Kinetic Analysis of Dehydropeptidase-I and Comparative *in Vitro* and *in Vivo* Stabilities of PS-5 Derivatives Modified at the C-3 Side Chain

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With partially purified kidney dehydropeptidase-I (DHP-I) preparations, the hydrolysis kinetics of glycyldehydrophenylalanine (Gly-dPh) by DHP-I were found to be completely non-Michaelian, whereas those of PS-5 and imipenem were composed of at least two-phase reactions which were also observed using *Bacillus cereus* type II β -lactamase. Thus the DHP-I stabilities of 34 PS-5 carbapenem derivatives which were synthesized by chemical modification at the C-3 side chain of PS-5 were examined *in vitro* using fresh mouse, dog and human DHP-I preparations, and are tentatively expressed in reference to the DHP-I stabilities of PS-5. The *in vitro* DHP-I stability of PS-5 was significantly improved by introduction of basic side chains and cysteines at C-3. More particularly, the D-cysteiny side chain was more stable relative to DHP-I than the L-cysteiny. *In vivo*, however, the degree of improvement of the DHP-I-stability by chemical modification at C-3 was not sufficient enough to establish therapeutically effective levels of serum concentrations and urinary recoveries of the carbapenem derivatives after parenteral administration.

Keywords carbapenem antibiotic; PS-5; dehydropeptidase-I; glycyldehydrophenylalanine; Michaelis-Menten equation; enzyme kinetic; *B. cereus* type II β -lactamase

In a previous paper,²⁾ the antimicrobial activities of 34 PS-5 carbapenem derivatives which possessed chemically-modified C-3 side chains were examined, revealing that the D-cysteiny and 4-pyridylthio side chains were the best for the antibacterial activities against gram-negative and gram-positive bacteria, respectively. For these PS-5 carbapenem derivatives to be clinically useful, their *in vitro* and *in vivo* stabilities to dehydropeptidase-I (DHP-I)³⁾ are also essential so that plasma levels may be kept satisfactorily high for a sufficient period of time after administration.

The present paper describes that the time course of hydrolysis of glycyldehydrophenylalanine (Gly-dPh)⁴⁾ by DHP-I did not fit to the Michaelian kinetics; and that the hydrolysis of carbapenems by DHP-I showed at least a two-phase reaction pattern, indicating the conventional kinetic evaluation was inapplicable to DHP-I. *Bacillus cereus* type II β -lactamase was also found to hydrolyze benzylpenicillin, cephalothin, PS-5 and imipenem in two-phase reaction patterns. Accordingly the DHP-I stabilities of the 34 PS-5 carbapenem derivatives and related carbapenem compounds were determined *in vitro* relatively by using reference substrate PS-5 and fresh mouse, dog and human DHP-I preparations; and *in vivo* by pharmacokinetic studies in mice after intravenous or subcutaneous administration.

Materials and Methods

Gly-dPh, PS-5 Carbapenem Derivatives and Related Carbapenem Compounds Gly-dPh, the standard substrate for DHP-I, was prepared according to the procedure of Price.⁴⁾ PS-5,⁵⁾ thienamycin (THM)⁶⁾ and the OA-6129 series of carbapenems⁷⁾ were produced by fermentation. The C-3 side chain-modified PS-5 derivatives were described in a previous paper (for chemical structures see Table I). Imipenem (IPM)⁸⁾ and 88617 (Fig. 7)⁹⁾ were synthesized as reported in previous papers.

Fresh Kidney DHP-I Homogenates Fresh mouse, dog, hog, monkey (crab eating macaque), and human kidneys were homogenized in 5 volumes of buffer (1/100 M phosphate buffer saline (PBS), pH 7.0) with a Teflon Potter homogenizer. Refrigerated centrifugation at 8000 $\times g$ for 20 min yielded the supernatant which was used as crude DHP-I or stored at -20°C .

***In Vitro* DHP-I Stability Tests by DHP-I Homogenates** Because no theoretical base of objective comparison is available (see the text), PS-5 was included as reference carbapenem substrate for relative comparison

of DHP-I stabilities in every run of the tests. Assay mixtures containing 0.1 ml of a test carbapenem solution (100 $\mu\text{g}/\text{ml}$) and 0.1 ml of a DHP-I homogenate were incubated at 37°C for 60 min. Reaction was terminated by heating at 100°C for 15 s. The concentration of the unreacted carbapenem in the assay mixture was bioassayed by the disc-agar diffusion method using *Comamonas terrigena* B-996 as assay organism. In numeric comparison, relative DHP-I stabilities of carbapenem compounds were expressed in relation to the DHP-I stability of PS-5 (1.0). Accordingly a ratio above 1.0 means that the carbapenem concerned is more stable than PS-5 to DHP-I and *vice versa*.

