Urol Res (1995) 22: 383–387

ORIGINAL PAPER

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Effect of prednisolone on ascending renal infection due to biofilm disease and lower urinary tract obstruction in rats

Received: 25 May 1994 / Accepted: 30 September 1994

Abstract A model of renal infection due to lower urinary tract obstruction and biofilm disease was constructed for the study of renal scarring by inserting glass beads coated with bacterial biofilm into the bladder of rats and then clamping the urethra. We previously reported the effect of antimicrobial therapy used in combination with the anti-inflammatory agent prednisolone to prevent renal scarring. In this study we investigated the effect of prednisolone on renal scar formation using our new model. Renal scarring could not be prevented in the group in which prednisolone was administered in the period during which the urethra was regularly being clamped. In contrast, scarring was prevented in the group that began to receive prednisolone after the period of clamping had ended. Therefore, in cases of lower urinary tract obstruction prednisolone should only be administered for the prevention of renal scarrring after the obstruction has been resolved.

Key words Lower urinary tract obstruction
Biofilm disease · Renal scarring · Prednisolone
Ciprofloxacin

Many animal models are used for the investigation of renal scarring, which is one of the main characteristics of reflux nephropathy. Glauser [2] and Slotki [14] both advised that antimicrobial agents should be given within 72 h in order to prevent renal scarring in rat models with direct intrarenal parenchymal inoculation of bacteria or retrograde renal infection due to intravesical injection of bacteria. Roberts [12] confirmed that it was possible to prevent renal scarring in a monkey model with retrograde transureteral infection by treatment with allopurinol, even if administration of the antimicrobial agent was delayed. We have also reported from studies using rats that MS pili (bacterial side) and superoxide or lysosomal enzymes produced by polymorphonuclear leukocytes (PMNLs) (host side) are important factors in renal scar formation, and prednisolone or antioxidants are effective in its prevention [4–8].

However, in these model renal infection was induced by a single bacterial inoculation. We mentioned that bacterial colony counts in the models using single intravesical injections of bacteria decreased in a linear manner and infection did not persist [5]. In terms of reflux pyelonephritis in humans, there are various clinical cases involving lower urinary tract obstruction (neurogenic bladder or posterior urethral valve), urolithiasis or an indwelling catheter disorder. Therefore, intrarenal reflux of bacteriuria occurs many times, which may result in persistent renal infection. In this study, we constructed a model of persistent infection specifically for the study of renal scarring or, more accurately, of human complicated pyelonephritis, by inserting glass beads coated with bacterial biofilm into the bladder of rats and then clamping the urethra.

We previously reported that, in models of single intravesical infection, bacterial colony counts in the kidney did not increase when prednisolone was administered, and renal scarring could be prevented by combination therapy comprising prednisolone and another antimicrobial agent [5]. In this study, we examined the effect of prednisolone on renal scarring and bacterial counts in the kidney using a new model of renal infection.

Material and methods

Experimental animals

Female Sprague-Dawley rats 6–8 weeks old and weighing 200–250 g were used. They were kept in specific pathogen-free conditions at room temperature. They received a diet for rats with tap water provided ad libitum.

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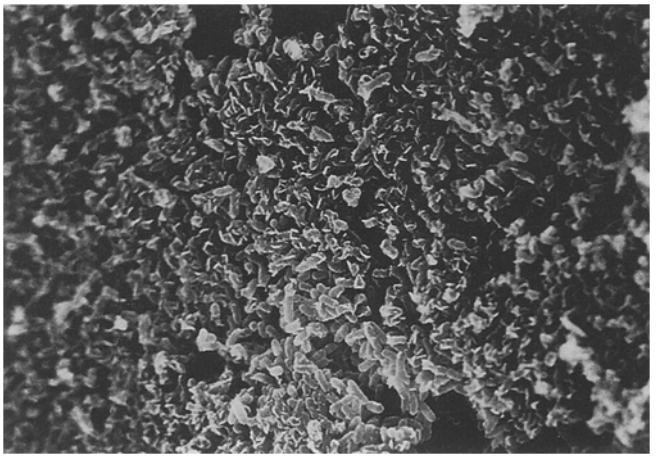

Fig. 1 Scanning electron micrograph shows the bacterial biofilm forming around the bead. Reduced from ×2000

Bacteria

Escherichia coli, HM32 strain, was isolated from a patient with urinary tract infection (UTI). This strain had both type 1 and P pili, which had been confirmed by a hemagglutination test, a P pili-specific PF test [16] and electron microscopy.

