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Concrement Formation in the Urinary Bladder in Rats Inoculated with *Ureaplasma Urealyticum*

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Summary. To study the concrement-forming ability of *Ureaplasma urealyticum* in the urinary tract, viable and heat-killed ureaplasmas as well as urease and non-urease-producing bacteria were inoculated into the bladder in rats. Viable ureaplasmas, in contrast to heat-killed, caused the formation of bladder stones with a frequency corresponding to urease-producing bacteria (*Proteus mirabilis*). It was not possible to reculture the inoculated ureaplasmas from the urinary tract. Non-urease producing microorganisms (*Escherichia coli* and *Mycoplasma hominis*) only occasionally induced stone formation. The results indicate that *U. urealyticum* can initiate stone formation, a property that appears to be associated with the urease activity of the organism.

Key words: Ureaplasma urealyticum, Infection concrements, Bladder concrements, Urinary tract infection, Rats.

Introduction

Ureaplasma urealyticum, a urease-producing mycoplasma, is frequently isolated from the lower urinary tract in man. A pathogenetic significance of the organism has been proved only for non-gonococcal urethritis in males [11] and for chronic cystitis in patients with hypogammaglobulinaemia [13]. *U. urealyticum* is not ordinarily present in the upper urinary tract [12] and has so far not been linked to any renal disease.

Infection with urease-producing bacteria has been considered a prerequisite for the formation of struvite and carbonate-apatite stones (infection stones) in the urinary tract. It was recently shown, however, that *U. urealyticum* is associated with the presence of infection-induced concrements in the upper urinary tract in man [8]. The ureaplasmas are not detected with conventional bacterial culture techniques, which may explain why infection concrements have not previously been associated with the organism. It has also recently been shown that *U. urealyticum* possesses the ability to cause concrement formation in synthetic urine in vitro, a process that can be prevented by urease inhibition [5]. Inoculation of *U. urealyticum* into the kidney or the bladder has been shown by Friedlander and Braude to induce the formation of bladder stones in rats [3]. The stone formation was not correlated to the frequency with which it was possible to reculture the inoculated ureaplasmas, however, and stones were also produced after inoculation with heat and acetone-killed ureaplasmas. The mechanism of stone induction by killed *U. urealyticum* in that study remains obscure.

This investigation was performed to study the stone-forming ability of *U urealyticum* when inoculated in rats and to compare it with that of urease and non-urease-producing bacteria.

Material and Methods

One hundred and ninety-five adult male Sprague-Dawley rats (Anticimex AB, Sweden), each weighing approximately 250 g, were used. The animals were kept in separate cages according to the microorganism inoculated. They were given water and a standard pellet diet ad libitum.

Microorganisms

One strain of *U. urealyticum* (serotype III) was kept in 1 ml samples at −70 °C in a broth described by Shepard and Lunceford (10). After thawing, one sample was transferred to 1,000 ml broth and incubated at 37 °C for 16 h followed by repeated centrifugation in aliquots of 250 ml. The pellets thus obtained were suspended in broth to a final *U. urealyticum* concentration of 10^4 colony-forming units · ml^{-1}.

The *Mycoplasma hominis* strain was grown for 48 h at 37 °C in a broth described by Graystone et al. [4]. The final concentration of *M. hominis* was $10^6 - 10^8$ colony-forming units · ml^{-1}.

One strain of *Proteus mirabilis* and urease-negative *Escherichia coli* were grown overnight at 37 °C in brain heart infusion broth, yielding a final concentration of 10^9 bacteria · ml^{-1}.

Table 1. Development of bladder stones after inoculation with different microorganisms in rats

Inoculate	No of rats	Stone frequency after various times				Stone weight per stone-bearing rat (mg)		
		2 weeks	4 weeks	6 weeks	Total	2 weeks	4 weeks	6 weeks
U. urealyticum	37	6/9	9/10	16/18	31/37 (84%)	24.6 ± 10.0	34.8 ± 7.7	111.3 ± 14.9
U. urealyticum (killed)	19	–	0/10	0/9	0/19	–	0	0
Ureaplasma broth ony	22	0/8	1/8	0/6	1/22 (5%)	0	16.4	0
P. mirabilis	51	6/17	10/15	15/19	31/51 (61%)	56.1 ± 16.2	107.5 ± 28.4	362.8 ± 57.2
M. hominis	27	0/9	0/9	1/9	1/27 (4%)	0	0	29.9
E. coli	22	1/9	1/7	1/6	3/22 (14%)	24.7	20.4	24.0

