Urol Res (1995) 23:335–338

ORIGINAL PAPER

P. J. du Toit · C. H. van Aswegen · J. A. Nel
P. L. Steyn · A. J. Ligthelm · D. J. du Plessis

In vivo effects of urease-producing bacteria involved with the pathogenesis of infection-induced urolithiasis on renal urokinase and sialidase activity

Received: 9 December 1994 / Accepted: 27 January 1995

Abstract Many hypotheses have been proposed for renal stone formation. It has been argued that with infection-induced renal stones the hydrolysis of urea by bacterial urease increases urinary pH, with consequent stone formation. Unfortunately, this theory is not applicable to the micro-organisms that do not produce urease (e.g. *Escherichia coli*). It has been recently reported that *E. coli* reduces the urinary urokinase activity of male rats, but does not influence the urinary sialidase activity. This study has now been expanded to the urease-producing bacteria *Proteus mirabilis*, *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa* and *Micrococcus luteus*. Subcutaneous injections with these bacteria were found to significantly ($P < 0.003$) reduce the UK activity of extrarenally obstructed kidneys. The urease-producing mammalian skin bacterium, *M. luteus*, was, however, the exception ($P = 0.1079$). In contrast to *S. epidermidis*, *P. aeruginosa* and *M. luteus* ($P < 0.0213$), *P. mirabilis* and *S. aureus* had no effect on renal sialidase activity ($P < 0.4047$). These results may explain why *Proteus* species are predominant in infection-induced renal stones. According to the urokinase-sialidase hypothesis, a decrease in urinary urokinase activity should increase the uromucoid levels, whilst no effect on the urinary sialidase activity should favour conversion of urinary uromucoid to mineralizable matrix. These conditions may lead to renal stone formation. An increase in urinary pH resulting from urease-producing micro-organisms will increase salt precipitation on the uromucoid. It is thus concluded that urease-producing bacteria may play a double role in renal stone formation.

Key words Bacteria · Pyelonephritis · Sialidase (Neuraminidase) · Urease · Urokinase · Uroliths

P. J. du Toit · C. H. van Aswegen (✉) · J. A. Nel · D. J. du Plessis
Department of Urology, HF Verwoerd Hospital, Pretoria 0001, Republic of South Africa

P. L. Steyn
Department of Microbiology,
University of Pretoria,
Pretoria, Republic of South Africa

A. J. Ligthelm
Department of Oral Pathology and Biology,
University of Pretoria,
Pretoria, Republic of South Africa

One of the many theories on the pathogenesis of renal stone formation is that urease-producing bacteria are involved in urolithiasis [6]. Hydrolysis of urea increases urinary ammonia, bicarbonate, carbonate and general alkalinity. The increased pH subsequently leads to precipitation of salts. Since this theory is not applicable to some micro-organisms, such as *Escherichia coli*, that do not produce urease but are present with infection-induced renal stones, the question arises as to whether the infection-inducing bacteria may be playing a double role in renal stone formation not only by producing urease, but also by affecting urokinase (UK) and sialidase (SA) activity. These urinary enzymes may be important in renal stone formation since it has been hypothesised that a decrease in UK and an increase in SA activity could lead to an increase in urinary uromucoid concentration, which would then favour renal stone formation, in accordance with the matrix theory [1, 2, 4].

Spectrophotometric studies have shown a strong correlation between bacteria present with renal stones and increased levels of inhibition or stimulation of UK or SA activity, respectively [1]. When pyelonephritis has been induced in rats by unilateral extrarenal urinary obstruction and subcutaneous injection of *E. coli*, the UK has been significantly reduced ($P = 0.0171$), whilst the SA activity remained the same [5]. Since

E. coli is urease-negative, it would be of interest to see whether a similar mechanism is present with urease-producing bacteria, in addition to the accepted splitting of urea and the biochemical changes that result in increased alkalinity. In order to investigate this, the rats with unilateral extrarenal urinary obstructions used in this study were subcutaneously injected with urease-producing bacteria.

Materials and methods

Animals: pyelonephritis in rats

Twelve-week-old male Sprague-Dawley rats (30) were housed in a temperature-controlled room where a constant 12-h light/12-h dark cycle was maintained. The rats had access to an Epol laboratory chow diet and water ad libitum. Infection of kidneys was achieved by extrarenal ureteric obstruction as reported [5, 8]. Each group consisted of five rats.

