization (19 *P. mirabilis*, 1 *P. vulgaris* and 1 *Morganella morganii*). Each had been catheterized for at least 9 months prior to the study. None of the patients had a history of non-infective renal calculi. Weekly urine samples were collected on the same day and at the same time each week for at least a 12-week period. Catheters were changed when blocked, and the period of time the catheters had been in situ recorded as the catheter lifespan. Catheters were examined when removed to confirm that the blockage was due to catheter encrustation. Those not blocked by encrustation were excluded from the analysis. Urine samples were tested for pH_v, pH_n and total calcium and magnesium concentrations weekly and mean figures used in the analysis.

The pH_v of each subject's urine was measured immediately on collection of the sample using a portable glass electrode pH meter (Checker 1, Hanna Instruments, Leighton Buzzard, UK). To measure the calcium and magnesium concentrations, urine was acidified to a pH of 4.0 using concentrated hydrochloric acid and centrifuged at 3,500 rpm for 3 min to remove insoluble particulate matter. The supernatant was diluted to 10% in 5% nitric acid. Concentrations were measured using atomic absorption spectroscopy on a PerkinElmer Instruments AAnalyst 200 spectrometer. Evaluation of pH_n was based on the method described by Choong et al. [7]. Experiments were performed in a water-jacketed chamber at 37°C. An electronic meter was used to measure the pH (FisherBrand Hydrus 300). Urinary pH was first reduced to 4.0 by adding aliquots of concentrated hydrochloric acid to the sample. The sample was then alkalinized in increments of 0.25 pH units with 10 M sodium hydroxide solution up to a pH of 10, or until sufficient data points had been collected above the supposed nucleation pH to allow a straight line to be plotted. At each increment the optical density was measured at 550 nm using a distilled water blank on a Cecil Model CE 1011 spectrophotometer. Concentrations of calcium and magnesium remaining in solution were also measured as before. The pH_n was determined from the resulting plot of pH versus optical density, or pH versus [Ca] and [Mg]. The pH_n was defined by an abrupt change in the slope of the graph showing either an increase in turbidity or a decrease in dissolved [Ca] or [Mg] caused by precipitation of crystalline Ca- and Mg-containing salts. Plotting pH versus [Ca], [Mg] or optical density produces two straight line segments which intersect at the pH_n. Regression lines were calculated by least squares analysis for these two portions of the graph and used to determine the pH at their intersection.

Results

The results presented in Fig. 1 show no correlation between the length of time a subject's catheter drained freely and the pH_v of their urine. In contrast, analysis showed a highly significant positive correlation between catheter lifespan and pH_n (Fig. 2). It is clear that among patients colonized with urease-producing bacteria, the catheters of those with a lower pH_n are more likely to block rapidly, not necessarily those with a higher pH_v.

The analysis of the data presented in Figs. 3 and 4 demonstrates a highly significant negative correlation between mean pH_n and mean urinary calcium and

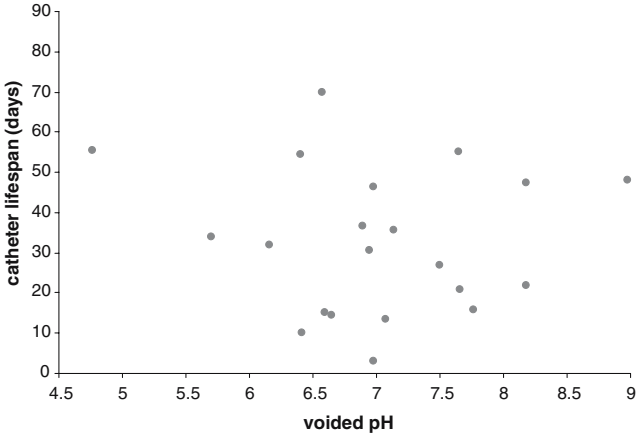

Fig. 1 A plot of mean catheter lifespan against mean voided pH. There is no correlation between the two variables ($r = -0.088$, $P = 0.704$)

