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

Pyelonephritis: renal urokinase and sialidase (neuraminidase) activity in rats fed a standard laboratory diet

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Abstract Renal stone formation can be caused by many different and varied disturbances, some of which are poorly understood. The relationship between urinary infection and renal stone formation has not been completely clarified. It is argued that renal stones form primarily as a consequence of the hydrolysis of urea by the bacterial enzyme urease. However, no explanation is given for microorganisms that produce urease only occasionally or not at all. The question arises as to whether the infection-induced microorganisms might not be playing a double role in renal stone formation by not only producing urease, but also by affecting in vivo urokinase (UK) and sialidase (SA) activity. With this in mind, the effect of *Escherichia coli* on renal UK and SA activity has been studied in male rats with a normal diet. The renal UK ($P=0.208$) and SA ($P=0.2135$) activities did not differ significantly between the two kidneys of the same rat. In contrast, when drainage from one kidney of a rat was externally obstructed, the UK and SA activities differed significantly between kidneys ($P<0.015$). An increase in UK ($r=0.6456$, $P<0.0001$) and SA ($r=0.7507$, $P<0.0001$) activity was observed over time in the obstructed kidney. Subcutaneous injections with *E. coli* reduced the UK activity of the obstructed kidney significantly ($p=0.0171$). However, the SA activity remained the same ($P=0.3929$). This decrease in the UK activity in the presence of microorganisms may result in an increase in the uromucoid concentration, leading to renal stone formation in the presence of increased salt precipitation on the uromucoid as caused by the urease producing microorganisms.

Key words Kidney · Pyelonephritis · Rat · Sialidase (neuraminidase) · Urokinase

C. H. van Aswegen (✉) · P. J. du Toit · J. D. Nel · D. J. du Plessis
Department of Urology, H. F. Verwoerd Hospital,
Private Bag X169, Pretoria 0001, South Africa

A. J. Ligthelm
Department of Oral Pathology and Biology, University of Pretoria,
H. F. Verwoerd Hospital, Pretoria, South Africa

There are many theories on the pathogenesis of renal stone formation. One is that specific microorganisms are involved in urolithiasis [5]. According to this theory, these microorganisms produce the urea-splitting enzyme, urease, which causes an increase in the excretion of ammonium ions and carbonate apatite in urine. The ammonium ions increase the pH with subsequent precipitation of salts. Unfortunately, this theory is not applicable to the microorganisms which do not produce urease (*Escherichia coli,* etc.). We therefore hypothesize that these microorganisms could affect urinary urokinase (UK) and sialidase (SA) activity. Sialidase may be responsible for the conversion of urinary mucosubstances to mineralizable matrix [3], whilst UK regulates the urinary uromucoid concentration [1, 2, 4]. It is anticipated that a decrease in UK activity would increase the uromucoid concentration. According to the matrix theory, an increase in uromucoid could promote renal stone formation. The results obtained with microorganisms are consistent with this hypothesis. Microorganisms associated with infection-induced stones, *Proteus mirabilis* and *E. coli,* respectively inhibit and stimulate the UK and SA activity [9]. In contrast, microorganisms not associated with infection stones (*Bacillus subtilis*) have significantly less effect on UK and SA activity. The effect of *E. coli* on rat kidney UK and SA activity has been studied in rats fed a standard laboratory diet, and the results are reported here.

Materials and methods

Reagents and chemicals

All reagents were of "Analar" grade. The reagents NADH, rabbit muscle lactate dehydrogenase (LDH) in ammonium sulphate solution, *N*-acetylneuraminic acid aldolase (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 lyophilized urokinase powder from

human kidney cells were obtained from Sigma (St. Louis, Mo.). Nutrient broth no. 2 was supplied by Oxoid (Basingstoke Hants., England). Epol (Johannesburg, SA) supplied the basic laboratory chow diet.

Microorganisms

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

Animals

Twelve-week-old male Sprague-Dawley rats 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.

Preparation of kidney cytosol

The rats were terminated with carbon dioxide. Kidneys were placed in 5 ml ice cold 0.1 mol/l sodium phosphate buffer, pH 7.5, containing 10 mmol/l EDTA and 0.1 g/l Triton X-100. The tissue was homogenized for 15 s at 9500 rpm with an Ultra Torrax T25 homogenizer (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.). 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. [10]. Briefly, 5 µl cytosol was added to 400 µl activator reagent and 423 µl 0.1 mol/l sodium phosphate buffer, pH 7.5, containing 10 mmol/l EDTA and 0.1 g/l Triton X-100. The activator reagent was composed of 1.0 µmol/l plasminogen and 0.6 mmol/l D-valyl-L-leucyl-L-lysine *p*-nitroanalide, dissolved in 0.1 mol/l sodium phosphate buffer. All additions were performed in ice. The total volume was 828 µl. The tubes were then placed in a waterbath 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 spectrophotometer. The molar absorptivity for *p*-nitrophenol was taken as 9620 mol/l^{-1} cm^{-1} [1].

