PS-6 AND PS-7, NEW β-LACTAM ANTIBIOTICS

IN VITRO AND *IN VIVO* EVALUATION

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Biological properties of two new β-lactam antibiotics, PS-6 and PS-7, containing the carbapenem nucleus were studied. The *in vitro* activities of PS-6 and PS-7 were tested against clinical isolates of Gram-positive and Gram-negative bacteria in comparison with those of cefazolin, ampicillin and PS-5. In general, the antibacterial spectra of PS-6 and PS-7 were similar to that of PS-5. PS-7 was slightly less active than PS-5 and ABPC against *Staphylococcus aureus*. Clinical isolates of Gram-negative bacteria were found to be 2- to 8-fold more resistant to PS-6 than to PS-5, while PS-7 was 2-fold more active than PS-5 against *Enterobacter*, *Klebsiella*, *Proteus*, *Serratia* and *Escherichia coli*. The therapeutic effect of PS-6 seemed slightly less than that of PS-5 in an experimental infection with *Staph. aureus* Smith in mice.

PS-6 and PS-7 are new members of the olivanic family[1] and are produced in less significant amounts together with PS-5 and related compounds by *Streptomyces cremeus* subsp. *auratilis* A271 and *S. flavogriseus* NRRL 8139. Fermentation, isolation and physico-chemical properties of PS-6 and PS-7 have been described in the preceding paper[2]. This paper describes the *in vitro* and *in vivo* evaluation of PS-6 and PS-7.

Materials and Methods

Antibiotics

The sodium salts of PS-5, PS-6 and PS-7 were prepared in our laboratories[2,3]. Cefazolin (sodium salt, CEZ) and ampicillin (sodium salt, ABPC) were obtained from Fujisawa Pharm. Co., Ltd. and Toyo Jozo Co., Ltd., respectively.

Bacterial strains

The standard strains were from the stock culture collection in our laboratories. Clinical isolates were supplied by Dr. NISHINO, Kyoto College of Pharmacy; Dr. YAMAGISHI, Faculty of Pharmaceutical Science, Chiba University, and Dr. KAWAGUCHI, Bristol-Banyu Research Institute, Ltd.

Determination of minimum inhibitory concentration (MIC)

Antimicrobial activity was measured by the agar dilution method[4]. Heart Infusion Agar (Difco) was used unless otherwise specified. An appropriate dilution ($10^6 \sim 10^8$ cells/ml) of a fresh overnight culture was prepared as inoculum. Plates were incubated at 35°C for 18 hours. MIC was defined as the lowest concentration of antibiotic that inhibited the development of visible growth of the test microorganism.

Bactericidal activity

Killing curves were drawn based on growth experiments in broth containing exponentially growing cells. The antibiotic to be tested was added to medium at the indicated concentrations. The mixture was inoculated and incubated at 37°C with shaking. Samples of the broth were taken at intervals, appropriately diluted and plated on Heart Infusion Agar for determination of the colony-forming unit of the broth.

Plasma levels in mice

Male *ddY* mice (aged 5 weeks, weighing 19~21 g) were used in groups of 5 mice each. A single dose of an antibiotic was given subcutaneously. Blood was taken from the retroorbital sinus at various time intervals using a heparinized capillary. Each blood sample was centrifuged at 1,500 *g* for 5 minutes and the supernatant was assayed by the disc-agar diffusion method with *Comamonas terrigena* B996[3] as the test organism.

Therapeutic effect on an experimental infection in mice

Groups of 5 mice each (male, *ddY*, aged 5 weeks; body weight 19~21 g) were used for the experiment. The challenge organism (*Staphylococcus aureus* Smith) was cultured overnight in Brain Heart Infusion Broth (Difco) at 37°C and suspended in 5% bacteriological mucin (Wilson Pharmaceutical and Chemical Co.). Two hours after intraperitoneal injection of 0.5 ml of the cell suspension (30~50× LD_{50}), a single dose of the test antibiotic was given subcutaneously. The mice were observed for 5 days. The therapeutic effect of the drug was expressed as ED_{50}(mg/mouse) calculated by the method of LITCHFIELD and WILCOXON[5].