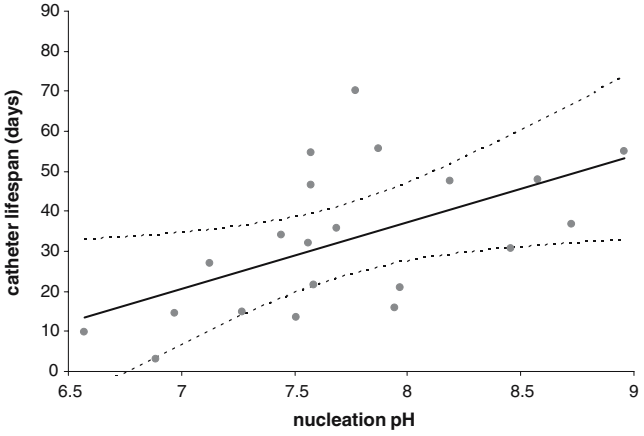

Fig. 2 Plot showing the fitted regression line (*solid*) with 95% confidence interval band (*dotted*) of the regression of mean catheter lifespan on mean nucleation pH ($r^2 = 0.315$). There is a highly significant positive correlation between the two variables ($r = 0.562$, $P = 0.004$)

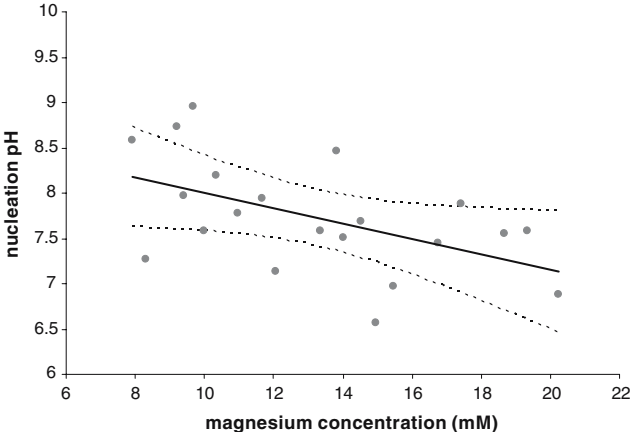

Fig. 4 Plot showing the fitted regression line (*solid*) with 95% confidence interval band (*dotted*) of the regression of mean nucleation pH on mean magnesium concentration ($r^2 = 0.267$). There is a highly significant negative correlation between the two variables ($r = -0.517$, $P = 0.008$)

magnesium concentrations of a patient's urine. Calculating the coefficient of determination r^2 for the regression of pH_n on calcium concentration indicated that almost half the variability in pH_n can be explained by changes in urinary calcium concentration alone, more than for magnesium concentration when considered alone. Both mean urinary calcium and magnesium concentrations are significant individual predictors of pH_n, with changes in calcium producing more marked changes in pH_n than those in magnesium.

Using an additive multiple linear regression model, [Mg] was not found to be a significant predictor of pH_n, allowing for the explanation of the variation in pH_n already predicted by the [Ca] variable. Additive models assume that the effects of changes in one variable are unaffected by the absolute level of the other variable. In other words, changes in calcium concentration would produce the same change in pH_n irrespective of the value of magnesium concentration. To examine the theory that the effect of one variable might change depending on the value of the other, an interaction term [Ca][Mg] was calculated, as the product of calcium and magnesium concentrations. In this model, all the terms are highly significant ($P < 0.001$ for [Ca], [Ca][Mg] and the intercept, and $P = 0.003$ for [Mg]). The linear regression prediction equation is

$$pH_n = 10.6 - 0.61\ [Ca] - 0.13\ [Mg] + 0.027\ [Ca][Mg].$$

For the model with interaction, $r = 0.875$, and so is higher than the r of 0.704 for the model with just [Ca] as a predictor, indicating that the model with interaction provides the best fit for the data. A plot of the pH_n results predicted by this equation against the actual results observed in the study participants is shown in Fig. 5. This model shows that changing the value of either calcium or magnesium concentration has less effect if the concentration of the other variable is already high.