Sialidase activity determination

Boehringer (Mannheim, FRG) supplied the method for the spectrophotometric determination of sialidase activity [3]. The reaction mixture consisted of 200 µl sialyllactose (0.15 mmol/l), 1890 µl Tris-buffer (50 mmol/l, pH 7.5), 30 µl NADH (10 mmol/l), 10 µl freshly prepared LDH (0.2 ml LDH in 0.8 ml distilled water), 20 µl 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^{-1} cm^{-1} [8].

Determination of UK and SA activity in kidneys with and without extrarenal obstruction

After unilateral extrarenal obstruction was performed, rats were allowed to recover for 2 weeks before nutrient broth (200 µl) was injected s.c. for measurement of vehicle baseline values. Intravenous perfusions were performed after 1 week of obstruction. Rats were terminated after fixed periods and the kidneys removed and immediately placed in ice. Urokinase and SA activity were determined on cytosol obtained from kidneys with and without external obstruction. Seven rats were used in each of these experiments.

Pyelonephritis in rats

Infection of kidneys was obtained by extrarenal ureteric obstruction and s.c. injection of bacteria. Unilateral ureteric obstruction was performed through a midline transperitoneal approach. The colon was mobilized and the upper third of the ureter isolated and ligated with 5/0 Dexon distal to the pelvi-ureteral junction. The abdomen was anatomically repaired. Nutrient broth (200 µl) containing 1.9×10^8 *E. coli* cells was injected after 2 weeks of recovery, as described for the baseline determinations [6]. The rats (four) were terminated after 3 weeks, and UK and SA activity of kidneys with and without extrarenal obstructions were determined. Kidney specimens were fixed in 10% formalin and prepared for light microscopical examination.

Statistical analysis

The nonparametric Wilcoxon signed ranks test was used to compare the renal UK and SA activity of the same rat. The rank test was also used to compare the enzyme activities of kidneys with and without extrarenal obstructions.

The unpaired Mann-Whitney test was done to compare the renal UK and SA activity in rats with and without pyelonephritis.

Results

The UK ($P = 0.2084$) and SA ($P = 0.2135$) activity of the kidneys from the same rat did not show a statistically significant difference ($n = 10$). However, a significant difference was found when one of the rat's kidneys was extrarenally obstructed and nutrient broth injected after 2 weeks of recovery. The UK and SA activities obtained over time after injection are illustrated in Figs. 1 and 2. Although the UK ($r = 0.1168$, $P = 0.5039$) and SA ($r = 0.2413$, $P = 0.1627$) activity of the non-obstructed kidneys remained basically the same, the UK ($r = 0.6456$, $P < 0.0001$) and SA ($r = 0.7505$, $P < 0.0001$) activity of the obstructed kidneys increased over time. A maximum value was obtained with both enzymes when the rats were terminated 3 weeks after the injection of nutrient broth. The UK activity in the extrarenally obstructed kidneys decreased after 5 weeks. The UK and SA activities in the obstructed kidneys differed significantly from those in the non-obstructed kidneys ($P = 0.015$).

When rats were injected with nutrient broth containing *E. coli,* the UK activity was significantly lower ($P = 0.0171$) in extrarenally obstructed kidneys from infected animals

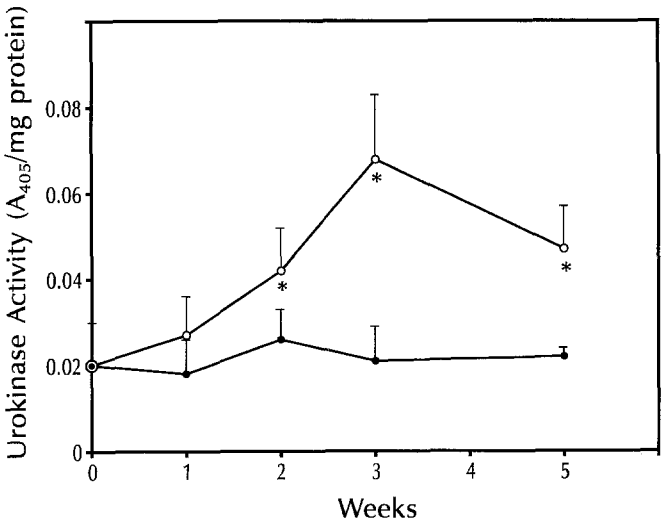

Fig. 1. Urokinase activity/time plot for extrarenally obstructed (○) and non-obstructed (●) kidneys. Each *point* is the average of 7 results (mean ± SD). *Significantly different from non-obstructed kidneys at the same time period ($P < 0.015$)

