Urol Res (1996) 24:345–348

ORIGINAL PAPER

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Ureaplasma urealyticum-induced urinary tract stones in rats

Received: 12 September 1995 / Accepted: 16 April 1996

Abstract This study investigated the possible role of *Ureaplasma urealyticum*, which is predominantly located in the urogenital tract, in the formation of infectious stones. A standardized *Ureaplasma urealyticum* broth culture isolated from a human urogenital specimen was inoculated into the renal medulla of five male rats (*Rattus norvegicus* L., Wistar C, weighing 170 ± 10 g) and the same amount of culture media was used for five identical control rats. Five days after the inoculation, the rats were killed and fresh preparations from the bladders and the inoculated kidneys of both groups were prepared. At the same time biochemical and histopathological analysis of the contents of the bladders and the inoculated kidneys of both groups was performed. Crystal formation within the bladders of the inoculated rats was demonstrated and biochemical analysis of the crystals showed calcium, magnesium and phosphate, which indicated the existence of infection-induced crystals. These findings were absent in the control rats. The role of *Ureaplasma* in the production of urinary tract infectious stones was thus demonstrated in vivo.

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Key words Ureaplasma urealyticum · Infectious stones
Urinary tract

Introduction

Ureaplasma urealyticum has been held responsible for chronic prostatitis, nongonococcal urethritis, epididymitis, pelvic inflammatory disease, postpartum fever, chorioamnionitis, infertility, spontaneous abortion and stillbirth, septic arthritis, chronic lung disease especially in low-birth-weight neonates and experimental pyelonephritis [1, 4, 9, 11, 14]. This microorganism is distinguished from all other Mollicutes by the possession of urease. This bacterial protein hydrolyzes urea in urine and converts ammonia to ammonium and hydroxyl ions. The increase in hydroxyl ions produces an increased amount of free phosphate ion and as a result struvite-magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) crystals form. Carbonate apatite-calcium phosphate [$Ca_{10}(PO_4)_6 \cdot CO_3$] crystals arise from further hydrolysis [5, 8].

In this study the possible role of *Ureaplasma urealyticum*, which is predominantly located in the urogenital tract, was investigated in the formation of infectious stones using an in vivo model.

Materials and methods

Experimental animals

Five male rats weighing 170 ± 10 g were used for the *U. urealyticum* inoculation and another five identical rats were used as the control group. All rats were *Rattus norvegicus* L. Wistar C F_7 generation and part inbred. The rats had been raised at 22 ± 2 °C at room temperature under laboratory conditions with 12 h dark and 12 h light at 55 ± 5% relative humidity moisture. The theoretical homogeneity was 99.20% and the research homogeneity was 74.40%.

Experimental microorganism and culture media

A strain of *U. urealyticum* recovered from a human urogenital specimen was used in the study. The medium used for the growth of the microorganism and inoculation was tripticase soy broth (Oxoid) containing 20.1% horse serum, 1.25% yeast extract, 0.1% urea, 0.002% phenol red and penicillin G 1000 U/ml (pH 5.5). A quantity of 200 ml *U. urealyticum* overnight culture medium at 36 °C containing 10^5 color-changing units of viable organisms was used for the inoculation of the rats as described previously [15].

