PHARMACOLOGICAL STUDIES ON CARBAPENEM ANTIBIOTICS

I. METABOLISM OF PS-5 IN ANIMAL TISSUES

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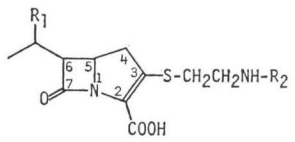
After injection into mice and dogs, PS-5 showed a very rapid decrease in its blood concentration, compared with cefazolin. Using *in vitro* experiments with tissue homogenates and acetone powder preparations, the kidney was found to be the primary site of PS-5-inactivation, although the extent of the inactivation varied depending on the species of animals. The comparative stability data of PS-5, NS-5 (deacetylated PS-5), thienamycin and *N*-formimidoylthienamycin in kidney homogenates of mouse, rabbit, dog and man are presented. Bilateral nephrectomy and the injection of ethylenediaminetetraacetate seemed to prolong the survival time of PS-5 in rats and mice respectively.

Carbapenem is a novel family of β-lactam compounds having the desthiacarbapenem nucleus.[1,2] Compared with the penicillins and cephalosporins, they have an extremely broad antimicrobial spectrum against Gram-positive and Gram-negative bacteria.[3,4] Furthermore their potent β-lactamase-inhibitory activity, combined with their intrinsic antibiotic activity, is expected to find a wide clinical utility in the therapy of infections caused by β-lactamase-producing pathogens.[5] Naturally occurring members of the carbapenem family, however, have been shown to be far more labile *in vivo* than penicillins and cephalosporins[6], which is now a serious problem to be solved before such compounds can find utility in the clinical treatment of infectious diseases. As is the case with cephalosporin C, chemical or biochemical modification of the naturally occurring carbapenem compounds is required to obtain therapeutically interesting derivatives.

PS-5 is a member of the carbapenem family produced by *Streptomyces cremeus* subsp. *auratilis* A271[2], *Streptomyces fulvoviridis*[7] and other streptomycetes. Fermentation, isolation and physicochemical and biological properties of PS-5 have been reported in previous papers.[2,4,5] Like other carbapenem compounds, PS-5 was rapidly metabolized *in vivo*, resulting in a very poor urinary recovery. Therefore PS-5 must also be modified by some chemical and/or biological methods to provide therapeutically useful carbapenem derivatives which have significantly improved chemical and biological stability.[8,9] Although very recently thienamycin and related compounds have briefly been reported to be susceptible to renal dehydropeptidase-I,[6] no

Fig. 1. Chemical structures of PS-5 and related carbapenem antibiotics.



	R_1	R_2
PS-5	–H	$-COCH_3$
NS-5	–H	–H
Thienamycin	–OH	–H
N-Formimidoyl-thienamycin	–OH	$-C(=NH)H$

Numbered according to the IUPAC method.

detailed paper is available on the metabolic fate of carbapenem compounds. Knowledge of the *in vivo* metabolism of carbapenem antibiotics is essential for evaluation of chemical and biochemical carbapenem derivatives for practical use.

The present paper deals with the pharmacokinetics of PS-5 in mice and dogs and the comparative stability of PS-5, NS-5 (deacetylated PS-5), thienamycin, *N*-formimidoylthienamycin and cefazolin in fresh kidney homogenates of mouse, rabbit, dog and man.

Materials and Methods

Antibiotics

PS-5 sodium salt and NS-5 were prepared as described in previous papers.[2,5] Thienamycin was produced by fermentation of *Streptomyces cattleya* NRRL 8057 in our laboratories.[1] *N*-Formimidoylthienamycin (MK0787) was a gift of Merck, Sharp and Dohme Research Laboratories.[10] Cefotaxime was supplied by Hoechst AG. Other penicillins, cephalosporins and 7-methoxycephalosporins were commercially available products.

Animals

The following animals were employed in this paper and were fed *ad libitum* throughout the experiments:

a) *dd*Y mice, male, aged 5 weeks, weighing 19~21 g.
b) Albino rabbits, weighing 2.1 kg.
c) Wistar rats, weighing 180~200 g.
d) Mongrel dogs, weighing 12 kg.

Antibiotic Assay

Concentrations of PS-5 in reaction mixtures and solutions were bioassayed by the disc-agar diffusion method using *Comamonas terrigena* B-996 as the detector organism.[2] Assay samples were kept unfrozen at 0~4°C until bioassay, because freezing and thawing significantly decomposes carbapenem compounds.