Solubilization of DHP-I from Kidney Acetone Powders The following kidney acetone powders were purchased from Sigma Chemical Co.: dog (K7625), horse (K7875), porcine type II (K7250), rat (K9125) and mouse (K7750). A kidney acetone powder (500 mg) was suspended in 50 ml of 1/20 M Tris-HCl buffer, pH 7.0, containing 20% *n*-BuOH, and stirred vigorously for 60 min at 5°C . After dialysis in the same buffer without *n*-BuOH, the suspension was centrifuged at 10000 $\times g$ for 20 min to give the supernatant or partially purified DHP-I preparation which was used for spectrophotometric enzyme kinetic studies.

Kinetic Analysis of DHP-I Computer-controlled ultraviolet (UV)-visible spectrophotometers (Hitachi double-beam UV-visible spectrophotometer 200-10 and Shimadzu UV-visible recording spectrophotometer UV-2100) equipped with thermostatted cuvette holders were employed. The assay was started by adding partially purified DHP-I to a substrate solution in a 3-ml cuvette; and the time course of absorbance (*A*) change at an assay wavelength was traced at preset time intervals. In this paper, the following differential molar extinction constants (ϵ) were employed: 8120 $\text{M}^{-1}\text{cm}^{-1}$ at 301 nm for PS-5, 14370 $\text{M}^{-1}\text{cm}^{-1}$ at 275 nm for Gly-dPh, 7600 $\text{M}^{-1}\text{cm}^{-1}$ at 300 nm for imipenem and 88617, 636 $\text{M}^{-1}\text{cm}^{-1}$ at 240 nm for benzylpenicillin, and 3840 $\text{M}^{-1}\text{cm}^{-1}$ at 280 nm for cephalothin. Absorbance readings collected were converted to molar substrate concentrations by the final-absorbance method that uses as zero the final absorbance reading at the completion of the assay.¹⁰⁾ The time-molar substrate concentration data were combined by the constant-difference, constant-point or all-combination method for calculation of one set of K_m and $V_{\max}$ by the integrated form of the Lineweaver-Burk plot,¹¹⁾ Scatchard plot,¹¹⁾ Hanes-Woolf plot,¹¹⁾ direct linear plot,¹²⁾ non-linear regression method¹³⁾ or Bannister method.¹⁴⁾

***Bacillus cereus* Type II β -Lactamase** Type II β -lactamase of *B. cereus* 569 (ATCC 27348) was purified by the method of Davis *et al.*¹⁵⁾

Enzyme Kinetic Data 1. Dog DHP-I [Gly-dPh $\epsilon = 14370 \text{ M}^{-1}\text{cm}^{-1}$ at 275 nm, 37°C , 0.1 M MOPS, pH 7.0; *A* 2.6079 (10 s), 2.0194 (110), 1.7794 (210), 1.5722 (310), 1.3757 (410), 1.1712 (510), 0.9563 (610), 0.7415 (710), 0.5340 (810), 0.3475 (910), 0.2108 (1010), 0.1304 (1110), 0.0971 (1210), 0.0862 (1310), 0.0834 (1410); final *A* = 0.0805].

2. Dog DHP-I [PS-5 $\epsilon = 8120 \text{ M}^{-1}\text{cm}^{-1}$ at 301 nm, 37°C , 0.1 M MOPS, pH 7; *A* 0.5998 (10.5 s), 0.4730 (20.17), 0.3731 (30.32), 0.3025 (40.48), 0.2580 (50.15), 0.2276 (60.3), 0.2089 (70.45), 0.1991 (80.13), 0.1928 (90.28),

0.1900 (100.43), 0.1891 (110.1); final $A=0.1887$].

3. Dog DHP-I [IPM $\epsilon=7600\text{ M}^{-1}\text{ cm}^{-1}$ at 300 nm, 37°C, 0.1 M MOPS, pH 7; A 0.9442 (61.02 s), 0.8737 (180.43), 0.7992 (300.32), 0.7361 (420.22), 0.6737 (540.10), 0.5828 (780.38), 0.5579 (900.25), 0.5298 (1020.15), 0.5024 (1140.03), 0.4766 (1260.42), 0.4546 (1380.28), 0.4343 (1500.15), 0.4168 (1620.03), 0.4011 (1740.38), 0.3886 (1860.27), 0.3767 (1980.13), 0.3672 (2100.52), 0.3591 (2220.38), 0.3521 (2340.23), 0.3466 (2460.12), 0.3418 (2580.47), 0.3387 (2700.83), 0.3356 (2821.18), 0.3338 (2940.08); final $A=0.3298$].