Bacterial inoculation

An *Escherichia coli* HM32 strain was cultivated in CF medium, which consisted of 1% casamino acid plus 0.15% $MgSO_4$ and 0.0005% $MnCl_2$. Glass beads, 2 mm in diameter, were also added and the culture was allowed to grow for 48 h at 37°C. Bacteria multiplied and formed biofilm around the beads (Fig. 1).

The rats were allowed free access to water. They were anesthetized with a 25-mg/kg (0.15 ml) i.p. injection of pentobarbital and the bladder was exposed through a midline abdominal incision. One of the glass beads coated with biofilm was introduced into the bladder of ten rats, and the urethra was then clamped for 1 h/day for 4 consecutive days, beginning 24 h after the operation. One of the sterile glass beads was introduced into the bladder of ten control rats, and then the urethra was clamped for 1 h/day for 4 consecutive days, beginning 24 h after the operation. In addition, one of the glass beads coated with biofilm was introduced into the bladder of a further ten control rats in which the urethra was not clamped.

Renal scarring

The rats were put to death 6 weeks after the bacterial inoculation and both kidneys from each rat were removed and observed both macroscopically and microscopically. Renal scarring occurred at various locations and involved various percentages of the kidney; histologically it consisted of chronic inflammatory cells and fibrosis. The absence or presence of scar formation within the kidney was determined by an investigator who remained unaware of the details of the experiment.

Administration of ciprofloxacin

Two groups were administered a 15-mg/kg dose of ciprofloxacin i.m. twice a day for 5 consecutive days. In the immediate treatment group, administration was started 6 h after the first clamping. In the delayed treatment group, administration was begun 72 h after the first clamping. Both experimental groups, and also a group of untreated controls, consisted of nine rats each.

Additional administration of prednisolone

Two groups were administered ciprofloxacin and prednisolone:

Early combination: A 15-mg/kg dose of ciprofloxacin was administered i.m. twice a day for 5 consecutive days, beginning 72 h after the first urethral clamping. In addition, a 2-mg/kg dose of prednisolone was administered s.c. once a day for 4 consecutive days, beginning 72 h after the first clamping (in the period during which the urethra was being regularly clamped).

Late combination: A 15-mg/kg dose of ciprofloxacin was administered i.m. twice a day for 5 consecutive days, beginning 72 h after the first urethral clamping. In addition, a 2-mg/kg dose of prednisolone was administered s.c. once a day for 4 consecutive days, beginning 120 h after the first clamping (after the period of regular clamping had ended).

These experimental groups, along with a group of untreated controls, consisted of nine rats each.

Determination of bacterial counts in the kidneys

The bacterial count in the kidneys of untreated rats was compared with that of the prednisolone-treated rats. A 2-mg/kg dose of prednisolone was administered s.c. once a day for 4 consecutive days, beginning 48 h after the first clamping. Kidneys were removed 1, 2, 4, 5, 8 and 14 days after infection had been induced and these were homogenized individually in 5 ml saline using Teflon homogenizers. The number of bacteria in each homogenized suspension was calculated using a serial dilution method. For each rat, the bacterial count of the kidney with the greater number of bacteria was used as the bacterial count. Each experimental group consisted of four rats.

Statistical analysis

Evaluation of data was performed by Fisher's exact test based on the renal scar formation in each experimental group. Student's *t*-test was also used to compare differences in the bacterial counts between each experimental group.

Results

Renal scarring

The ten rats which received glass beads coated with biofilm followed by urethral clamping were compared with the controls. The kidneys were observed both macroscopically and microscopically 6 weeks later.

Of the ten rats which received glass beads coated with biofilm followed by urethral clamping, eight were observed to have scarring in one or both kidneys. In contrast, no kidneys were observed to have scarring in either of the control groups. The differences between the two control groups and the group of rats which received glass beads coated with biofilm followed by urethral clamping were both statistically significant ($P<0.01$) (Table 1).

Table 1 Appearance of renal scarring in a new ascending pyelonephritis model in rats

Bacteria	Clamping[a]	Scarring[b] (number of rats)	Fisher's exact test	Scarring (number of kidneys)	Fisher's exact test
HM32	Done	8/10		14/20	
HM32	None	0/10	$P<0.01$	0/20	$P<0.01$
None	Done	0/10	$P<0.01$	0/20	$P<0.01$

[a] Urethras were clamped for 1 h/day for 4 consecutive days
[b] Renal scarring was observed macroscopically and microscopically 6 weeks after bacterial inoculation

Effect of ciprofloxacin

Rats were left untreated, or were treated with ciprofloxacin alone for 5 days beginning either 6 h after the first clamping (immediate treatment group) or 72 h later (delayed treatment group). Each group comprised nine rats. Two rats treated with ciprofloxacin alone (one each from the immediate and delayed treatment groups) died following complications involving anesthesia administration. The kidneys were observed both macroscopically and microscopically 6 weeks later.