Stability Tests in Tissue Homogenates

Fresh organs (kidneys, lungs, livers, spleens and hearts) were homogenized in 5 volumes of 1/50 M phosphate buffer, pH 7.5, or 1/50 M tris-HCl buffer, pH 7.5, with a Teflon® Potter homogenizer. After refrigerated centrifugation at 8,000×*g* for 20 minutes, one volume of the supernatant solution was mixed with one volume of an antibiotic solution (including an additive, if indicated) and then incubated at 37°C for 60 minutes. Enzymes contained in the reaction mixture were inactivated by heating at 100°C for 15 seconds at the end of incubation. The concentration of the antibiotic remaining in the reaction mixture was determined by bioassay using *Comamonas terrigena* B-996.

Solubilization of the PS-5-inactivating Agent from Kidney Acetone Powders

All the kidney acetone powders employed in this study were purchased from Sigma Chemical Company (dog kidney acetone powder Cat. No. K7625; horse kidney acetone powder Cat. No. K7875; eel kidney acetone powder Cat. No. K8375; mouse kidney acetone powder Cat. No. K7750; porcine kidney acetone powder type I Cat. No. K3875; porcine kidney acetone powder type II Cat. No. K7250). They were suspended in 1/50 M tris-HCl buffer, pH 7.5, containing 5% Triton X-100, 5% Tween 80 or 1% sodium dodecyl sulfate. After centrifugation at 5,000×*g* for 20 minutes, clear extract solutions were used for the PS-5-inactivation test. Mixtures of the extracts with PS-5 were incubated at 37°C for indicated periods of time with or without additive. Following heat-treatment at 100°C for 15 seconds, the amounts of unchanged PS-5 were disc-assayed as described above.

Plasma Levels and Urinary Recoveries of PS-5

(1) Mouse: Before drug administration, mice were forced to urinate by suprapubic manipulation. A single subcutaneous or intravenous dose of β-lactam was given to groups of 5 mice. Blood specimens were collected from the retroorbital sinus at indicated times with heparinized capillary tubes and

the tubes were sealed at one end with Iatroseal® and centrifuged at 3,000 rpm for 5 minutes. At 6 hours after drug administration, urine samples were recovered from bladders with injection syringes. The concentrations of β-lactam in sample solutions were disc-assayed as antimicrobial activity against *Comamonas terrigena* B-996.

When examining the protective effect of ethylenediaminetetraacetate (EDTA), 400 mg/kg of EDTA was injected intravenously to mice immediately after subcutaneous administration of 100 mg/kg of PS-5.

(2) Rat: Under anesthesia with ethyl ether, two test rats were bilaterally nephrectomized, while two control rats were laparotomized without nephrectomy. PS-5 (50 mg/kg) was injected subcutaneously to the rats 3.5 hours after the operation. Blood samples (0.1 ml) were collected from the cervical vein with hypodermic syringes. The amounts of unchanged PS-5 in plasmas were bioassayed as described above.

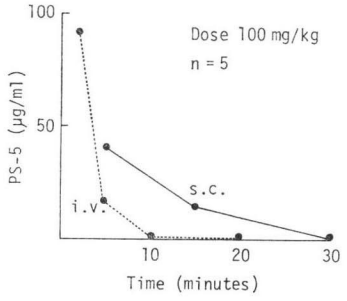
(3) Dog: A single dose of 50 mg/kg of PS-5 or cefazolin in 1/100 M phosphate buffer, pH 7.5, was injected intramuscularly to mongrel dogs. Blood specimens (1 ml) were taken from the cephalic vein without anesthesia by using hypodermic syringes. The concentrations of β-lactam in plasmas were measured by the bioassay method.

Results

Plasma Levels of PS-5 in Mice and Dogs

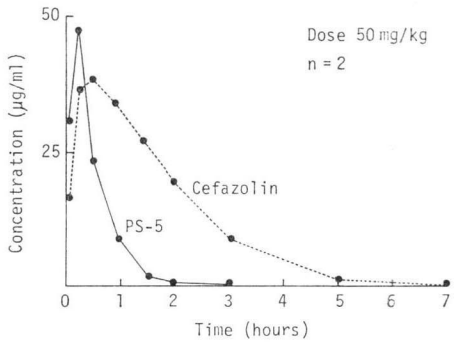
Fig. 2 shows the mean plasma concentrations of PS-5 after a single subcutaneous or intravenous injection of 100 mg/kg to mice. The half-life of PS-5 in mice, as calculated from the regression curve of log antibiotic concentration on elapsed time, was 7.5 minutes for the subcutaneous injection and 1.5 minutes for the intravenous injection, assuming that the peak plasma level was observed at 5 minutes (41.0 μg/ml) for the former route and at 2 minutes (90.8 μg/ml) for the latter route. The urinary recoveries of PS-5 in the period of 0~6 hours were less than 0.2% for both routes. In the control experiment using cefazolin at the same dose, the half-life of cefazolin was found to be 26.5 minutes (C_{max} = 156.5 μg/ml at 15 minutes) for the subcutaneous administration and 11 minutes (C_{max} = 556 μg/ml at 2 minutes) for the intravenous administration; urinary recoveries were over 65% in the first 6-hour period.