Preparation of kidney cytosol

The rats were killed with carbon dioxide. Kidneys were placed in 5 ml ice cold 0.1 M sodium phosphate buffer, pH 7.5, containing 10 mM ethylene diaminetetra-acetic acid (EDTA) and 0.1 g/l Triton X-100. The tissue was homogenised for 15 s at 9500 rpm with an Ultra Torrax T25 homogeniser (Janke & Kunkel, IKA-Labortechnik, Staufeni). The homogenate was centrifuged at 1700 g for 30 min at 4 °C, and the supernatant stored in ice. Protein concentration was determined with the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif, USA). Bovine albumin was used as the standard. Absorbance was measured at 595 nm in a Hitachi 150-20 spectrophotometer (Tokyo, Japan).

Urokinase activity determination

Urokinase activity was assayed according to a modified method of Wiman et al. [12]. Briefly, 5 µl cytosol was added to 400 µl activator reagent and 423 µl 0.1 µM sodium phosphate buffer, pH 7.5, containing 10 mM EDTA and 0.1 g/l Triton X-100. The activator reagent was composed of 1.0 µM plasminogen and 0.6 mM D-valyl-L-leucyl-L-lysine *p*-nitroanalide, dissolved in 0.1 M sodium phosphate buffer. All additions were performed in ice. The total volume was 828 µl. The tubes were then placed in a water bath at 37 °C for 90 min. After the desired incubation period, the reaction was stopped by placing the tubes in ice and adding 0.1 ml 50% acetic acid to each tube. The difference in absorbance between the blank and control (containing cytosol) was measured at 405 nm with a Hitachi 150-20 spectrophotometer. The molar absorptivity for *p*-nitrophenol was taken as 9620 mol/l per centimetre [1].

Sialidase activity determination

Sialidase activity was spectrophotometrically determined as described before [3, 11]. The reaction mixture consisted of 200 µl sialyllactose (0.15 mM), 1890 µl TRIS buffer (50 mM, pH 7.5), 30 µl nicotinamide adenine dinucleotide, reduced (NADH, 10 mM), 10 µl freshly prepared lactate dehydrogenase (LDH) (0.2 ml LDH in 0.8 ml distilled water), 20 µl *N*-acetyl neuraminic acid (NANA)-aldolase (0.4 U) and 20 µl cytosol. The total reaction volume was 2170 µl. NADH, the measured variable, was monitored at 334 nm in a Hitachi spectrophotometer at 37 °C, connected to a data processor. The molar absorptivity for NADH was taken as 6220 mol/l per centimetre [10].

Reagents and chemicals

The reagents NADH, rabbit muscle LDH in ammonium sulphate solution, NANA-aldolase from *E. coli* and bovine colostrum *N*-acetylneuraminosyl-D-lactose (sialyllactose) were obtained from Boehringer (Mannheim, Germany). Merck (Darmstadt, Germany) and BDH (Poole, Dorset, England) supplied sodium phosphate, EDTA and Triton X-100. The substrates plasminogen (human plasma) and D-valyl-L-leucyl-L-lysine *p*-nitroanalide as well as lyophilised urokinase powder from human kidney cells were obtained from Sigma (St. Louis, Mo., USA). Nutrient broth No. 2 was supplied by Oxoid (Basingstoke, UK). Epol (Johannesburg, South Africa) supplied the basic laboratory chow diet.

Micro-organisms

Nutrient broth medium (10 times diluted with water) was used as growth medium. The inoculated flasks were incubated overnight at 37 °C, centrifuged (1700 g, 30 min, 5 °C), suspended in the diluted nutrient broth and counted on a haemocytometer (Neubauer).

Statistical analysis

The non-parametric Wilcoxon's signed rank test was used to compare the renal UK and SA activities of kidneys with and without extrarenal obstructions. The unpaired Mann-Whitney U test was performed to compare the renal UK and SA activity in rats with and without pyelonephritis.

