Urol Res (1991) 19:141–144

Urological Research

Experimental bacterial prostatitis in rats

T. Goto, M. Kawahara, K. Kawahara, S. Mahinose, Y. Mizuma, N. Sakamoto, and Y. Ohi

Department of Urology, Faculty of Medicine, Kagoshima University, Kagoshima, Japan

Accepted: June 1, 1990

Summary. Experimental acute bacterial prostatitis in rats was induced by four different routes of bacterial inoculation. The most simple and reproducible method of producing bacterial prostatitis was to instil the bacterial suspension into the prostatic urethra after the administration of an appropriate antibiotic to prevent associated pyelonephritis.

Key words: Experimental – Bacterial – Prostatitis – Rat – Antibiotics

For many years now the prostatitis syndrome has perplexed clinicians due to the extreme difficulty in obtaining a definite diagnosis and the lack of successful treatment. The technique for localization of lower tract infection described by Meares and Stamey [6] and a new classification system for the various forms of prostatitis proposed by Drach et al. [1] have provided a more sound scientific basis for investigation; however, some important aspects of the pathogenesis and treatment of prostatitis syndrome have not been clearly defined [8].

The purpose of this study was to find the best method of producing bacterial prostatitis in rats since the rat model may be useful for the study of the human bacterial prostatitis.

Materials and methods

Bacterial strains

Escherichia coli NIHJ JC-2 and a clinical strain of *Entrococcus faecalis* KATO isolated from the urine of a patient with acute bacterial prostatitis were used in this study. These two strains were chosen as the challenging organisms because they are the pathogens most often responsible for bacterial prostatitis [8]. Inocula for animal challenge were prepared by subculturing the organisms in trypticase-soy broth (TSB, Eiken Chemical Co., Ltd., Tokyo, Japan) at 37°C for 20 h and washing three times in phosphate-buffered saline (PBS), pH 7.2.

Antibiotics and antimicrobial susceptibility testing

Amikacin (AMK) was kindly provided by the Banyu Pharmaceutical Company (Tokyo, Japan). The minimal inhibitory concentrations (MIC) were determined by standard agar dilution methods described by the Committee of Japan Society of Chemotherapy [10]. Mueller-Hinton agar or broth (Difco Lab., Detroit, USA) was used as medium.

Animals and experimental design

A total of 60 male Wistar rats weighing 250–350 g were used in this experiment. Rats were anesthetized with ether. In the first experiment the development of bacterial prostatitis was examined in four groups of animals; each group had been inoculated with bacteria via a different route. In the second experiment it was determined whether the administration of an antibiotic before bacterial inoculation prevented the development of pyelonephritis. In the last experiment, the distribution of the carbon particles in the prostate was examined histologically after the instillation of India ink into the prostatic urethra or bladder to learn how the bacteria reached the prostate.

Experiment 1. Rats were divided into four groups (A, B, C, and D) according to route of bacterial inoculation. Each group consisted of seven rats. After inoculation of *E. coli* NIHJ JC-2 using a 26-gauge needle attached to a tuberculin syringe, the animals were returned to their cages and cared for normally. As controls two animals from each experimental group received sterile PBS instead of the bacterial suspension. All animals were killed 48 h after the inoculation. Neither macroscopic nor microscopic lesions were observed in the prostates of any of the control animals.

Group A: 0.05 ml of the bacterial suspension (10^8 cells/ml) was injected into the right ventral prostate which was exposed aseptically through a midline abdominal incision, then the wound was closed.

Group B: 0.1 ml of the bacterial suspension was instilled into the right vas deferens, which was exposed aseptically through inguinal incision toward the seminal vesicle, then the wound was closed.

Group C: After the urethral meatus was clamped, any residual urine was aspirated from the bladder, which was exposed aseptically through a midline abdominal incision. Bacterial suspension (1 ml) was slowly infused into the bladder and the wound was closed. The urethral meatus was opened 1 h after the bacterial infusion.

Group D: 1 ml of bacterial suspension was instilled into the prostatic urethra through an indwelling polyethylene tube (0.7 mm in OD, 5 cm in length).

