

EFFICACY OF A NOVEL INJECTABLE CEPHALOSPORIN, CEFCLIDIN®, ON THE EXPERIMENTAL COMPLICATED URINARY TRACT INFECTIONS WITH URINARY STONES CAUSED BY *PSEUDOMONAS AERUGINOSA* AND *PROTEUS MIRABILIS*

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We evaluated the effects of a novel cephalosporin, cefclidin (CFCL) and imipenen (IPM), on the eradication of bacteria from the urine, bladder stones and the kidneys, and also on the prevention of the infection stone formations, in our polymicrobial urinary tract infection model of rats associated with bladder stones using IPM-sensitive or IPM-resistant *Pseudomonas aeruginosa* and *Proteus mirabilis* as a causative pathogen.

CFCL completely eradicated *P. mirabilis* from the urine and the stone in the short-term regimen (5 days). CFCL completely eradicated both IPM-sensitive *P. aeruginosa* and *P. mirabilis* from the urine, the stones and the kidneys as compared to IPM in the long-term regimen (11 days), reflecting the superior antibacterial activity of CFCL. CFCL also significantly prevented the development of infection stones as compared to IPM in the long-term regimen. There was no significant difference in the blood urea nitrogen (BUN) values between the CFCL or IPM-treated and the non-treated groups.

The cumulative recovery rate of unchanged CFCL reached 47.3% of the total dosage (20 mg/kg) within 8 hours.

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Key words: *Pseudomonas aeruginosa*, Urinary tract infections

INTRODUCTION

Pseudomonas urinary tract infections associated with urinary stones, indwelling catheter and/or obstructive uropathy are well known refractory and resistant to various antipseudomonal chemotherapies. In terms of the bacteriological feature, most of *Pseudomonas* complicated urinary tract infections are noted to be polymicrobial infections. Accordingly, antipseudomonal agents with a broad spectrum are preferable in the management of *Pseudomonas* complicated urinary tract infections. Therefore, imipenem (IPM), which shows a very broad spectrum is one of the most useful antimicrobial agent in controlling *Pseudomonas* urinary tract infection^{1,2)}. However, emergence of resistant *P. aeruginosa* to IMP

was recently noted on a patient with complicated urinary tract infections³⁾. Some recent reports also indicated that resistance of *P. aeruginosa* to IPM was acquired in the course of the actual treatment^{4,5)}, probably due to the decrease in the permeability of IPM toward the outer membrane of *P. aeruginosa*⁶⁾.

CFCL, a novel injectable cephalosporin, manifests a broad antibacterial spectrum and especially exhibits prominent activity toward various clinical isolates of *P. aeruginosa* including IPM-resistant *P. aeruginosa*^{7,8)}. Therefore, we evaluated the prospective usefulness of CFCL in the management of *P. aeruginosa* induced complicated urinary tract infections, using our polymicrobial urinary tract infection model associated with urinary stones⁹⁾.

MATERIALS AND METHODS

Bacteria

Proteus mirabilis (E05106), IPM-sensitive (MIC of IPM $< 3.13 \mu\text{g/ml}$) *Pseudomonas aeruginosa* (E030033) and IPM-resistant (MIC of IPM $> 6.25 \mu\text{g/ml}$) *Pseudomonas aeruginosa* (E030400) were isolated from patients with urinary tract infections.

Antibiotics

CFCL was synthesized at Eisai Co., Ltd. and its purity was more than 98%. IPM preparation (Banyu Pham., Co., Ltd., Japan) was purchased.

Animals

Female Sprague Dawley strain rats (specific pathogen free, 7 weeks old, each weighing 180 to 220 g, Charles River Japan Inc.) were used in all experiments.

The experimental procedures for the preparation of our polymicrobial infection model in rats

The fundamental experimental procedures were conducted according to the method of Satoh *et al.* (1984). Namely, rats were intraperitoneally anesthetized with sodium pentobarbital. A sterile zinc ring (4 mm in diameter) was surgically implanted into the bladder followed by the administration of aminobenzyl-penicillin to prevent the infection associated with surgery.

Seven days after surgery, the first bacterium, *P. mirabilis* (10^7 cfu/rat) was transurethraally inoculated through a polyethylene tube (PE-10), and then the second test bacterium, *P. aeruginosa* (10^8 cfu/rat) was inoculated in the same manner 5 days after the first inoculation.

Three types of experiments were performed:

Experiment 1 [the short-term regimen for the IPM-sensitive *P. aeruginosa* (E030033) plus *P. mirabilis* (E05106) infection]. The minimum inhibitory concentration (MIC) of CFCL for *P. mirabilis* (E05106) and *P. aeruginosa* (E030033) was $0.4 \mu\text{g/ml}$ and $0.8 \mu\text{g/ml}$, respectively, and the MIC of IPM for *P. mirabilis* and *P. aeruginosa* was $6.25 \mu\text{g/ml}$ and $1.56 \mu\text{g/ml}$, respectively. CFCL was administered intramuscularly twice a day at the dosage of 20 mg/kg for

5 days beginning from the 4th day after infection of *P. aeruginosa*. IPM was administered intramuscularly twice a day at the dosage of 10 mg/kg under the same condition.