Fig. 2. Mean plasma concentration of PS-5 after a single s.c. or i.v. administration to mice.



	s.c.	i.v.
C_{max} (μg/ml)	41.0	90.8
$t_{1/2}$ (minutes)	7.5	1.5
Urinary recovery (0~6 hours)	0.2%	0.1%

Fig. 3. Mean plasma concentrations of PS-5 and cefazolin after a single i.m. administration to dogs.



	PS-5	Cefazolin
C_{max} (μg/ml)	48.0	38.3
$t_{1/2}$ (minutes)	14.0	92
Urinary recovery (0~24 hours)	0.1%	86.1%

Table 1. Stability of PS-5 in the fresh tissue homogenates of various mouse organs.

Organ	Relative stability of PS-5 (%)
1/50 M Phosphate buffer, pH 7.0	100.0 (Control)
Kidney	0.43
Liver	90.6
Spleen	89.4
Lung	13.6
Heart	43.8

Comparative pharmacodynamic properties of PS-5 and cefazolin in dogs are summarized in Fig. 3 after a single intramuscular injection of 50 mg/kg.

Although the urinary recovery of PS-5 was very poor in both species, the rate of metabolism of PS-5 seemed to be more rapid in mice than in dogs.

Table 2. Stability of penicillins and cephalosporins in the mouse liver and kidney homogenates.

β-Lactam	Relative stability*	
	Liver (%)	Kidney (%)
PCG	104	104
ABPC	98	98
PEPC	92.5	92.5
DMPPC	100	100
ACPC	90	90
SBPC	100	100
PIPC	100	112
CEZ	100	100
CTX	62	62
CEX	100	100
CFX	100	100
CMZ	100	100
PS-5	88	1.5

* Relative to the control in 1/50 M phosphate buffer, pH 7.0, in %.

Abbreviations: PCG=benzylpenicillin; ABPC=ampicillin; PEPC=phenethicillin; DMPPC=methicillin; ACPC=ciclacillin; SBPC=sulbenicillin; PIPC=piperacillin.

CEZ=cefazolin; CTX=cefotaxime; CEX=cephalexin; CFX=cefoxitin; CMZ=cefmetazole.

Stability of PS-5 in Mouse Tissue Homogenates

As a clue to the explanation of the short life of PS-5 *in vivo*, the stability of PS-5 in fresh homogenates of various mouse organs was examined (Table 1). It is apparent that PS-5 is inactivated drastically in the kidney homogenate and slowly in the lung and heart homogenates, while it is relatively stable in the spleen and liver homogenates.

Some of the semisynthetic penicillins and cephalosporins are known to be labile *in vivo*. For example, cephalothin is deacetylated by renal esterase to provide a less active product.[11] Thus, it seemed interesting to characterize the instability of PS-5 in comparison with those of commercially available penicillins and cephalosporins. Table 2 presents the comparative stability of 7 penicillins, 5 cephalosporins and PS-5 in the mouse liver and kidney homogenates.

Among the tested β-lactam compounds, cefotaxime was unstable in both homogenates, which is reportedly due to esterase.[12] In contrast, PS-5 was inactivated to a far greater extent in the kidney homogenate than in the liver homogenate.

Comparative Stability of PS-5, NS-5, Thienamycin, *N*-Formimidoylthienamycin and Cefazolin in the Fresh Kidney Homogenates of Various Animal Species

The type and nature of the C-3 and C-6 side chains of carbapenem derivatives have been proved to have marked influences on their antimicrobial and β-lactamase-inhibitory activities.[13] Using PS-5, NS-5 (deacetylated PS-5), thienamycin and *N*-formimidoylthienamycin (the last three compounds have basic C-3 side chains), the effect of the nature of the C-3 side chain on stability in kidney homogenates was studied by using kidneys from mouse, rabbit, dog and man (Table 3).