Table 1. Bacterial counts in prostate, urine, and kidney 48 h after inoculation of *E. coli* NIHJ JC-2

Group of rats	Route of inoculation	Inoculum size[a]	No. of animals	Log of viable counts (Mean ± SD)		
				Prostate (/g)	Urine (/ml)	Kidney (/g)
A	Prostate	0.05	5	7.8 ± 0.2	2.0 ± 4.5	0
B	Vas deferens	0.10	5	6.0 ± 3.5	2.6 ± 8.8	0
C	Bladder	1.00	5	7.4 ± 0.8	4.0 ± 2.5	2.2 ± 5.2
D	Urethra	1.00	5	7.2 ± 0.7	3.2 ± 3.6	1.2 ± 3.2

[a] Bacterial solution of 10^8 cfu/ml

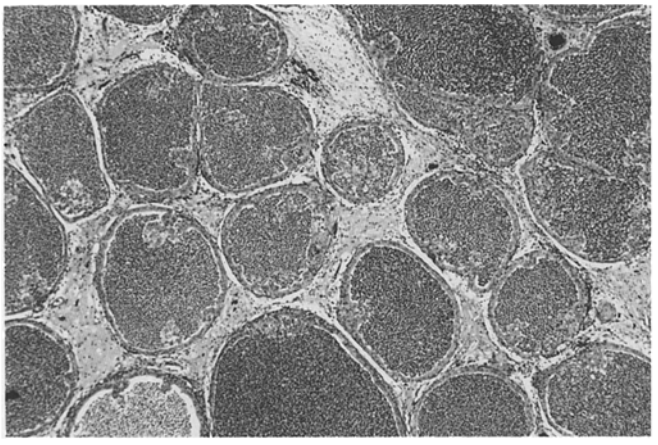

Fig. 1. Ventral prostate 48 h after intraurethral infection of *E. coli* NIHJ JC-2 (group D). Extensive infiltration of polymorphonuclear leukocytes into glandular acini and interstitium (×40) can be seen

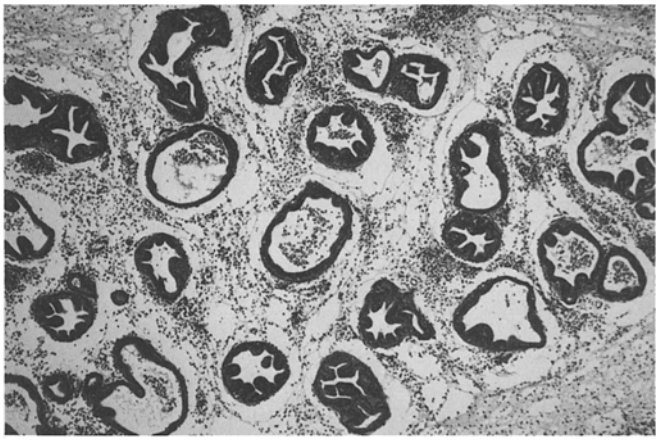

Fig. 2. Intense periglandular edema in the ventral prostate in group A (×40)

Experiment 2. AMK (5 mg/kg) was administered intraperitoneally 1 h before urethral inoculation of *E. coli* NIHJ JC-2 or *E. faecalis* KATO into two groups of 10 rats. Animals were killed 2 days and 7 days, respectively, after the inoculation.

Experiment 3. The distribution of carbon particles was examined after instilling India ink into the bladder or urethra. 1 ml of India ink was introduced into the bladder, which was exposed through a midline abdominal incision after clamping the urethral meatus, or into the prostatic urethra through an indwelling polyethylene tube in two rats of each group. They were killed 15 min after instillation, and the distribution of carbon particles was examined grossly and microscopically.

Bacterial count

The kidneys and prostate with the bladder, urethra, seminal vesicle, epididymis, and testis were removed en bloc aseptically from five of the seven animals in each experimental group. A portion of these tissues was separated by sterilized knives and scissors, weighed, and homogenized in a sterile glass tissue grinder. Urine and tissue homogenates were separately cultured quantitatively by the pour plate technique, using 10-fold dilution in PBS. The evaluation of colony forming units (cfu) of bacteria in each sample with this technique allowed detection of a minimum of 10 cfu/ml of urine and 20 cfu/g of tissue homogenates. Each sample was considered sterile if no organisms were detected by this method. Results were expressed as the mean log of cfu per milliliter or gram ± standard deviation of the mean.

Histopathological examination

The kidneys, bladder, and prostate removed from the two animals in each group were preserved in 10% buffered formalin, pH 7.2, and embedded in paraffin. The sections were cut at 5 μ, stained with hematoxylin and eosin, and examined microscopically.