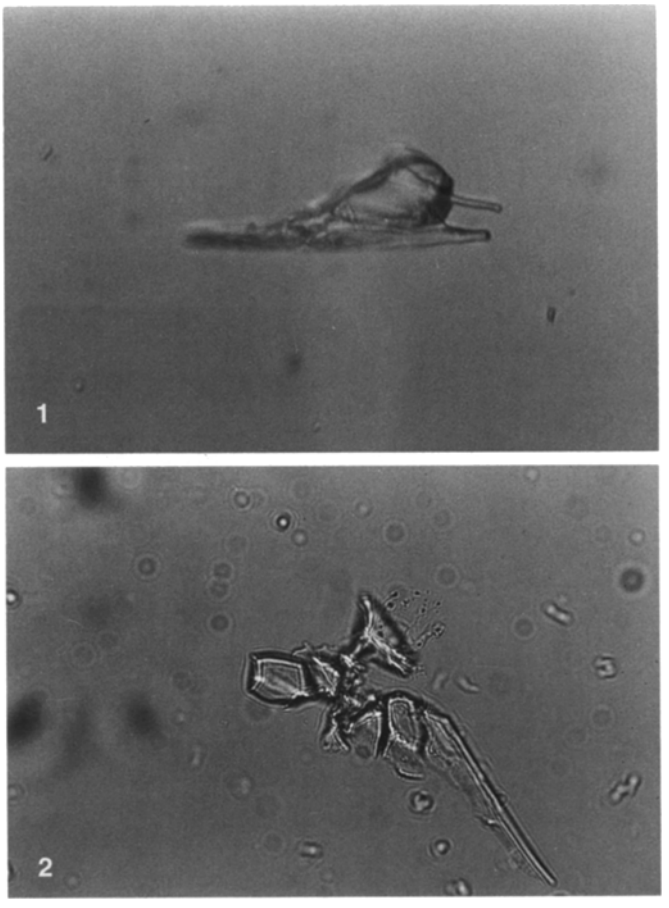
Experimental procedures

The rats were anesthesized by cetamine [di-2-(*O*-clorophenil)-2-(methylamino) cyclohecsanon hydrochlorine] and their abdomens were opened by midline incisions. The kidneys of the rats were then exposed and 200 µl *U. urealyticum* broth culture was injected into the renal medulla of five male rats. The same amount of culture media without *U. urealyticum* was inoculated into the control rats following the same procedure.

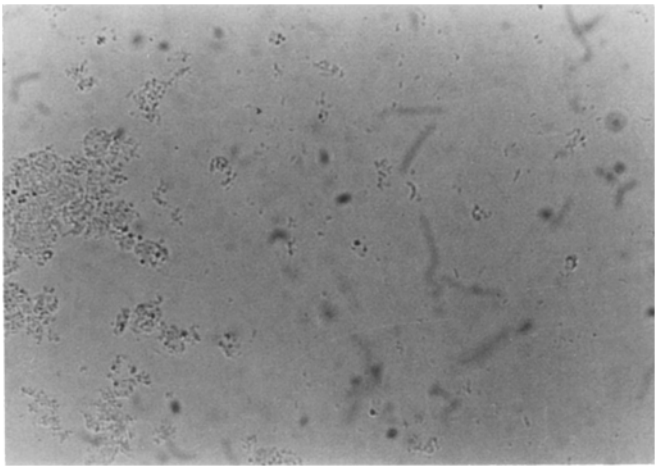
After 5 days both inoculated and control rats were killed and their bladders and kidneys were examined after opening. Fresh preparations of the bladder and inoculated kidney contents were made and biopsies from kidneys and bladders were taken for histopathological examination. The insides of the opened kidneys and bladders were washed with distilled water and centrifuged at 3000 rpm for 30 min. Biochemical analysis of the sediment was done as previously described by Varley [7]. Supernatant fluids were biochemically assayed in duplicate with a Tehnicon RA 1000 autoanalyzer.

Statistical analysis

Statistical analysis of the autoanalyzer values of calcium, magnesium and phosphorus of both inoculated and control rats was done by the Mann-Whitney U-test [12] using the SPSS for Windows (version 5.0.1) program.



Figs. 1, 2 Microscopic view of clustered crystals in fresh preparations of the bladder contents of inoculated rats



Figs. 3 Microscopic view of a fresh preparation of the bladder contents of control rats

Results

Five days after the inoculation, the rats were killed and bladders and kidneys were observed after opening. No macroscopic stone formation was seen in the bladders and kidneys of the inoculated rats.

Clustered crystals were observed in the fresh preparations of the bladder contents of the *U. urealyticum*-inoculated rats (Figs. 1, 2), while no crystal formation was seen in the preparations of the bladders contents of the control rats (Fig. 3). No crystal formation was observed in the preparations of the kidneys of either inoculated or control rats.