Table 2. Positive cultures after bladder inoculation with different microorganisms

Inoculate	No of rats	Positive cultures after various times				Positive cultures per stone-bearing rat
		2 weeks	4 weeks	6 weeks	Total	
U. urealyticum	37	0/9	0/10	0/18	0/37	0/31
U. urealyticum (killed)	19	–	0/10	0/9	0/19	0/0
Ureaplasma broth only	22	0/8	0/8	0/6	0/22	0/1
P. mirabilis	48[a]	13/17	10/15	13/16	36/48	28/28
M. hominis	27	0/9	0/9	0/9	0/27	0/1
E. coli	22	4/9	4/7	0/6	8/22	1/3

[a] Three rats with bladder stones that died within 4–6 weeks were not cultured

Experimental Procedure

The surgical procedures were performed aseptically under ether anaesthesia. The bladder was exposed through a short lower midline abdominal incision and urine aspirated for culture and pH measurement with a thin needle (external diameter 0.9 mm). The microorganisms were then injected into the bladder lumen in a volume of 0.1 ml. The puncture canal was closed with a resorbable ligature of the tissue around the needle, which resulted in the development of a small diverticulum.

Six groups of rats with the following inoculates were used: 1. *U. urealyticum* (40 rats), 2. *U. urealyticum,* killed by heat (100 °C, 15 min) (20 rats), 3. ureaplasma broth without microorganisms (23 rats), 4. *P. mirabilis* (56 rats), 5. *M. hominis* (29 rats), 6. *E. coli* (27 rats). Rats that died within 7 days postoperatively were excluded. The number of excluded rats varied from 0 to 5 in each group.

When the rats were killed, after 2, 4 or 6 weeks, urine was aspirated from the bladder for culture and pH measurement. Bladder stones were collected, weighed and analysed. In the ureaplasma-inoculated rats, cultures were also performed of the stones as well as of specimens from the bladder wall, the prostate and the kidneys.

Analyses

Culture for *U. urealyticum* and *M. hominis* was performed according to the methods described by Shepard and Lunceford [10] and Graystone et al. [4]. Cultures from the bladder wall, prostate and kidneys were performed after dilution with isotonic saline. Bacteria were cultured with conventional bacterial methods. The urine pH was measured by means of a surface pH electrode (Orion Research Corp. USA, model 91–35). The bladder stones were analysed for their contents of calcium, magnesium and phosphate with conventional chemical methods [9, 14]. Representative stones from ureaplasma and proteus-inoculated rats were also analysed by infrared spectroscopy and optical crystallography (Arawak, Laboratories, USA)

Statistical Methods

Conventional statistical methods were used to calculate the means and standard error of the means. Student's t-test was used to study differences between paired observations.

Results

In rats inoculated with *U. urealyticum,* 31 out of 37 developed bladder stones. The weight of the stones increased with time between two and six weeks after inoculation (Table 1). Bladder stones were found in 31 out of 51 rats infected with *P. mirabilis.* Three of these 31 rats died 4–6 weeks after the inoculation; all of them had bladder stones obstructing the upper urinary tract. The weight of the stones in the proteus group also increased with time but the average weight of the stones was about three times higher than that in the ureaplasma group. In the rats inoculated with heat-killed *U. urealyticum,* ureaplasma broth, *M. hominis* or *E. coli,* the incidence of bladder stones was zero or very low.

All stones recovered in this study contained 8–12% magnesium. Only minimal amounts of calcium were detected, which indicates that the stones were composed of almost pure struvite. These results were verified by infrared spectroscopy and optical crystallography.

Urine cultures for ureaplasmas and bacteria were negative before inoculation in all rats and contaminating microorganisms were never found at sacrifice. After inoculation with *U. urealyticum,* 2, 4 and 6-week cultures of bladder urine, as well as of specimens from the bladder wall, prostate, kidneys and stones, were all negative, though 31 rats in this group developed bladder stones (Table 2). All rats inoculated with heat killed *U. urealyticum,* ureaplasma broth or *M. hominis* also had negative cultures for bacteria and mycoplasmas. The total rate of positive cultures in the proteus group was 36 out of 48 rats, all stone-bearing rats having cultures positive for *P. mirabilis.* Of the 22 rats infected with *E. coli,* eight had positive cultures. No rat in this group was infected after six weeks. Only one of the three stone-bearing *E. coli* rats had positive culture.