Results

Experiment 1

Gross lesions on the ventral prostate (swelling and/or abscess) were observed in all animals except two of five in group B, and inoculated bacteria were recovered from the prostate of all animals. The mean bacterial count 48 h after inoculation of *E. coli* NIHJ JC-2 in the urine, prostate, and kidneys is shown in Table 1. Histologically intense infiltration of polymorphonuclear leukocytes into glandular acini and interstitium was observed in the prostates (Fig. 1). Generally, inflammation was most prevalent in the ventral and lateral prostate, being diffuse in groups C and D and focal in groups A and B. Severe periglandular edema was seen in the prostate in group A (Fig. 2). Although inoculated *E. coli* were isolated from urine samples of most of the animals, histological evi-

Table 2. Bacterial counts in prostate, urine, and kidney 2 days and 7 days after intraurethral inoculation

Inoculated strain[a]	MIC of AMK (μg/ml)	Days post-infection	No. of animals	Log of viable counts (Mean ± SD)		
				Prostate (/g)	Urine (/ml)	Kidney (/g)
E. coli	0.78	2	5	7.6 ± 0.3	2.4 ± 3.3	0
E. coli	0.78	7	5	7.8 ± 0.2	4.0 ± 3.5	0
E. faecalis	25	2	5	7.4 ± 0.3	6.0 ± 1.0	4.0 ± 3.5
E. faecalis	25	7	5	7.8 ± 0.2	6.8 ± 1.2	6.4 ± 1.3

MIC, minimum inhibitory concentration; AMK, amikacin
[a] *E. coli; E. coli* NIHJ JC-2; *E. faecalis; E. faecalis* KATO

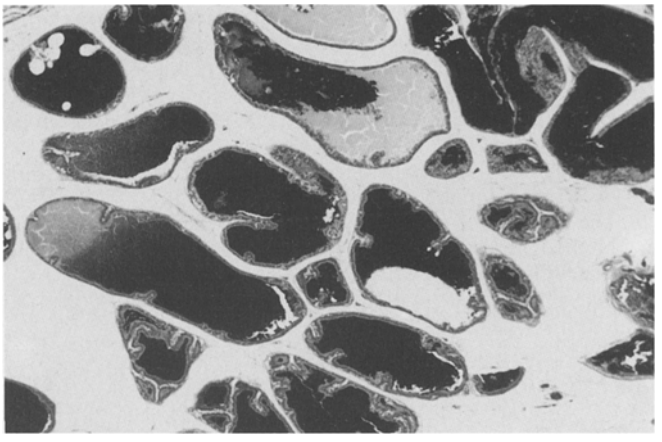

Fig. 3. Carbon particles into glandular acini in lateral prostate (×40)

dence of cystitis was observed only in animals in group C. Inoculated bacteria were recovered from the kidneys in four and three of five animals, respectively, in groups C and D; however, histological examination did not reveal pyelonephritis in these kidneys.

Experiment 2

The mean bacterial count in the prostate, urine, and kidneys is shown in Table 2. Acute bacterial prostatitis developed in all animals after inoculation of *E. coli* NIHJ JC-2. However, the bacterium was not recovered from the kidneys after intraperitoneal administration of 5 mg/kg of AMK 1 h before intraurethral inoculation. Macroscopically and microscopically, no difference in the occurrence of prostatitis was observed between the two groups with or without administration of AMK, but *E. faecalis* KATO was recovered from the kidneys in spite of prior administration of AMK. The difference in MIC of AMK against these two strains could be the reason why *E. coli* NIHJ JC-2 was not but *E. faecalis* KATO was recovered from the kidneys. When *E. faecalis* KATO was inoculated, inflammatory lessions in the vental prostate were more intense on day 7 than on day 2, and at histological examination pyelonenphritis was observed in 3 of 10 animals.

Experiment 3

Upon administration of India ink, carbon particles were observed in the ventral and lateral portions of the prostate but not in the dorsal prostate regardless of the route of administration (Fig. 3).

Discussion

The purpose of this study was to find the simplest and most reproducible method of producing bacterial prostatitis in rats. In 1972, Frielander and Baunde [3] and Freedman [2] reported acute bacterial prostatitis in rats. They inoculated bacterial solution into the bladder or directly into the ventral prostate which was exposed through an abdominal incision. Direct injection showed high reproducibility of prostatitis, suggesting that this might be a useful model of prostatitis to examine in the pathogenicity of various bacterial strains [4]. However, an operation was needed to expose the ventral prostate, and the inflammatory lesions in the prostate were focal and segmental. Intravesical instillation also required surgical exposure of the bladder, and it was associated with pyelonephritis due to vesicoureteral reflux [9]. Moreover, the reproducibility of prostatitis was not fully acceptable.