Table 3 indicates that, among the four carbapenem derivatives tested, PS-5 was the most susceptible. In common to the four compounds, the mouse kidney exhibited the strongest inactivating activity,

Table 3. Comparative stability of PS-5, NS-5, thienamycin, *N*-formimidoylthienamycin and cefazolin in the fresh kidney homogenates of various animal species.

Species	Relative stability*				
	PS-5	NS-5	Thienamycin	*N*-Formimidoyl thienamycin	Cefazolin
Buffer**	100.0	100.0	99.2	96.0	100.0
Mouse	0.5	6.7	22.4	32.0	100.0
Rabbit	2.7				100.0
Dog	8.5	44.8	22.4	46.4	100.0
Man	20.0	89.6	82.4	96.0	100.0

* Relative to the control in 1/50 M phosphate buffer, pH 7.5 without incubation at 37°C (in %).
** In 1/50 M phosphate buffer, pH 7.5; after incubation at 37°C for 60 minutes.

Table 4. Influences of divalent cations on stability of PS-5 in the detergent-solubilized fractions of kidney acetone powders.

Origin	Detergent	Amount of unchanged PS-5		
		None (%)	1 mM Zn^{++} (%)	1 mM Mg^{++} (%)
Mouse	Triton X-100	0	0	0
	Tween 80	0	0	0
	S.D.S.*	0.4	0.4	0.6
Porcine type I	Triton X-100	0	0	0
	Tween 80	7.2	0.35	0.51
	S.D.S.*	22.0	9.2	8.1
Porcine type II	Triton X-100	0	0	0
	Tween 80	0.7	0	0
	S.D.S.*	1.6	1.8	1.2
Eel	Triton X-100	127.5	112.7	127.5
	Tween 80	127.5	100	127.5
	S.D.S.*	100	112.7	127.5
Dog	Triton X-100	0	0	0
	Tween 80	0	0	0
	S.D.S.*	0.8	0.6	0.7
Horse	Triton X-100	37.5	25.5	28.5
	Tween 80	54	62	80
	S.D.S.*	62	37.5	90

* S.D.S.=sodium dodecyl sulfate.

whereas the human kidney did the weakest. It is important to note that the basic nature of the C-3 side chain leads to improved stability in the kidney, because the deacetylation of PS-5 to NS-5 results in significantly increased resistance to the kidney homogenate.

Stability of PS-5 in Kidney Acetone Powders and the Protective Effect of Ethylenediaminetetraacetate (EDTA)

Since a preliminary experiment showed that a PS-5-inactivating agent was poorly soluble in water, commercially available kidney acetone powders of mouse, pig, eel, dog and horse were subjected to deter-

gent-solubilization. The stability data of PS-5 in detergent-solubilized fractions of the kidney acetone powders with or without 1 mM of zinc or magnesium ions are summarized in Table 4.

The eel kidney acetone powder had no inactivating activity. The other powders inactivated PS-5 to a more or less significant extent depending on the animal species. The addition of Zn^{++} and Mg^{++} seemed to promote inactivation in the porcine type I powder.

Using the detergent-solubilized fractions of kidney acetone powders, the effect of EDTA, a metal-ion chelating agent, was examined with respect to stability of PS-5 (Table 5).

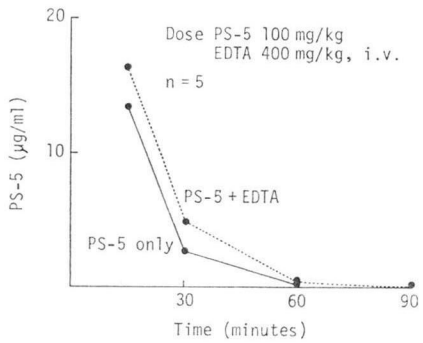
Except for the mouse kidney acetone powder, it is clearly seen that 1 mM of EDTA significantly protects PS-5 from breakdown. The protective effect of EDTA was also observed in fresh kidney homogenates of rat and mouse at a concentration as low as 10^{-2} mM. In addition, when the chelating agent was preincubated with fresh kidney homogenates without PS-5, its beneficial effect became more pronounced (data not shown).

Table 5. Effect of EDTA on stability of PS-5 in the detergent-solubilized fractions of various kidney acetone powders.