Results and Discussion

Table 1 summarizes the MICs of PS-6, PS-7 and related antibiotics.

PS-6, having the isopropyl side chain at C-6, was less active than PS-5. PS-7, which is structurally dehydro-PS-5[2], showed better activity against Gram-negative bacteria and poorer activity against Gram-positive bacteria, than PS-5[6,7,8].

Against some typical bacteria, the antimicrobial activities of PS-6 and PS-7 were compared with those of PS-5, CEZ and ABPC using the cumulative inhibition percentage. PS-6, PS-7 and the other test drugs were of similar activity against *staphylococci* (Fig. 1).

In contrast, against Gram-negative bacteria, a clear advantage with PS-7, and, to a less extent, PS-6 and PS-5 over ABPC and CEZ was observed at two inoculum levels. Against β-lactam-resistant

Fig. 1. Growth-inhibitory activity of PS-7, PS-5, cefazolin (CEZ) and ampicillin (ABPC) against clinical isolates of Gram-positive bacteria.

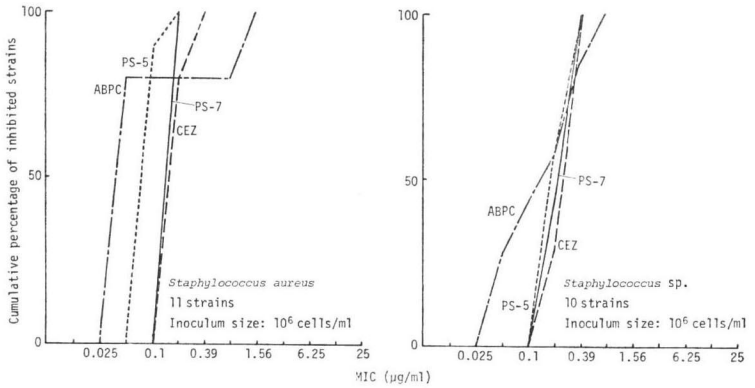


Table 1. *In vitro* activity of PS-6, PS-7, PS-5, cefazolin and ampicillin.

Microorganism	MIC (μg/ml)				
	PS-6	PS-7	PS-5	CEZ	ABPC
Bacillus subtilis ATCC 6633	0.78	0.39	0.10	0.10	0.05
Sarcina lutea	0.39	0.20	0.10	0.78	<0.013
Staphylococcus aureus FDA 209P	0.10	0.39	0.024	0.10	<0.013
Staph. aureus Smith	0.20	0.39	0.20	0.20	0.05
Staph. aureus Russell	0.20	0.39	0.20	0.20	25
Staph. epidermidis	0.39	0.20	0.20	0.20	0.024
Alcaligenes faecalis A1	1.56	0.78	0.78	3.13	3.13
Citrobacter freundii GN 346	12.5	3.13	3.13	>400	>400
Comamonas terrigena B 996	0.10	0.05	0.024	0.39	0.05
Enterobacter aerogenes E 19	25	3.13	6.25	>400	>400
Ent. cloacae 45	25	3.13	6.25	>400	>400
Enterobacter sp. E 8	6.25	1.56	3.13	3.13	6.25
Escherichia coli K-12	6.25	0.78	3.13	1.56	3.13
E. coli RGN 823	6.25	1.56	3.13	200	>400
Klebsiella pneumoniae K 13	25	3.13	6.25	400	>400
Proteus mirabilis P 6	12.5	6.25	12.5	6.25	3.13
P. rettgeri P 7	6.25	1.56	12.5	>400	>400
P. vulgaris GN 76	12.5	12.5	25	>400	>400
Proteus sp. P 22	25	12.5	25	>400	>400
Providencia sp. P 8	6.25	1.56	6.25	100	400
Pseudomonas aeruginosa IFO 3445	50	12.5	25	>400	>400
Ps. aeruginosa NCTC 10490	50	6.25	25	>400	>400
Serratia marcescens S 18	25	1.56	6.25	>400	200
Ser. marcescens T 55	50	3.13	6.25	>400	50

Inoculum size: 10^5 cells/ml

clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*, PS-7 and, less significantly, PS-6 and PS-5 were more active than ABPC and CEZ (Fig. 2).