Experiment 2 [the short-term regimen for the IPM resistant *P. aeruginosa* (E030400) plus *P. mirabilis* (E05106) infection]. The MIC of CFCL and IPM for *P. aeruginosa* (E030400) was $0.2 \mu\text{g/ml}$ and $6.25 \mu\text{g/ml}$, respectively. The antibiotics were administered under the same condition as described in experiment 1.

Experiment 3 [the long-term regimen for the IPM-sensitive *P. aeruginosa* (E030033) plus *P. mirabilis* (E05106) infection]. The antibiotics were administered for 11 days under the same condition as described in experiment 1.

All the tested rats were killed to make the stipulated determinations 24 hours after the final dosing.

Bacteriological determinations

At the time of death, the bladder urine was aspirated with a tuberculin syringe, and then diluted with a sterile saline. Both kidneys were removed and homogenized. The bladder stones were removed from the bladder at autopsy and weighed, then immersed in a 70% alcohol solution for 10 minutes, followed by twice washing with a sterile saline and cultured after grinding them. Our preliminary examinations clarified that live bacteria on the surface of the bladder stone were eradicated by the immersion in 70% alcohol for 10 minutes, which had no effect on the live bacteria inside the bladder stone. The specimens were cultured after inoculation onto both BTB and NAC agar media (Eiken Co., Ltd., Japan).

Blood urea nitrogen (BUN) determinations

At the time of death, the blood specimens from the aorta abdominals were collected to determine the blood urea nitrogen (BUN) according to the method of Seligson¹¹⁾.

Determination of urinary recovery rates of antibiotics

The amounts of unchanged CFCL and

IPM in the urine were determined by the thin layer disc method using *Escherichia coli* E01174 and *Bacillus subtilis* ATCC 6633 as a test bacterium.

RESULTS

Effects of CFCL and IPM on the polymicrobial infection of *P. aeruginosa* plus *P. mirabilis* in rats

In experiment 1 (the short-term regimen for the IPM-sensitive *P. aeruginosa* plus *P. mirabilis* infection), CFCL completely eradicated *P. mirabilis* from the urine and the stones, and also *P. aeruginosa* from the stone. IPM eradicated *P. aeruginosa* from the stones, but could not eradicate *P. mirabilis* from the urine and the stones, and *P. aeruginosa* from the urine.

Both CFCL and IPM showed marked efficacy in preventing the development of

infection bladder stones. There was no significant difference in BUN values or the incidence of kidney abscess between the treated and non-treated groups (Table 1).

In experiment 2 (the short-term regimen for the IPM-resistant *P. aeruginosa* plus *P. mirabilis* infection), CFCL more effectively eradicated IPM-resistant *P. aeruginosa* from the urine and the stone as compared to IPM, reflecting its superior *in vitro* antipseudomonal activity and CFCL also completely eradicated *P. mirabilis* from the urine and the stones. Furthermore, CFCL significantly prevented the development of infection bladder stones as compared to IPM. There was no significant difference in the incidence of abscess formation among the three groups and all the BUN values in the three groups were in the normal range (Table 2).

In experiment 3 (the long-term regimen

Table 1. Effects of the short-term regimen (5 days) with CFCL and IPM on viable cells in both the urine and the stone, and on the infection stone formation caused by IPM-sensitive *P. aeruginosa* + *P. mirabilis*

Group	Incidence of positive culture ^{a)}				Stone weight (mg) (Mean ± S.E.)	BUN (mg/dl) (Mean ± S.E.)
	Urine		Stone			
	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>		
CFCL	0/9	2/9	0/9	0/9	56.7 ± 9.6 ^{b)} **	27.4 ± 1.8
IPM	5/8	4/8	2/8	0/8	66.4 ± 4.0**	28.5 ± 2.8
Non-treated	12/12	12/12	10/12	11/12	134.3 ± 9.2	27.1 ± 1.8

a) Number of rats showing a positive culture judged by the following criteria :

Urine ; >2 × 10² cfu/ml. Stone ; >40 cfu/stone. Kidney ; >40 cfu/kidneys.

b) ** : P < 0.01. significantly different from the non-treated group by *t*-test.

c) †† : P < 0.01, † : P < 0.05. significantly different from the IPM-treated group or the non-treated group by X²-text.

Table 2. Effects of the short-term regimen (5 days) with CFCL and IPM on viable cells in both the urine and the stone, and on the infection stone formation caused by IPM-resistant *P. aeruginosa* + *P. mirabilis*.