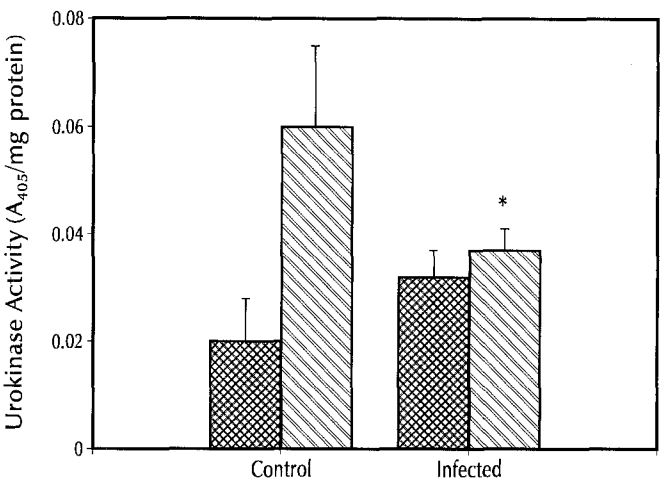

Fig. 3. Comparison of UK activity in rat kidneys with and without infection. Each determination was performed four times (mean ± SD). *Significantly different from control ($P < 0.018$). ▩ Non-obstructed; ▧ obstructed

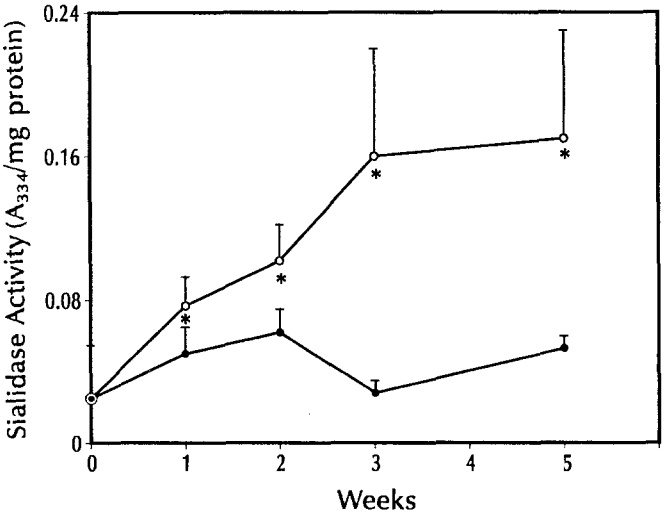

Fig. 2. Sialidase activity/time plot for extrarenally obstructed (○) and non-obstructed (●) kidneys. Each *point* is the average of 7 results (mean ± SD). *Significantly different from non-obstructed kidneys at the same time period ($P < 0.015$)

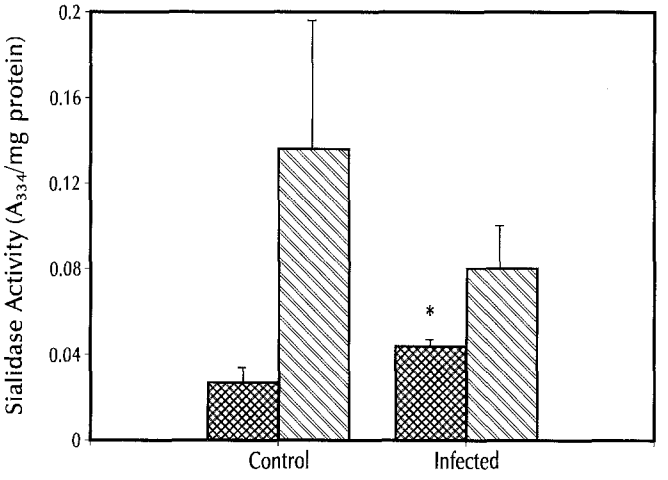

Fig. 4. Comparison of SA activity in rat kidneys with and without infection. Each determination was performed four times (mean ± SD). *Significantly different from control ($P < 0.011$). ▩ Non-obstructed; ▧ obstructed