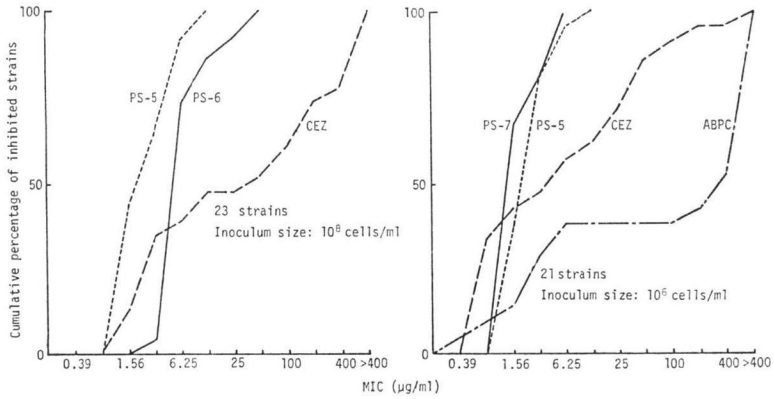
β-Lactam-resistant strains of *Proteus vulgaris, Proteus morganii, Enterobacter cloacae, Enterobacter aerogenes, Alcaligenes faecalis* and *Serratia marcescens* were found to be susceptible to PS-7, PS-6 and PS-5, but practically resistant to ABPC and CEZ (Fig. 3).

The bactericidal effects of PS-7, PS-6, PS-5 and CEZ were studied by counting viable cells of the test organism over an incubation period of 5~6 hours in the presence of the drug. Against *Staph. aureus* Smith, all the β-lactam compounds tested showed a similar pattern of killing, resulting in a 3-log reduction of viable cell counts after 6-hour incubation (Fig. 4A). When *Enterobacter cloacae* 45 was employed as the test organism, a marked difference was observed between the PS-compounds and CEZ. At a concentration of 4×MIC, PS-6 and PS-5 were highly bactericidal, while a certain regrowth was seen with PS-7. CEZ, in contrast, was found to be the least bactericidal even at a high concentration of 800 μg/ml (Fig. 4B).

Table 2 shows the mean plasma levels and the plasma-half-lives of PS-5, PS-6 and CEZ after a single subcutaneous dose of 2 mg/mouse in groups of 5 mice. The plasma levels of PS-5, PS-6 and CEZ were 43.0 μg/ml, 51.3 μg/ml and 168.8 μg/ml at 5 minutes, 5 minutes and 15 minutes after injection respec-

Fig. 2. Growth-inhibitory activity of PS-6, PS-7, cefazolin (CEZ) and ampicillin (ABPC) against clinical isolates of Gram-negative bacteria.

A. *Escherichia coli*



B. *Klebsiella pneumoniae*

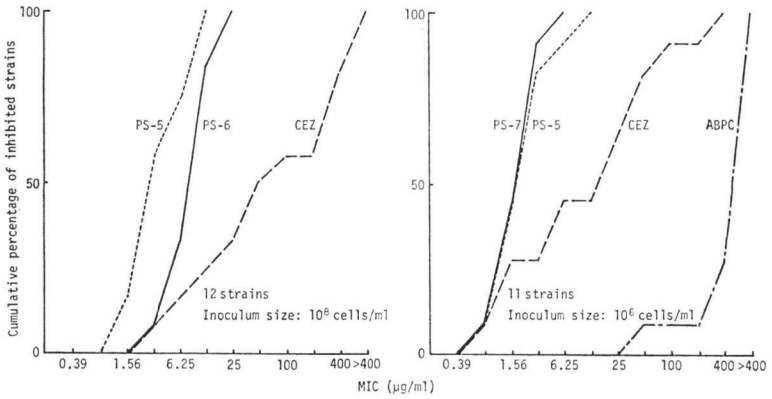


Table 2. Mean plasma levels of PS-6, PS-5 and CEZ after a single subcutaneous administration in mice ($n=5$).