Origin	Detergent	Amount of unchanged PS-5	
		no EDTA (%)	1 mM EDTA (%)
Mouse	Triton X-100	0	0
	Tween 80	0	0
	S.D.S.*	0.4	0.3
Porcine type I	Triton X-100	0	25.1
	Tween 80	7.2	37.5
	S.D.S.*	22.0	42.0
Porcine type II	Triton X-100	0	17.6
	Tween 80	0.7	22.1
	S.D.S.*	1.6	42.0
Dog	Triton X-100	0	28.4
	Tween 80	0	39.2
	S.D.S.*	0.8	12.5

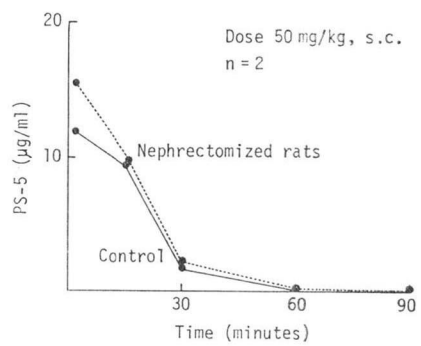
* S.D.S.=sodium dodecyl sulfate.

Fig. 4. Effect of EDTA on the plasma concentration of subcutaneously given PS-5 in mice.



	PS-5 only	PS-5+EDTA
Time 15 minutes	13.3±3.5	16.3±2.0 μg/ml
30	2.7±1.1	4.9±1.5
60	0.1±0.1	0.3±0.3
90	0	0
Urinary recovery (0~6 hours)	0.05%	0.34%

Fig. 5. Influence of nephrectomy on the plasma concentration of PS-5 in rats.



	Nephrectomized	Control
Peak plasma concn.	15.5 μg/ml	11.8 μg/ml
Biological half-life	14.0 minutes	15.5 minutes

In Vivo Effect of Ethylenediaminetetraacetate (EDTA)

Immediately after subcutaneous administration of 100 mg/kg of PS-5, 400 mg/kg of tetrasodium EDTA was intravenously injected to mice. The plasma concentrations of PS-5 measured by antimicrobial activity are presented in Fig. 4.

Although the extent of improvement in the plasma level was less than expected from the *in vitro* findings, the beneficial effect of EDTA was confirmed *in vivo*.

Influence of Nephrectomy

As the primary site of metabolism for PS-5 was found to be the kidney, the influence of nephrectomy on the plasma level of PS-5 was examined in rats and mice. Fig. 5 shows the results in rats. The mean blood level of PS-5 was marginally higher in the nephrectomized rats than in the control ones. Similar results were also obtained in mice.

Discussion

Although the hydrolysis of the β-lactam ring of penicillins and cephalosporins by microbial β-lactamases has extensively been studied in view of drug resistance, there are only few available papers describing vaguely the breakdown of the β-lactam ring in animals. For example, BIRNER isolated penicilloic acid from the urine after oral administration of penicillin V.[14] The differences of penicillin derivatives in the blood level were discussed by ROSENBLATT *et al.* on the basis of the degrees of metabolism and of stability in the liver.[15] KIND *et al.* described the metabolism of benzylpenicillin and ampicillin in the rat liver.[16] However, no direct proof has been presented for the *in vivo* opening of the β-lactam ring of penicillins and cephalosporins by animal enzymes.

Except for the very rare involvement of penicillin acylase, deacylase and amidase,[16] it has generally been accepted that most of chemically-modified β-lactam antibiotics presently in clinical use are stable *in vivo*, providing adequate blood levels and urinary recoveries.

In contrast to the conventional penicillins and cephalosporins, the carbapenem family of β-lactam compounds such as PS-5 and thienamycins are labile *in vivo*, resulting in poor blood levels and urinary recoveries.[6] The experimental findings that PS-5 was inactivated more rapidly in the kidney than in the other organs; that the degree of PS-5-inactivation in kidney homogenates varied markedly among the species of animals; and that the renal PS-5-inactivating agent may be activated by Zn^{++} and inhibited by ethylenediaminetetraacetate seem to suggest that the carbapenem family of β-lactam antibiotics may be inactivated *via* a hitherto unknown metabolic route presumably by action of enzymes for hydrolysis, oxido-reduction, conjugation or sulfation.

The effect of bilateral nephrectomy on the blood level of PS-5 was far less than expected from the *in vitro* experimental results (Fig. 5). Thus it seems possible that other organs such as lung and heart are also involved in the inactivation of PS-5. Very recently Merck scientists have reported the blood level of *N*-formimidoylthienamycin with or without MK0791 in man.[17]

The following paper will describe the isolation of renal dipeptidase as a responsible enzyme for PS-5-inactivation.

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