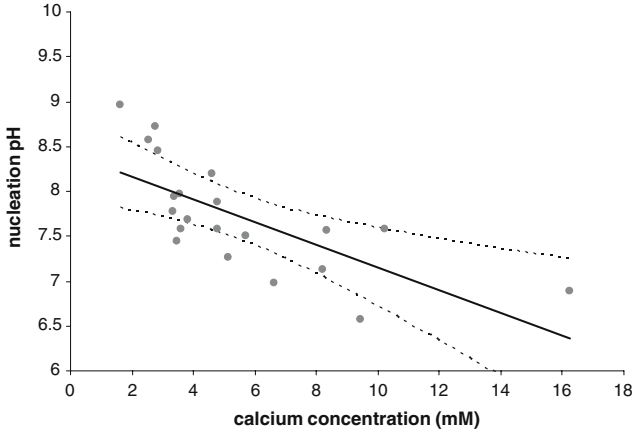

Fig. 3 Plot showing the fitted regression line (*solid*) with 95% confidence interval band (*dotted*) of the regression of mean nucleation pH on mean calcium concentration ($r^2 = 0.495$). There is a highly significant negative correlation between the two variables ($r = -0.704$, $P < 0.001$)

Discussion

It is inevitable that a long-term catheter will be colonized by bacterial biofilm [11]. These structured communities of multiple bacterial species form layers over the surfaces of the catheter [12]. Any urease-positive bacteria present encourage the precipitation of struvite and carbonate apatite crystals into the biofilm. The secreted extracellular polysaccharide matrix protects both the bacteria from the effects of antibacterials [13] and the crystals from any subsequent decrease in urinary pH which would ordinarily cause them to re-dissolve [14]. Chronically acidifying the urine is also impractical [15]. The only alternative treatment currently available for catheter encrustation is to reduce the action of bacterial

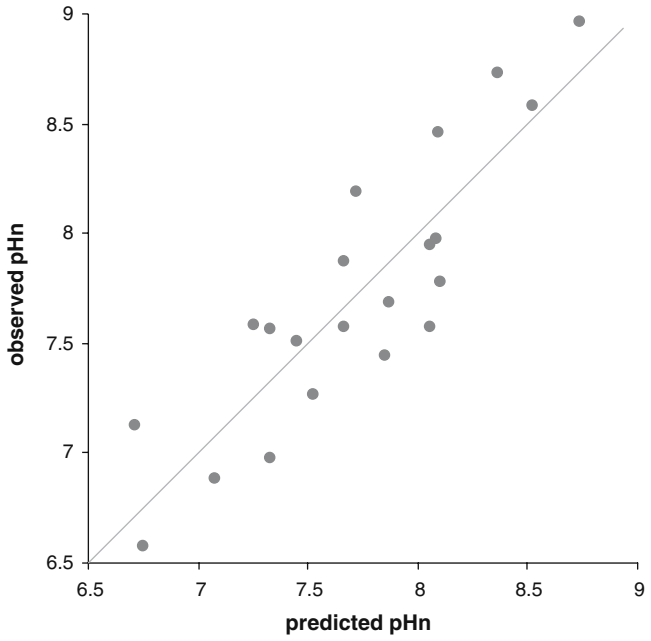

Fig. 5 Plot of observed values of mean nucleation pH against nucleation pH predicted from mean [Ca] and [Mg] values using the multiple linear regression equation. The *45°* grey line shows where points would lie if the prediction were perfect

urease. Urease inhibitors such as acetohydroxamic acid and fluorofamide have been shown to reduce the rate of catheter encrustation in an in vitro model [16]. Unfortunately they have substantial side effects which limit their long-term clinical use in catheter users, who often have considerable co-morbidity [17].