The biochemical analysis of the crystals in the sediment of the wash fluids of the bladders and kidneys showed calcium, phosphate and magnesium. These elements were not present in the sediment of wash fluids of control rats. The biochemical analysis of the supernatants of the wash fluids of the bladders and kidneys of both inoculated and control rats is shown in Table 1.

The difference between the magnesium, calcium and phosphorus values of the supernatants of the wash fluids of the bladders of the inoculated and control rats were statistically significant. Magnesium, calcium and phosphorus showed U and P values of $U = 0.0$, $P = 0.0090$; $U = 1.0$, $P = 0.0147$; $U = 0.0$, $P = 0.0090$, respectively. At the same time the magnesium, calcium

Table 1 Median magnesium, calcium and phosphorus values of the supernatants of wash fluids of bladders and kidneys of inoculated and control rats

	Inoculated rats			Control rats		
	Magnesium (mmol/l)	Calcium (mg/dl)	Phosphorus (mg/dl)	Magnesium (mmol/l)	Calcium (mg/dl)	Phosphorus (mg/dl)
Bladder	1.6	1.3	7.3	0.7	0.6	1.8
Kidney	1.5	1.3	7.8	0.2	0.3	2.6

and phosphorus values of the supernatants of the wash fluids of the kidneys of the two groups were also significantly different. (The U and P values for magnesium, calcium and phosphorus were $U = 1.5$, $P = 0.208$; $U = 1.0$, $P = 0.0163$; $U = 2.0$, $P = 0.0283$, respectively.)

The histopathological analysis of both kidneys and bladders of the *U. urealyticum*-inoculated rats showed light-refracting crystals under polarized light, which were especially localized in the calix renalis within the kidneys, and an amorphous granular sediment formation was observed inside the tubules of the kidneys. Light-refracting crystals and sediment formation was not detected in the bladders and kidneys of the control rats. A moderate and patchy subacute interstitial inflammatory infiltrate was observed in the *U. urealyticum*-infected kidneys. The interstitial infiltrate was predominantly composed of mononuclear cells. Some tubules had an intraluminal hyaline cast formation, which gave rise to a typical thyroid-like appearance. Some tubules also had leukocytic casts. Similar changes were not seen in the uninfected kidneys. An inflammatory infiltrate was not observed within the bladders of either *U. urealyticum*-infected or control rats.

Discussion

Ureaplasma urealyticum has been found in mid-stream urines of healthy people, demonstrating its existence in the urinary tract [3], and this bacteria, which is predominantly located in the urogenital system, is therefore likely to cause disease.

Struvite and carbonate apatite (calcium and magnesium ammonium phosphate) stones are considered to be infectious stones [10, 16]. Because of the activity of urease, *U. urealyticum*, like other urease producers, catalyzes the hydrolysis of urea and elevates ammonia levels, causing the crystallization of struvite and apatite as a result of elevated urinary ammonium ion levels [6].

Ureaplasma urealyticum has been isolated from patients with urolithiasis [2, 10, 18]. This phenomenon has been demonstrated by showing the formation of phosphate- and magnesium-rich struvite and white lockite precipitate after the inoculation of *U. urealyticum* into fresh human urines in an in vitro model [13, 17].

We explored the possible role of *U. urealyticum* in the formation of infectious stones in an in vivo model and showed the presence of magnesium-, calcium- and phosphate-rich crystals in the bladder of rats. Our results are similar to those of Texier et al. [15], except that these authors were able to observe macroscopic stones in the bladders of the rats as soon as 3–6 days after *U. urealyticum* injection. We did not observe such bladder calculi, which may be due to the strain we used or the length of the incubation period, whereas we were able to demonstrate calcium, magnesium and phosphate crystals, indicating the induction of infection within the bladders of the inoculated rats, which did not take place in the control group.

We have thus demonstrated the role of *U. urealyticum* in the production of infectious urinary tract stones in a rat model, which could reflect the events occurring humans with urolithiasis.

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