Time (minutes)	Mean±S.D. (μg/ml)		
	PS-6	PS-5	CEZ
5	51.3±8.8	43.0±2.0	N.T.
15	11.2±1.1	11.7±4.4	168.8±12.5
30	3.2±1.1	5.2±1.5	96.0± 5.7
60	0.6±0.6	0.6±0.5	34.0±12.7
90	0	0	7.3± 3.4
Biological half-life (min.)	4.5	5.5	20
Urinary recovery (0~6 hours) (%)	1.6	0.2	65

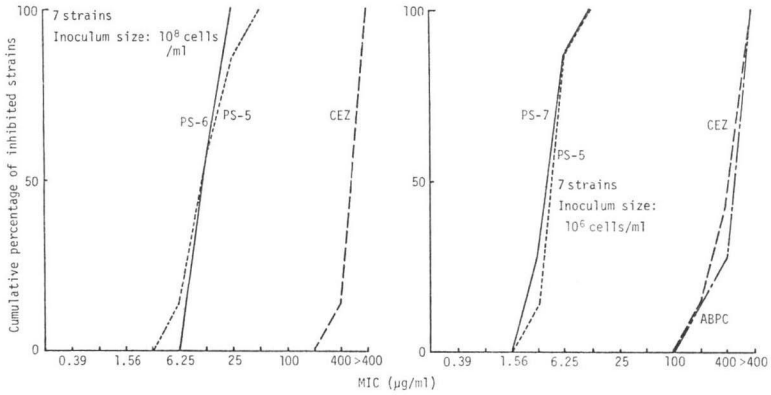
N.T.: not tested. dose: 2 mg/mouse

Table 3. Therapeutic effect of PS-6, PS-5 and CEZ against an experimental infection of *Staphylococcus aureus* Smith in mice.

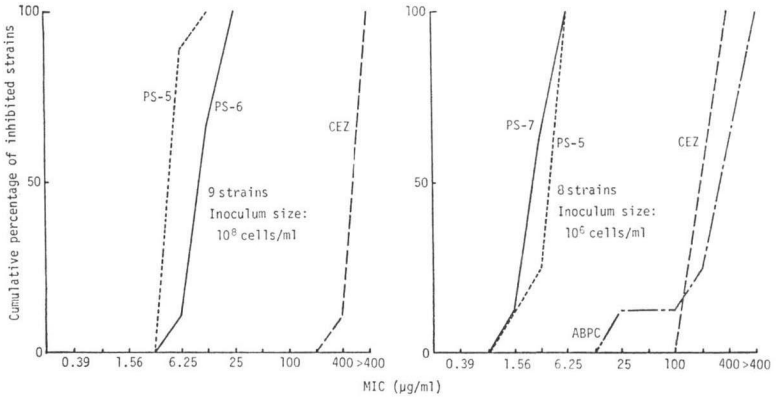
Drug	MIC (μg/ml)	ED_{50} (mg/mouse)
PS-6	0.20	1.6
PS-5	0.20	0.24
CEZ	0.20	0.0087

Fig. 3. Growth-inhibitory activity of PS-6, PS-7, cefazolin (CEZ) and ampicillin (ABPC) on clinical β-lactam-resistant isolates of Gram-negative bacteria.