4. *B. cereus* type II β -lactamase [benzylpenicillin $\epsilon=636\text{ M}^{-1}\text{ cm}^{-1}$ at 240 nm, 30°C, 0.1 M Tris-HCl, pH 7.0; A 0.8010 (30.08 s), 0.7407 (90.45), 0.6848 (150.32), 0.6319 (210.18), 0.5824 (270.05), 0.5358 (330.42), 0.4916 (390.30), 0.4524 (450.17), 0.4166 (511.00), 0.3840 (570.38), 0.3561 (630.27), 0.3324 (690.13), 0.3138 (750.48), 0.3001 (810.35), 0.2904 (870.23); final $A=0.2768$].

Comparative Time Course Assays of Relative DHP-I Stabilities of Carbapenem Derivatives As the routine application of the Michaelis-Menten equation to the kinetic DHP-I stability assay of carbapenem compounds was disapproved (see the text), the time course of absorbance change was graphically shown for ease of visual comparison of the relative DHP-I stability.

A carbapenem substrate in 0.1 M Tris-HCl, pH 7.0, and a partially purified DHP-I preparation were preincubated at 37°C for 2 min, and then mixed at a ratio of 1:1 in a quartz cuvette of 1 mm lightpath. The time course of absorbance change at an assay wavelength was traced at 37°C at the indicated time intervals in a UV-visible spectrophotometer. For convenience of visual comparison, several time courses of absorbance change were drawn on one sheet of graph paper, referring to the start absorbance readings as 100%.

Plasma Concentrations and Urinary Recoveries of Carbapenem Compounds in Mice A single dose of a carbapenem compound was given subcutaneously or intravenously to a group of 5 male ddY mice (aged 5 weeks, weighing 19–21 g). Blood specimens were collected from the retroorbital sinus at the indicated times with heparinized capillary tubes, and centrifuged at 5000 rpm for 5 min. At 6 h after drug administration, urine samples were recovered from the metabolic cages and bladders. The concentrations of the carbapenem compound in urine and plasma samples were disc-assayed for antimicrobial activity against *Comamonas terrigena* B-996.

Results and Discussion

Optimization of Reaction Conditions for Hydrolysis of PS-5 by DHP-I As previously reported,¹⁶ DHP-I, which is a key enzyme in the renal metabolism of carbapenem compounds, opens the β -lactam ring of carbapenem by the same reaction mechanism as is known for β -lactamase with penicillins and cephalosporins.¹⁷ For quantitative structure-activity relationship studies of carbapenem antibiotics, it is theoretically preferable to use the Michaelian parameters such as K_m , K_i and $V_{\max}$ for objective and precise comparison of their DHP-I stabilities. With this purpose in mind, the DHP-I assay conditions for PS-5 were optimized with respect to assay buffer and animal species. 1/10 M Tris-HCl, TES (*N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonate), MOPS (3-[*N*-morpholino]propanesulfonate) and 1/100 M phosphate, pH 7.0, were compared by spectrophotometry, using PS-5 and partially purified hog DHP-I. Figure 1 shows that phosphate is clearly inferior to Tris-HCl, TES and MOPS. As zinc is the cofactor for DHP-I, it seems reasonable that phosphate deprives DHP-I of zinc, resulting in the decreased DHP-I activity.

DHP-I enzymes were solubilized from acetone powders of dog, hog, mouse, rat and horse kidneys, and were comparatively assayed with PS-5 and IPM. The assay results revealed that the dog kidney acetone powder had the highest DHP-I activity among the tested preparations.

Reexamination of the Valid Applicability of the Michaelis-Menten Equation to the Kinetic Analysis of Hydrolysis of

Fig. 1. Comparative Rates of PS-5 Hydrolysis by Hog DHP-I in PBS, Tris-HCl, TES and MOPS (pH 7.0).

—○—, PBS (1/100 M); —▲—, TES (1/10 M); —●—, Tris-HCl (1/10 M); —△—, MOPS (1/10 M).