Group	Incidence of positive culture ^{a)}				Stone weight (mg) (Mean ± S.E.)	BUN (mg/dl) (Mean ± S.E.)
	Urine		Stone			
	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>		
CFCL	0/8	2/8	0/8	0/8	51.4 ± 4.8	25.6 ± 2.4
IPM	8/9	6/9	5/9	1/9	77.9 ± 8.5	14.4 ± 1.2
Non-treated	11/12	10/12	8/12	6/12	123.7 ± 7.4	16.4 ± 1.5

a) Number of rats showing a positive culture judged by the following criteria :

Urine ; >2 × 10² cfu/ml. Stone ; >40 cfu/stone. Kidney ; >40 cfu/kidneys.

b) ** : P < 0.01. * : P < 0.05. significantly different from the IPM-treated group or the non-treated group by *t*-test.

c) †† : P < 0.01, † : P < 0.05. significantly different from the IPM-treated group or the non-treated group by X²-text.

Table 3. Effects of the long-term regimen (11 days) with CFCL and IPM on viable cells in the urine, the stone and the kidney, and on the infection stone formation caused by IPM-sensitive *P. aeruginosa* + *P. mirabilis*

Group	Incidence of positive culture ^{a)}						Stone weight (mg)	BUN (mg/dl)
	Urine		Stone		Kidney			
	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	Mean ± S.E.	Mean ± S.E.
CFCL	0/11	0/11	0/11	0/11	0/11	0/11	24.4 ± 4.2	19.9 ± 0.6
IPM	6/11	4/11	2/11	0/11	0/11	2/11	47.6 ± 6.8	20.7 ± 0.7
Non-treated	10/10	10/10	10/10	10/10	10/10	9/10	180.7 ± 25.0	43.5 ± 14.4

a) Number of rats showing a positive culture judged by the following criteria :

Urine ; $>2 \times 10^2$ cfu/ml. Stone ; >40 cfu/stone. Kidney ; >40 cfu/kidneys.

b) ** : $P < 0.01$. * : $P < 0.05$. significantly different from the IPM-treated group or the non-treated group by *t*-test.

c) †† : $P < 0.01$, † : $P < 0.05$. significantly different from the IPM-treated group or the non-treated group by χ^2 -text.

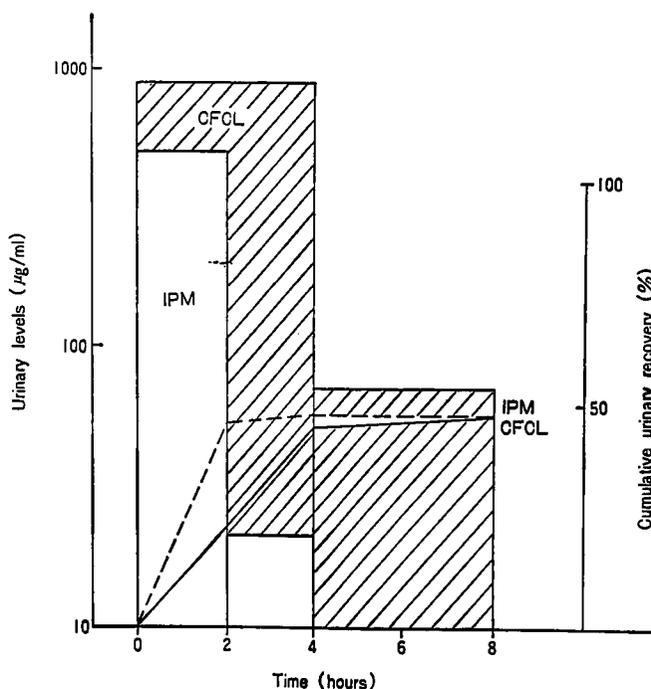


Fig. 1. Urinary levels of CFCL and IPM in rats with infection stones caused by *P. mirabilis* plus *P. aeruginosa*. CFCL and IPM were administered intramuscularly 13 days after infection of *P. mirabilis* (8 days after infection of *P. aeruginosa*, CFCL; 20 mg/kg, IPM; 10mg/kg).

for the IPM-sensitive *P. aeruginosa* plus *P. mirabilis* infection), CFCL completely eradicated both *P. aeruginosa* and *P. mirabilis* from the urine, the stones and the kidneys. CFCL was significantly superior to IPM in the prevention of the development of infection bladder stones (Table 3). Kidney

abscesses were observed in 6 of the 12 rats in the non-treated groups and in 1 of 8 rats in the IPM-treated groups. Meanwhile, non of the rats in the CFCL-treated group had kidney abscesses. The cumulative recovery rate of unchanged CFCL reached 47.3% of the total dosage (20 mg/

kg) within 8 hours, comparable to that of IPM (48.0%). Meanwhile, CFCL showed a urinary excretion pattern different from that of IPM, with a more prolonged urinary level (Fig. 1).