In experiment 1, we used four different routes of challenge, and acute bacterial prostatitis was produced in each case. However, in terms of reproducibilty of bacterial prostatitis and simplicity of bacterial inoculation, urethral inoculation was thought to be the best mode. We found no bacterial contamination in animals which were inoculated via the urethra in this experiment. Nonetheless attention should be paid to avoid contamination, because diphteroides or *Proteus spp.* are often isolated from the urethra of normal rats [7].

In experiment 2, an antibiotic was administered before urethral inoculation to prevent pyelonephritis. Acute bacterial prostatitis was produced without pyelonephritis developing when AMK was administered 1 h before the inoculation of *E. coli* NIHJ JC-2. However, *E. faecalis* KATO was recovered from the kidneys in spite of prior administration of AMK. The different MIC of AMK against these two bacterial strains may account for *E. faecalis* KATO but not *E. coli* being recovered from the kidney. Therefore, the antibiotics administered should

have sufficient antibacterial activity against the inoculated strain. Generally, β-lactam antibiotics or aminoglycosides are useful because of the pharmacodynamics; renal excretion and poor distribution to normal prostatic tissue [8].

We used a polyethylene tube to inoculate the bacteria into the prostatic urethra. After disinfecting the external urethral orifice with 70% alcohol, the tube was inserted about 3 cm into the urethral orifice. The length of the urethra from the external orifice to the prostatic urethra was measured to be about 3 cm according to specimens from rats after cystourethrectomy in a preliminary study.

From the studies of Kirby et al. on human prostates [5] and of Müntzing et al. on rat prostates [7], reflux of infected urine into the prostatic ducts is thought to be an important mode of infection. We examined the distribution of carbon particles in rat prostate after intravesical or intraurethral instillation of India ink in experiment 3. The distribution of carbon particles in the prostate was affected by the volume of ink rather than by the route of instillation. Carbon particles were easily distributed to ventral and lateral portions of the prostate where the inflammatory lesions predominated in experiment 2. These results suggest that the increase in intraurethral pressure by the instillation of the bacterial solution allows the bacteria easier access to the prostatic ducts.

Prostatitis was produced by instilling bacterial solution into the lower urinary tract via various routes. However, in terms of reproducibility and simplicity, intraurethral inoculation is thought to be the best method of producing acute bacterial prostatitis in rats. Moreover, antibiotics which are sufficiently antibacterial against the inoculated strain should be administered before bacterial inoculation in order to prevent pyelonephritis from developing.

References

1. Drach GW, Meares EM Jr, Fair WR, Stamey TA (1978) Classification of benign disease associated with prostatic pain: prostatis or prostatodynia. J Urol 120:266
2. Freedman LR (1972) Experimental pyelonephritis XVIII *P. aeruginosa* and *E. coli* prostatic infection in the rat and the effect of water diuresis. Yale J Biol Med 45:559
3. Frielander AM, Baunde AI (1972) Experimental prostatitis, relationship to pyelonephritis. J Infect Disease 126:645
4. Kamidono S, Fujii A, Harada A, Umezu K, Kataoka N, Arakawa S, Ishigami J (1985) Experimental studies on bacterial prostatitis. 1. Pathogenicity of various clinical isolates. Nishinihon J Urol 47:1083
5. Kirby RS, Lowe D, Bultitude MI, Shuttlerworth KED (1982) Intraprostatic urinary reflux: an etiological factor in abacterial proatatitis. Br J Urol 54:729
6. Meares EM, Stamey TA (1968) Bacteriological localization patterns in bacterial prostatitis and urethritis. Invest Urol 5:492
7. Müntzing J, Sufrin G, Murphy GP (1979) Prostatitis in the rat. Scand J Urol Nephrol 13:19
8. Orland SM, Hanno PM, Wein AJ (1985) Prostatitis, prostatosis and prostatodynia. Urology 25:439
9. Sommer JL (1961) Experimental pyelonephritis in the rat with observations on ureteral reflux. J Urol 86:375
10. The Committee of Japan Society of Chemotherapy (1981) Methods for determination of minimal inhibitory concentration. Chemotherapy (Tokyo) 29:76

T. Goto, MD
Department of Urology
Faculty of Medicine
Kagoshima University
1208-1, Usuki-cho
Kagoshima
890 Japan