Gly-dPh by DHP-I DHP-I was discovered by Campbell *et al.*¹⁸ and has been kinetically studied in details by the differential form of the Lineweaver-Burk plot. There are a variety of parameters of DHP-I's reported in the literature. For example, the K_m value of hog DHP-I for Gly-dPh scatters in a range from 0.6 to 2.0 mM,^{19,20} and that for imipenem from 1.38 to 16.9 mM.^{21,22}

Recently the present authors have devised a spectrophotometric progress curve assay of enzymes such as β -lactamase using a microcomputer.^{12,23} As Gly-dPh is the standard substrate for DHP-I, the progress curve data of hydrolysis of Gly-dPh by hog DHP-I which was reported by Campbell *et al.*²⁴ was subjected to analysis by the integrated form of the Michaelis-Menten equation (Figs. 2C, C'). In addition, Gly-dPh was assayed by the computer-controlled spectrophotometric assay using mouse DHP-I (Fig. 2A) and dog DHP-I (data set 1; Fig. 2B). Figure 2 compares the Lineweaver-Burk plots of the four assays (Figs. 2A, B, C and D), where the kinetic data are combined at a constant difference of 1 by the constant-difference method. Figure 2A (mouse DHP-I; $S_0=0.12\text{ mM}$ Gly-dPh) and Fig. 2B (dog DHP-I; $S_0=1.75\text{ mM}$ Gly-dPh) apparently deny the application of the Michaelian kinetics, whereas Fig. 2C (hog DHP-I; $S_0=0.06\text{ mM}$ Gly-dPh, Campbell *et al.*) and Fig. 2D (dog DHP-I, $S_0=0.88\text{ mM}$ Gly-dPh) seemingly fit to the Michaelis-Menten equation. When the same kinetic data as employed in Figs. 2C and 2D are displayed in the -45° -rotated version of the Eadie-Hofstee space (unpublished work; Figs. 2C' and 2D') (less suitably, in the Scatchard space, too), the application of the Michaelian kinetics to these data sets is also unambiguously disapproved.

It is important to note that the single use of the Lineweaver-Burk plot sometimes results in the wrong conclusion on account of the error-concealing property of the Lineweaver-Burk transformation which is specifically ascribed to undue weighting on data points. As is the case with Figs. 2B and 2D, D', the 2-fold difference in the initial

Fig. 2. Kinetic Analysis of Hydrolysis of Gly-dPh by Mouse, Dog and Hog DHP-I

A) Lineweaver-Burk: Constant difference=1, $K_m = -72.44 \times 10^{-3}$, $V_{\max} = -31.60 \times 10^{-6}$, $r = 96.54 \times 10^{-2}$. B) Lineweaver-Burk: Constant difference=1, $K_m = -22.74 \times 10^{-2}$, $V_{\max} = -14.47 \times 10^{-4}$, $r = 96.58 \times 10^{-2}$. C) Lineweaver-Burk: Constant difference=1, $K_m = 67.66 \times 10^{-3}$, $V_{\max} = 24.36 \times 10^{-6}$, $r = 96.56 \times 10^{-2}$. C') Eadie-Hofstee: Constant difference=1, $K_m = 88.74 \times 10^{-3}$, $V_{\max} = 28.92 \times 10^{-6}$, $r = -70.12 \times 10^{-2}$. D) Lineweaver-Burk: Constant difference=1, $K_m = 13.09 \times 10^{-2}$, $V_{\max} = 18.92 \times 10^{-4}$, $r = 99.69 \times 10^{-2}$. D') Eadie-Hofstee: Constant difference=1, $K_m = 47.63 \times 10^{-2}$, $V_{\max} = 54.60 \times 10^{-4}$, $r = -79.30 \times 10^{-2}$.

substrate concentration of Gly-dPh leads to the exactly opposite conclusion. There will be several plausible reasons for the complete deviation of DHP-I from the Michaelian kinetics. One reason which seems probable based on the concave progress line in Fig. 2B is that dehydrophenylalanine, one of the reaction products, is spontaneously converted to the secondary product, accompanied by concomitant inactivation of DHP-I.

Hydrolysis Curves of PS-5 and Imipenem by DHP-I Subsequently PS-5 (data set 3; $S_0 = 0.051$ mM) and IPM (data set 4; $S_0 = 0.081$ mM) were subjected to the spectrophotometric dog DHP-I assay, followed by the progress curve analysis. As is seen with Gly-dPh, the lineweaver-Burk plots (Figs. 3A and 3B) seem to show the valid applicability of the Michaelian kinetics to analysis, whereas the Eadie-Hofstee plots (Figs. 3A' and 3B') clearly reveal the invalidity.