As catheter encrustation is brought about by urease activity and elevation of the urinary pH, it would seem likely that the higher the urinary pH generated by the biofilm, the more rapid the blockage of catheters. The results presented in Figs. 1 and 2, however, suggest a more subtle relationship between the urinary pH and the encrustation process. Analysis of the data shows that in patients infected with urease-producing bacteria, pH_n is the important factor predicting the rate of catheter encrustation. Patients with high pH_n values are more likely to encrust their catheters slowly. The results of our recent prospective observational study [10] on catheter encrustation in patients infected with *Proteus* species showed that while there were individuals with consistently high or low pH_n values, in some subjects this value varied considerably from week to week. These observations suggested that it may be possible to manipulate pH_n as a therapeutic approach. This work also demonstrated that it is possible to have a *Proteus* infection leading to a rise in pH_v above the levels at which encrustation would normally be assumed to occur, but to have few problems as a result of having urine possessing a very high pH_n. One avenue for future treatment is to accept that pH_v cannot be lowered in the presence of urease-positive organisms, that urease-positive organisms may not be eradicated and that urease itself may not be safely inhibited, but that pH_n may be raised sufficiently to alleviate the problem of encrustation.

It would seem intuitive that the concentrations of calcium and magnesium in the voided urine would correlate with a urease-positive catheter user's propensity to form crystals of struvite and carbonate apatite. As expected, in a sterile in vitro study using human urine incubated with purified jack bean urease, Hugosson et al. [18] found a positive relationship between the precipitation of magnesium ammonium phosphate and calcium phosphate and the urinary concentration of magnesium and calcium, respectively. However, in studies comparing the urine of catheter 'blockers' and 'non-blockers', no differences in calcium or magnesium concentrations were found by Choong et al. [7] or Getliffe [8], but significant differences in urinary calcium were found by Kunin et al. [9] and Burr and Nuseibeh [6]. These contradictory results may be because the 'non-blockers' in these studies were not colonized by urease-positive organisms and so were unlikely to encrust irrespective of their urinary composition. It is doubtful that studying those catheter users who lack the major cause of encrustation will lead to strategies to manage this condition.

The data presented in Figs. 3 and 4 show that in patients with urease-producing bacteria in their urine, the concentrations of both calcium and magnesium are significant predictors of pH_n. The concentration of urinary calcium affects the propensity of the urine to precipitate crystals of carbonate apatite, which is reflected in the nucleation pH. The causative relationship is shown by the addition of calcium to samples of uninfected human urine, which has been shown to decrease pH_n [19]. The same effect was seen with the addition of magnesium. In our current study, the multiple linear regression analysis showed that both calcium and magnesium concentrations were important determinants of pH_n in this group of patients. Calcium was found to have a greater influence than magnesium on pH_n. Certainly, calcium-containing carbonate apatite crystals precipitate at lower pH than the magnesium-containing struvite [7] and so could be expected to have more influence on the pH above which encrustation occurs. There may be considerable therapeutic value in altering the concentration of calcium, or those urinary constituents which chelate calcium. The regression model also seems to show that the effects of calcium reduction will only be pronounced if urinary magnesium is also low, presumably because otherwise struvite precipitation will continue to occur.

Diluting the urine will result in lowering the concentration of both these constituents and therefore increasing fluid intake may be the simplest way to achieve maximum benefit. The recent study by Suller et al. [19] found that diluting urine by increasing fluid intake has a profound effect on pH_n in healthy subjects. For example, increasing an individual's normal fluid intake by just 750 ml resulted in the pH_n rising from 6.48 to 8.22. A study of encrustation of catheters using

P. mirabilis infected human urine in a catheterized in vitro bladder model also showed that the times taken by catheters to block can be extended significantly by increasing the fluid intake of those donating the urine [20]. All these results are consistent with the conclusion that increasing the fluid intake to dilute the urine will raise pH_n and increase the lifespan of the catheter. It should be noted, however, that substantially increasing the fluid intake of the elderly and disabled populations which make up the majority of long-term catheter users is not always easy to achieve in practice, and innovative approaches may be required.

In summary, for those truly at risk of catheter blockage, the rate at which catheters encrust can be seen to be related not to the pH of a catheter user's urine but to that urine's nucleation pH. Urinary pH_n is in turn related to the concentrations of calcium and magnesium in the urine. Decreasing the concentration of these urinary constituents by increasing the fluid intake should elevate pH_n to levels that will ensure little deposition of crystalline material on catheters. However, it may be that measures to reduce calcium and magnesium output or the availability of these substances to form crystals in the urine will also be needed in some patients.

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