A. *Proteus vulgaris*



B. *Proteus morganii*



C. *Enterobacter cloacae*

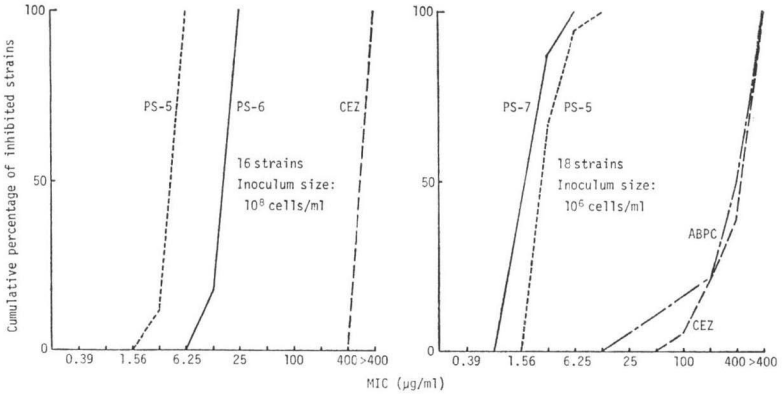
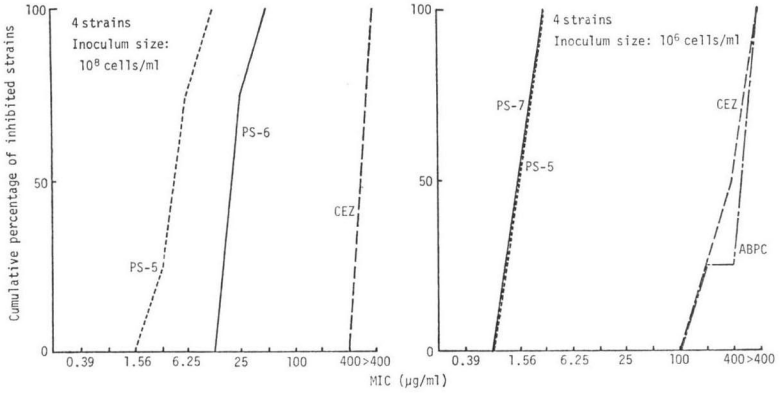
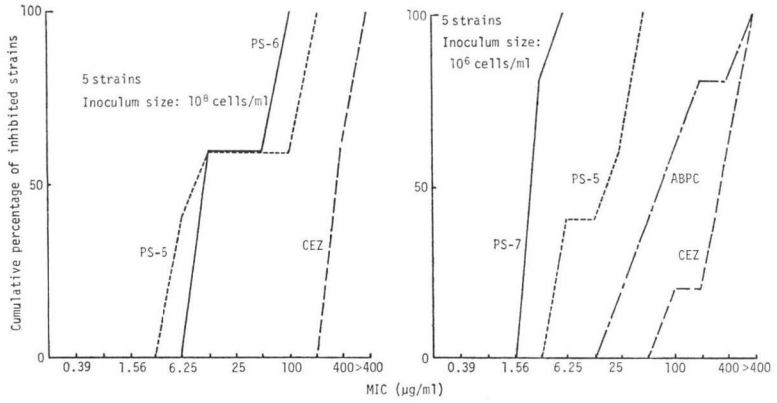


Fig. 3. (continued)