Results

To determine the effect of urease-producing bacteria on the UK and SA activity in male rats, various urease-producing bacteria were investigated (Table 1). Except for *Micrococcus luteus*, all the bacteria tested have been reported to be associated with renal stone formation

Table 1 Characteristics of micro-organisms (+, − occasionally)

Organism	Producing urease [6, 9]	Gram classification [9]	Present with stones [6, 7, 9]
Proteus mirabilis	+	−	Yes
Staphylococcus aureus	+	+	Yes
Staphylococcus epidermidis	+, −	+	Yes
Pseudomonas aeruginosa	+, −	−	Yes
Micrococcus luteus	+, −	+	No

[6, 7, 9]. Extrarenal obstruction of kidneys resulted in histological changes associated with pyelonephritis. The degree and extent of these changes varied from moderate to severe chronic inflammatory cell infiltration, interstitial fibrosis, pronounced tubular changes including atrophy, dilatation and degeneration as well as marked vascular changes. Neutrophil infiltration with suppuration and abscess formation in some cases were more prominent in the bacterially infected cases than in the controls. Although differences in the severity of the above changes were seen within groups, the acute inflammatory changes were more extensive in animals infected with *Proteus mirabilis*. The non-obstructed kidneys revealed normal renal structures and total absence of any of the above-mentioned features. These results are consistent with the microscopic examination of kidneys infected with *E. coli* [5].

The UK ($P = 0.02334$) and SA ($P = 0.02334$) control activities of the five obstructed kidneys were significantly increased (Figs. 1, 2). The UK and SA activity of the non-obstructed kidneys from infected animals, five rats in each category, did not differ significantly from the control value ($P > 0.051$). The only exceptions were *Staphylococcus epidermidis* (UK) and *Pseudomonas aeruginosa* (UK and SA), which were significantly different from the control ($P < 0.021$). The UK activities of externally obstructed kidneys from rats infected with *P. mirabilis*, *P. aureus*, *S. epidermidis* and *P. aeruginosa* showed a statistically significant reduction ($P < 0.003$). In contrast, the UK activity of *M. luteus* did not differ significantly from that of the control ($P = 0.1079$). *P. mirabilis* ($P = 0.5215$) and *S. aureus* (P = 0.4047) had no significant effect on the SA activity of obstructed kidneys in contrast to *S. epidermidis*, *P. aeruginosa* and *M. luteus*, which significantly decreased the SA activity ($P < 0.0213$).

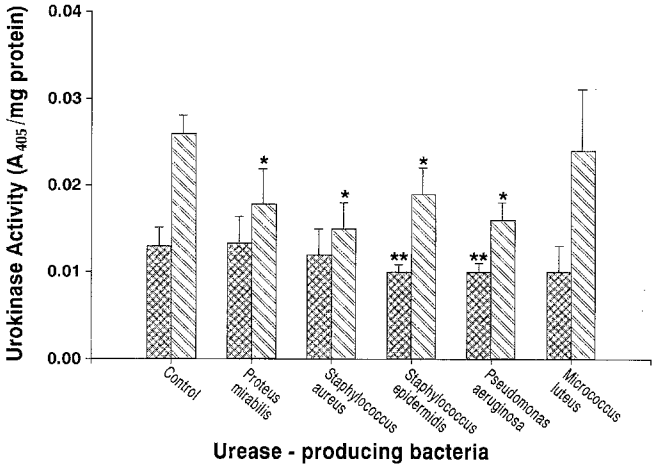

Fig. 1 Effect of urease-producing bacteria on renal urokinase activity. Data represent the mean ± SD of five kidneys. Significantly different from control: * $P < 0.003$, ** $P < 0.01$. ▩ Non-obstructed; ▨ obstructed

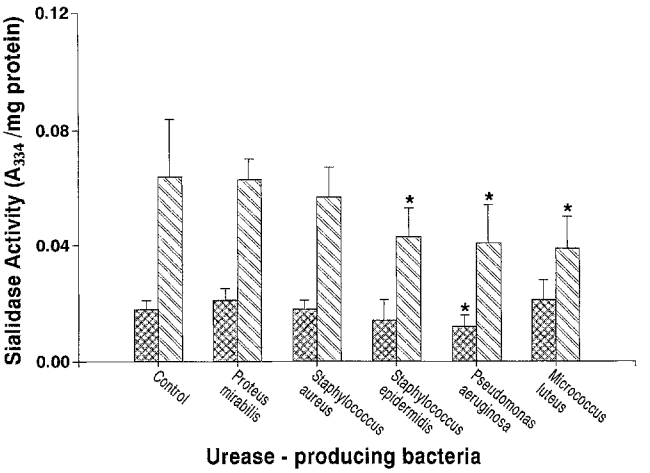

Fig. 2 Effect of urease-producing bacteria on renal sialidase activity. Data represent the mean ± SD of five kidneys. Significantly different from control: *$P < 0.0213$. ▩ Non-obstructed; ▨ obstructed