The initial mean pH of the bladder urine was 6.7 ± 0.1. It remained virtually unchanged in all rats, except the *P. mirabilis*-infected rats that developed stones. In these rats the urine pH increased to 7.8 ± 0.1 after 2 week and remained at this level throughout the observation period ($p < 0.01$).

Discussion

Inoculation of viable *U. urealyticum* in rats was followed by the formation of bladder concrements composed of struvite. The frequency with which stone formation occurred corresponded to that observed after inoculation with *P. mirabilis,* a potent urease producer and a notorius stone-forming agent in both humans and animals. Rats inoculated with *U. uralyticum* and *P. mirabilis* mostly produced multiple stones but the total weight of the ureaplasma induced stones was only one-third of that seen after *P. mirabilis* inoculation.

Inoculation by simple bladder puncture mostly failed to induce infection and stone formation in a pilot study. With the method developed, however, where a small bladder diverticulum was created, it was possible to achieve an infection rate of 80% after inoculation with *P. mirabilis.*

In all *P. mirabilis*-inoculated rats that developed bladder stones and an alkaline urine, *P. mirabilis* was recultured at sacrifice. In the *U. urealyticum*-infected rats, however, it was not possible to reculture the organism from urine, stones, bladder wall, prostate or kidneys. Urinary tract tissues and blood are known to possess mycoplasmacidial activity, which could explain the negative cultures. This activity is, however, said to be diminished by dilution [7], a procedure which was performed, but nevertheless failed to reculture the organism.

The presence of bacterial urease activity in the urinary tract has been considered an obligate condition for the formation of infection concrements in man [6]. As no urease-producing bacteria were isolated in the *U. urealyticum*-infected rats neither at the time of inoculation nor later, *U. urealyticum* appears to be linked to the stone formation despite the fact that it was not recultured from any level of the urinary tract. The inoculation of L-forms of *P. mirabilis* has also been found to cause formation of bladder stones though it was not possible to reculture the organism except for the first few days after inoculation [1]. The stone formation in that study was prevented by the administration of antibiotics after inoculation. These observations were taken to indicate that living organisms were present during stone formation despite negative cultures. The progressive stone growth noted in our study may also indicate that ureaplasmas were present, but that we failed to detect them. On the other hand, the absence of an alkalinisation of the urine in the *U. urealyticum*-infected rats after 2–6 weeks, indicating a low or absent urease activity, contradicts the theory that viable *U. urealyticum* were present after 2–6 weeks. It is interesting to note in this context that both the L-forms and the ureaplasmas are cell wall-deficient microorganisms.

Another explanation for the stone formation in this study is that the urinary solution-equilibrium differs in rats and man, and that struvite formation can continue without urease activity on already formed struvite nuclei in rats. Such nuclei could have been formed in connection with the inoculation with *U. urealyticum.* That the urease activity of *U. urealyticum* is of importance is demonstrated by the fact that inoculation with non-urease-producing microorganisms such as *E. coli, M. hominis* or heat-killed *U. urealyticum* failed to cause stone formation at the same rate as viable *U. urealyticum.* It is however, plausible that microorganisms, irrespective of their urease activity, may to a limited extent act as nuclei for stone formation a theory which could explain the few cases of bladder stones in the *E. coli* and *M. hominis* rats [2].

In the *P. mirabilis*-inoculated rats, urease activity was continuously present, as reflected by the alkaline urine. This urease activity may explain why these rats had a more rapid stone-growth than the *U. urealyticum*-infected rats, where urease was probably only present in close connection with the inoculation.

In a recent investigation, *U. urealyticum* was linked to the presence of infection stones in the upper urinary tract in man [5]. Our study confirms the ability of *U. urealyticum* to induce the formation of urinary tract stones. It was necessary to inoculate the rats with viable ureaplasmas, however, in contrast to what has previously been claimed [3], but it was not possible to reculture the ureaplasmas in spite of progressive stone growth.

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