Renal scarring occurred in one or both kidneys in seven of the nine untreated rats. Of the eight rats which received immediate treatment with ciprofloxacin alone, only one rat was observed to have scarring, and this was in only one kidney. In contrast, of the eight rats which received delayed treatment with ciprofloxacin alone, four were observed to have scarring in one or both kidneys. The difference between the immediately treated group and the untreated control group was statistically significant ($P<0.01$). However, the total numbers of rats and kidneys affected by renal scarring in the delayed-treatment group were comparable to those of the untreated controls (Table 2).

Table 2 Effect of the immediate or delayed treatment of ciprofloxacin on renal scarring in a new ascending pyelonephritis model in rats (*NS*, not significant)

Bacteria	Treatment[a]	Scarring[b] (no. of rats)	Fisher's exact test	Scarring (no. of kidneys)	Fisher's exact test
HM32	None	7/9		9/18	
HM32	Ciprofloxacin (immediate)	1/8	$P<0.01$	1/16	$P<0.01$
HM32	Ciprofloxacin (delayed)	4/8	NS	5/16	NS

[a] Immediate treatment of ciprofloxacin: a 15-mg/kg dose of ciprofloxacin was administered i.m. twice a day for 5 consecutive days, beginning 6 h after the first urethral clamping.
Delayed treatment of ciprofloxacin: 15-mg/kg dose of ciprofloxacin was administered i.m. twice a day for 5 consecutive days, beginning 72 h after the first urethral clamping
[b] Renal scarring was observed macroscopically and microscopically 6 weeks after bacterial inoculation

Table 3 Effect of the early or late combination of prednisolone and ciprofloxacin on renal scarring in a new ascending pyelonephritis model in rats (*NS*, not significant)

Bacteria	Treatment[a]	Scarring[a] (no. of rats)	Fisher's exact test	Scarring (no. of kidneys)	Fisher's exact test
HM32	None	7/9		10/18	
HM32	Early combination	4/8	NS	5/16	NS
HM32	Late combination	1/9	$P<0.05$	1/18	$P<0.05$

[a] Early combination: a 15-mg/kg dose of ciprofloxacin was administered i.m. twice a day for 5 consecutive days, beginning 72 h after the first urethral clamping. In addition, a 2-mg/kg dose of prednisolone was administered s.c. once a day for 4 consecutive days, beginning 72 h after the first clamping.
Late combination: a 15-mg/kg dose of ciprofloxacin was administered i.m. twice a day for 5 consecutive days, beginning 72 h after the first urethral clamping. In addition, a 2-mg/kg dose of prednisolone was administered s.c. once a day for 4 consecutive days, beginning 120 h after the first clamping.
[b] Renal scarring was observed macroscopically and microscopically 6 weeks after bacterial inoculation

Effect of the addition of prednisolone on the delayed treatment with ciprofloxacin

Rats were left untreated, or else they were treated with the early combination or late combination treatment. Nine rats were inoculated with bacteria in each group. One rat treated with the early combination treatment died following complications involving anesthesia administration. The kidneys were observed both macroscopically and microscopically 6 weeks later.

Renal scarring occurred in one or both kidneys in seven of the nine untreated rats. Of the eight rats which received early combination treatment, four were observed to have scarring in one or both kidneys. In contrast, of the nine rats which received late combination treatment, only one was observed to have scarring, and this was in only one kidney. The total numbers of rats and kidneys affected by renal scarring in the early combination group were comparable to those of the untreated controls. However, the difference between the late combination group and the untreated control group was statistically significant ($P<0.01$) (Table 3).

Bacterial count in the kidneys

Viable bacteria were quantified using kidneys from the untreated and prednisolone treatment group. In the untreated control group, bacterial counts were at the 10^2 colony-forming unit (cfu) level on the 1 day after bacterial inoculation (before the first clamping) and at the 10^7 cfu level on the 2nd day after bacterial inoculation (1 day after the first clamping); counts were maintained at levels above 10^6 cfu for 3 days (during the period of regular clamping), then decreasing gradually. In the