Emergence of resistant strains of *P. aeruginosa* toward CFCL and IPM during the present treatment was not observed.

DISCUSSIONS

We evaluated the efficacy of CFCL and IPM against the polymicrobial *Pseudomonas* urinary tract infections, a representative refractory urinary infection, using our polymicrobial urinary tract infection model associated with urinary stones. The short-term regimen (5 days) with CFCL was insufficient to eradicate IPM-sensitive *P. aeruginosa* (MIC of CFCL; 0.8 $\mu\text{g}/\text{ml}$) from the urine as compared with the corresponding long-term regimen (11 days). This suggests that the long-term regimen should be used for the satisfactory management of *Pseudomonas* complicated urinary tract infections, even if a potent antipseudomonal agent such as CFCL is used. Meanwhile, CFCL more effectively eradicated IPM-resistant *P. aeruginosa* (MIC of CFCL; 0.2 $\mu\text{g}/\text{ml}$) from the urine and stones than IPM in the short-term regimen. This may be attributable to the difference in antipseudomonal activity and the urinary excretion pattern between CFCL and IPM.

Concerning the correlation between the MIC values and eradication actions to *P. aeruginosa* from the urine, the kidney and the stones, an interesting finding has been reported; both the short-term and long-term regimen (11 days) with indicating MIC for the tested *P. aeruginosa* of 1.56 $\mu\text{g}/\text{ml}$ were insufficient to eradicate *P. aeruginosa* from the urine, the stone and the kidney in the same experimental conditions¹²⁾. Similarly, the present study showed that IPM with an MIC of 1.56 $\mu\text{g}/\text{ml}$ for *P. aeruginosa* was not sufficient for the eradication of *P. aeruginosa* in experimental 3 (the long-term regimen). Accordingly, the MIC values of a therapeutic agent toward *P. aeruginosa* must be below 0.8 $\mu\text{g}/\text{ml}$, in order to fulfill satisfactory eradication of *P.*

aeruginosa from the urine, the kidney and associated infection stones.

In the present experiment, *Enterococcus faecalis* infections were observed in CFCL-treated, IPM-treated and non-treated groups, although, the rats had not been inoculated with *E. faecalis*. Our preliminary examinations clarified that these *E. faecalis* infections were inevitably induced by the transurethral cannulation of the polyethylene tube *via* the ascending route. Although IPM had potent antibacterial activity toward *E. faecalis*¹³⁾, *E. faecalis* could not be eradicated from the urine, the stones or the kidneys. The insufficient eradication effects of IPM might be attributable to its rapid excretion from the urine.

Our present findings indicate that the associated *P. mirabilis* must primarily be eradicated for the complete eradication of *P. aeruginosa* because infection stones act as a sanctuary to hide bacteria from chemotherapy. Accordingly, it is basically important to remove the associated urinary stones from the urinary tract for the treatment of urinary tract infections accompanied with urinary stones. Recently, ultrasonic lithotripsy or extracorporeal shock wave lithotripsy has been widely used for the treatment of urinary stones. In lithotripsy regimen, the bacteria existing in the urinary stones are released and induce fever and bacteremia, so that the combination regimen of a powerful antibacterial agent such as CFCL and lithotripsy is strongly recommended as an intensive therapy in the management of *Pseudomonas* complicated urinary tract infections accompanied with urinary stones.

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(迅速掲載)

和文抄録

感染結石を伴う *Pseudomonas aeruginosa* と *Proteus mirabilis* による実験的複雑性尿路感染に対する新規注射用セファロスポリン“セフクリジン”の効果

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Imipenem (IPM) 感受性および耐性の *Pseudomonas aeruginosa* と *Proteus mirabilis* による感染性膀胱結石を伴う新規ラット複数菌尿路感染モデルを用いて、尿、膀胱結石および腎からの除菌および感染性膀胱結石の形成抑制に対する新規セファロスポリン・セフクロジン (CFCL) およびチェナム (IPM) の効果を評価した。

CFCL は、5日間 (20 mg/kg, b.i.d) の短期投与で尿および結石中から *Proteus mirabilis* を完全に除菌

した。CFCL は、その優れた抗菌力を反映して11日間の長期投与で IPM と比較した時、尿、膀胱結石および腎から IPM 感受性および耐性の *Pseudomonas aeruginosa* を完全に除菌した。また、CFCL は、長期投与で IPM と比較して、感染結石の成長を有意に抑制した。BUN 値は、CFCL 投与群、IPM 投与群および非投与群の間に有意の差はなかった。ラット (20 mg/kg, 筋注) における未変化体 (活性体) の尿中回収率は、8時間で47.3%に達した。

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