Discussion

It has been postulated that urinary UK and SA may play important roles in renal stone formation. With this in mind, the effect of *E. coli* on stone formation in rats has been investigated [5]. It has been reported that the UK and SA enzyme activities are similar in both kidneys of the same rat and that external renal obstruction results in increased UK and SA activities. The urease-negative bacterium *E. coli* significantly reduces the UK activity of the obstructed kidney ($P = 0.0171$). However, the SA activity remains unchanged ($P = 0.3929$). Similar results were obtained with the most dominant bacterium associated with infection-induced stones, namely *Proteus*. *P. mirabilis* and *S. aureus* conform to the UK/SA hypothesis on renal stone formation. It can be speculated that in the presence of *P. mirabilis* or *S. aureus* UK activity should be reduced with consequent increased urinary uromucoid concentrations, whilst the unchanged SA activity should result in the conversion of urinary uromucoid to mineralizable matrix. It is expected that *S. epidermidis* and *P. aeruginosa* will be less potent renal stone formers than *P. mirabilis* and *S. aureus*, since they decrease the SA activity. It is interesting to note that the urease-producing organism *M. luteus*, which is not associated with renal stones, showed characteristics opposite to those expected from a renal stone-promoting bacterium. For example, *M. luteus* had no effect on the UK activity, but reduced SA activity. These conditions would not favour the matrix theory.

The above results may explain the importance of urease and non-urease-producing organisms in renal stone formation. It can be argued that urease-producing and non-producing bacteria primarily affect the urinary UK and SA activity in renal stone formation. The increase in pH with certain bacteria is probably

a secondary effect. Thus urease-producing bacteria could be playing a dual role in renal stone formation. Whatever the cause(s) of urinary renal stone formation, the pathogenesis of this multifactorial disease remains complex.

Acknowledgements The authors would like to thank the Department of Physiology of the University of Pretoria for the use of their facilities. This work was supported by the University of Pretoria and the Medical Research Council of South Africa.

References

1. Aswegen CH van, Neitz AWH, Becker PJ, Plessis DJ du (1988) Renal calculi: urate as a urokinase inhibitor. Urol Res 16:143
2. Aswegen CH van, Hurter P, Merwe CA van der, Plessis DJ du (1989) The relationship between total urinary testosterone and renal calculi. Urol Res 17:181
3. Aswegen CH van, Merwe CA van der, Plessis DJ du (1990) Sialic acid concentrations in urine of men with and without renal stones. Urol Res 18:29
4. Aswegen CH van, Dirksen van Sckalkwyk JC, Toit PJ du, Verster L, Franz RZ, Plessis DJ du (1992) The effect of calcium and magnesium ions on urinary urokinase and sialidase activity. Urol Res 20:41
5. Aswegen CH van, Toit PJ du, Nel JA, Ligthelm AJ, Plessis DJ du (1994) Pyelonephritis: renal urokinase and sialidase (neuraminidase) activity in rats fed a standard laboratory diet. Urol Res 22:57
6. Backman U, Danielson BG, Ljunghall (1985) Renal stones. Almqvist and Wiksell, Stockholm, p 54
7. Bruce RR, Griffith DP (1981) Retrospective follow-up of patients with struvite calculi. In: Smith L, Robertson WG, Finlayson B (eds) Urolithiasis: clinical and basic research. Plenum, New York, p 191
8. Freedman LR, Kaminkas E, Beeson PB (1960) Experimental pyelonephritis. V11. Evidence on the mechanisms by which obstruction of urine flow enhances susceptibility to pyelonephritis. Yale J Biol Med 33:65
9. Griffith DP, Bruce RR, Fishbein WN (1980) Infection (urease)-induced stones. In: Coe FL, Brenner BM, Stein JH (eds) Nephrolithiasis. Churchill Livingstone, New York, p 230
10. Segel IH (1976) Biochemical calculations. Wiley, New York, p 416
11. Toit PJ du, Aswegen CH van, Steyn PL, Pols A, Plessis DJ du (1992) Effects of bacteria involved with pathogenesis of infection-induced urolithiasis on the urokinase and sialidase (neuraminidase) activity. Urol Res 20:393
12. Wiman B Mellbring G, Randby M (1983) Plasminogen activator release during venous stasis and exercise as determined by a new specific assay. Clin Chim Acta 127:279