D. *Enterobacter aerogenes*



E. *Alcaligenes faecalis*



F. *Serratia marcescens*

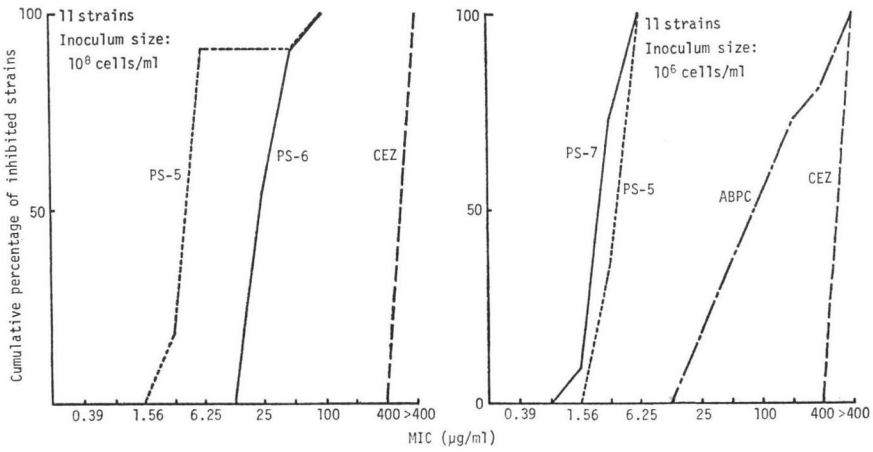


Fig. 4-A. Bactericidal effects of PS-6, PS-7, PS-5 and CEZ on *Staphylococcus aureus* Smith.

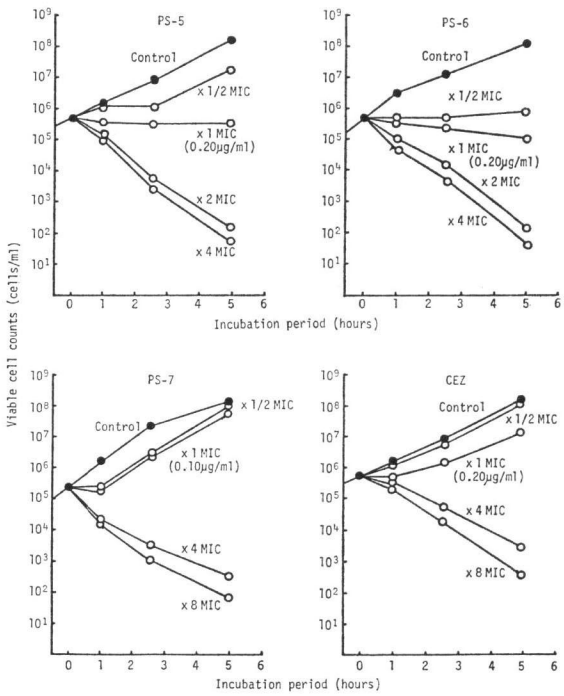
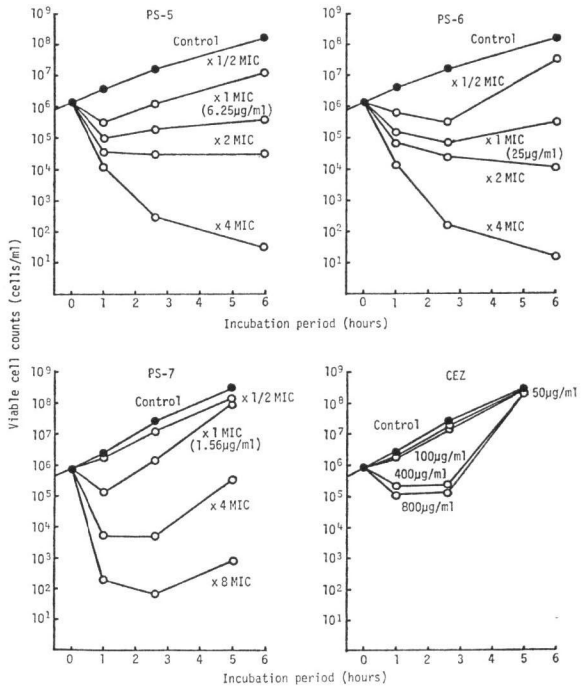


Fig. 4-B. Bactericidal effects of PS-6, PS-7, PS-5 and CEZ on *Enterobacter cloacae* 45.



tively. PS-6 and PS-5 had shorter half-lives than CEZ. The 6-hour urinary recovery rates of PS-5 and PS-6 were as low as 0.2% and 1.6% respectively.

The therapeutic effect of PS-6 on an experimental infection of *Staph. aureus* Smith in mice was compared with those of PS-5 and CEZ. As is shown in Table 3, PS-6 was 6-fold less active than PS-5.

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