than in kidneys obtained from control animals (Fig. 3). In contrast, the SA activity did not differ significantly ($P = 0.3929$) between these two groups (Fig. 4). Comparison of the UK (Fig. 3) and SA (Fig. 4) activities of the non-obstructed kidneys from the control and infected animals revealed no significant difference for the UK activities ($P = 0.0582$), in contrast to the SA activities ($P = 0.0106$). Microscopical examination of the kidneys that were extrarenally obstructed showed changes indicative of pyelonephritis. The degree and extent of these changes were pronounced and included moderate to severe inflammatory cell infiltration, extensive interstitial fibrosis, tubular atrophy, dilatation and/or degeneration, and marked vascular changes (Fig. 5). The cases with *E. coli* infection, however showed more neutrophil infiltration, with abscess formation in some cases. The non-obstructed kidneys, revealed normal renal structures with total absence of any of the above-mentioned features (Fig. 6).

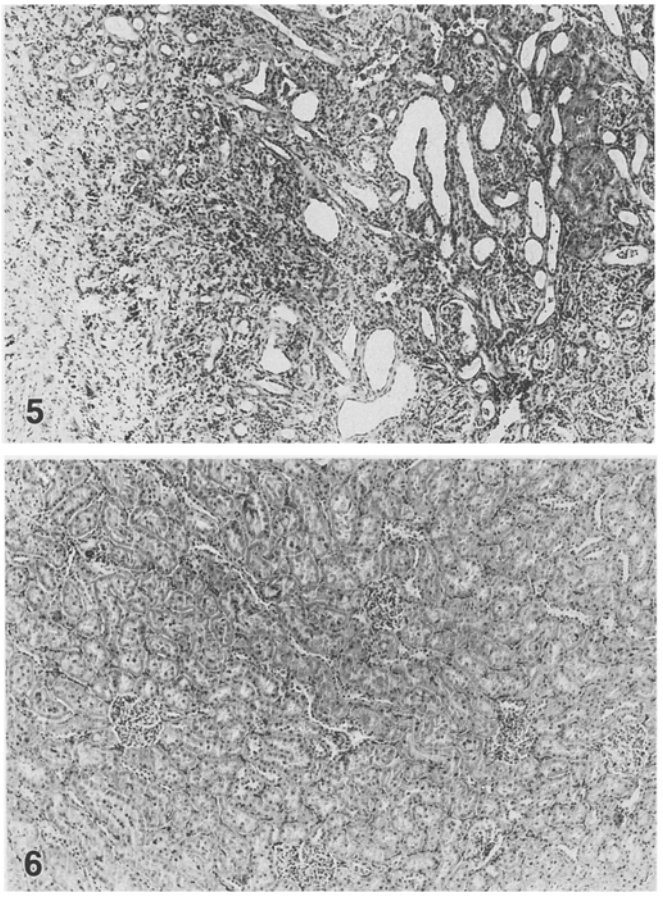

Fig. 5. Extrarenally obstructed kidney showing extensive changes of pyelonephritis. HE, 100×

Fig. 6. Non-obstructed kidney revealing normal renal structures HE, 100×

Discussion

Because the renal UK and SA activities differed between rats, within-rat comparisons of the enzyme activities in the two kidneys were first done. Statistically they were identical in UK and SA activity and therefore the experiments were devised so that one kidney could serve as the control whilst the other was the experimental kidney.

To obtain acute infection throughout the entire kidney, it was necessary to obstruct the ureter [7]. The microscopical changes observed in the obstructed kidneys were extensive in all instances, irrespective of secondary infection. The mere presence of total obstruction seemed to be responsible for the extent of changes, with more pronounced acute inflammatory cell infiltration where infection was also present. Urinary obstruction allows bacteria to multipy more readily in the renal parenchyma and serves to transmit infection from one part of the kidney to the other. The effect of obstruction on renal UK and SA activities was first studied when rats had received a general chow diet. As illustrated, both UK and SA activity increased. Although we cannot explain the increase in SA activity, the increase in UK activity may be attributed to a fibrinolytic effect.

In the presence of infection the UK activity significantly decreased. This in vivo effect of bacteria on the UK activity is in accordance with the spectrophotometric observations, namely that the bacteria causing infection-induced renal stones inhibit the UK activity. In consequence, this low UK activity could be causing an increase in uromucoid concentration, which would then increase the tendency for renal stone formation. Although these results may help to explain the role of non-urease-producing microorganisms in infection-induced renal stones, the probability that urease-producing microorganisms may have a dual role in renal stone formation is not excluded and requires further investigation.

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