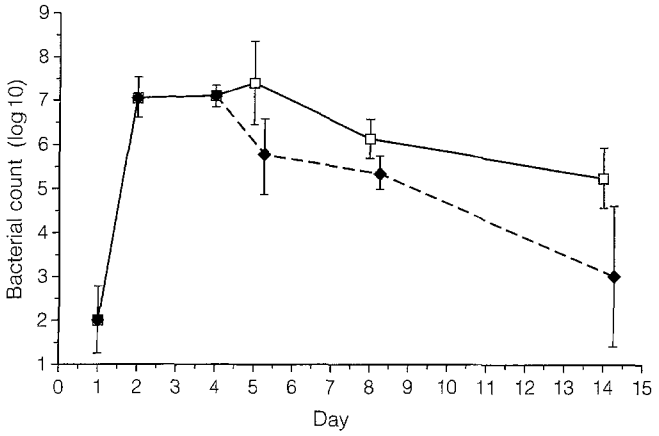

Fig. 2 Bacterial counts in the kidneys are shown for untreated and prednisolone-treated groups. Bacterial counts in the prednisolone-treated group were significantly larger than those of the control on days 5, 8 and 14, □ prednisolone-treated, ◆ control

prednisolone-treated rats, bacterial counts were significantly greater than those of the controls on days 5, 8 and 14 (Fig. 2).

Discussion

Functional or anatomical lower urinary tract obstruction is an important risk factor involved in renal scarring because of the appearance or the deterioration of vesicoureteral reflux [10, 11]. It is necessary to maintain urination pressure at a low level by using intermittent catheterization in these cases. However, a few patients are catheterized with an indwelling catheter such as for cystostomy or have complications of urolithiasis. In these cases, a persistent or have complications of urolithiasis. In these cases, a persistent urinary tract infection is likely to develop because of biofilm formation. Moreover, if lower urinary tract obstruction due to bladder stones or catheter obstruction were to occur, the risk of renal scarring would increase.

It is necessary for any model of such conditions to possess high pressure reflex and persistent infection. Therefore, we constructed a new model by inserting glass beads coated with bacterial biofilm into the bladder of rats and then clamping the urethra for 1 h/day for 4 consecutive days. We previously used an experimental model in which renal scarring was induced by a single injection of bacteria in the bladder followed by clamping of the urethra for 4 h. In this model, the colony counts within the kidney decreased in a linear manner to the 10^6 level at 1 day after clamping, to the 10^4 level on the 7th postoperative day and to the 10^3 level on the 14th postoperative day [5]. Our new experimental model using beads coated with bacterial biofilm seemed to be more appropriate for providing the necessary conditions than conventional models since the rats showed counts at the 10^7 level 1 day after the first clamping and then maintained a count level of more than 10^6 for 3 days.

Hagberg et al. [3] reported a model of ascending, unobstructed urinary tract infection in mice which they used to monitor the initial stage of the infectious process. However, in our study, in those rats whose urethras were not clamped, no kidneys were observed to have scarring. Therefore, in order to study renal scar formation specifically, it is necessary to design an obstructed ascending renal infection model.

Direct adoption of these data to the clinical situation in human should only be carried out very carefully because of interspecies differences in both anatomy and immunological function. Thus, before clinical use, it is necessary for prednisolone to be investigated in other animal models besides the rodent model.

It has been experimentally [1–3] and clinically [1, 9, 13, 15] established that antimicrobial agents should be given during an early stage (within 72 h) to prevent renal scarring after the onset of reflux pyelonephritis. In a previous experiment using a model of single intravesical infection, we reported that renal scarring could be prevented by combination therapy including prednisolone, even if the antimicrobial therapy was delayed. Moreover, there were no effects of prednisolone on the colony counts [5]. In this study, however, renal scarring could not be prevented in the group to which prednisolone was first administered during the period of regular clamping. In contrast, such scarring was prevented in the group that received the first administration of prednisolone after the clamping period had ended. The colony counts in the kidney were significantly increased by the administration of a 2-mg/kg dose of prednisolone for 4 consecutive days, which began during the period of regular clamping. These results lead to the conclusion that renal scarring could not be prevented in the early treatment group because the administration of prednisolone during the period of urethral clamping reduced the activity of PMNLs in the kidney and decreased the defense of the host, thus permitting bacteria to multiply at a greatly increased rate; in contrast, scarring could be prevented in the late treatment group because the administration after the period of clamping had ended, reducing the activity of PMNLs at that point, although not enough to prevent phagocytosis from eradicating bacteria, with the result that at that time a critical concentration of bacteria was not present and thus the critical inflammatory response did not take place.

Early antimicrobial therapy for renal infection is the most effective way to prevent renal scarring. If it is delayed, prednisolone would be more effective, and should be given after the obstruction has been cleared (intermittent catheterization, calculus elimination or catheter exchange) in those cases where there is lower urinary tract obstruction.

Acknowledgements We thank Mr. Takade for his technical assistance with the electron micrographs. This work was supported in part by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science and Culture, Japan. We wish to thank Miss K. Miller (Royal English Language Center, Fukuoka, Japan) for correcting the English used in this manuscript.

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