Hydrolysis Curves of Benzylpenicillin and PS-5 by *B. cereus* Type II β -Lactamase *B. cereus* type II β -lactamase also requires zinc as the cofactor for enzyme activity, and

hydrolyzes carbapenem by the same reaction mechanism as DHP-I. Comparative studies on this β -lactamase and DHP-I's are expected to shed light on the reaction specificity of DHP-I. Figure 4 compares the hydrolysis curves of benzylpenicillin ($S_0 = 0.82$ mM; data set 5; Figs. 4A and 4A') and PS-5 ($S_0 = 0.07$ mM in 0.1 M MOPS, pH 7.0; Figs. 4B and 4B') by the type II β -lactamase using the integrated forms of the Lineweaver-Burk and Eadie-Hofstee spaces. This β -lactamase was also concluded not to conform to the Michaelian kinetics.

There is no description available in the literature which indicates the possibility that *B. cereus* type II β -lactamase might not fit the Michaelis-Menten equation in kinetic analysis of hydrolysis of benzylpenicillin, cephalothin and other β -lactam compounds.

Insusceptibility of Gly-dPh to *B. cereus* Type II β -Lactamase Although the type II β -lactamase and DHP-I share several common properties, the finding that Gly-dPh is not susceptible to the β -lactamase suggests some critical difference in the detailed reaction mechanism between these

Fig. 3. Hydrolysis of PS-5 (A, A') and Imipenem (B, B') by Dog DHP-I

A) Lineweaver-Burk: Constant difference=1, $K_m=11.91 \times 10^{-3}$, $V_{\max}=90.33 \times 10^{-5}$, $r=98.53 \times 10^{-2}$. A') Eadie-Hofstee: Constant difference=1, $K_m=21.28 \times 10^{-3}$, $V_{\max}=12.57 \times 10^{-4}$, $r=-91.65 \times 10^{-2}$. B) Lineweaver-Burk: Constant difference=1, $K_m=12.51 \times 10^{-2}$, $V_{\max}=40.90 \times 10^{-5}$, $r=98.81 \times 10^{-2}$. B') Eadie-Hofstee: Constant difference=1, $K_m=53.18 \times 10^{-2}$, $V_{\max}=12.09 \times 10^{-4}$, $r=-67.05 \times 10^{-2}$.

Fig. 4. Hydrolysis Curves of Benzylpenicillin (A, A') and PS-5 (B, B') by *B. cereus* Type II β -Lactamase

Lineaweaver-Burk: Constant difference=1, $K_m=15.61 \times 10^{-2}$, $V_{\max}=16.10 \times 10^{-4}$, $r=99.70 \times 10^{-2}$. A') Eadie-Hofstee: Constant difference=1, $K_m=19.36 \times 10^{-2}$, $V_{\max}=17.97 \times 10^{-4}$, $r=-97.26 \times 10^{-2}$. B) Lineaweaver-Burk: Constant difference=1, $K_m=38.00 \times 10^{-4}$, $V_{\max}=20.91 \times 10^{-5}$, $r=99.07 \times 10^{-2}$. B') Eadie-Hofstee: Constant difference=1, $K_m=61.92 \times 10^{-4}$, $V_{\max}=26.18 \times 10^{-5}$, $r=-94.34 \times 10^{-2}$.

enzymes.

Precise Analysis of the Reaction Progress Curves of PS-5 and Benzylpenicillin by DHP-I and *B. cereus* Type II β -Lactamase It seemed thus useful to comparatively analyze the reaction patterns of PS-5 and benzylpenicillin by these

two types of enzymes in detail so that some reason for unapplicability of the Michaelian kinetics to the enzymes might hopefully be found. Figure 5 shows the results of pattern analysis of the four representative cases. The time courses of hydrolysis of PS-5 ($S_0=0.12$ and 0.74 mm in

Fig. 5. Precise Analysis of the Reaction Progress Curves of DHP-I and *B. cereus* Type II β -Lactamase (-45° -Roated Version of the Eadie-Hofstee Plot)

A, PS-5: hog DHP-I; B, PS-5: dog DHP-I; C, PS-5: β -lactamase; D, PCG: β -lactamase. 5 Stages of a progress curve: Substrate inhibition (SI), initial Michaelian phase (IM), transition (TR), secondary Michaelian phase (SM) and unsaturated enzyme (UE).

Tris-HCl, pH 7.0, at 37°C) by hog and dog DHP-I's are shown in Figs. 5A and 5B, and those of PS-5 ($S_0 = 0.067\text{ mM}$ in 0.1 M TES, pH 7.0, containing 1 mM ZnSO_4 , at 30°C) and benzylpenicillin ($S_0 = 0.82\text{ mM}$ in 0.1 M Tris-HCl, pH 7.0, at 30°C) by the type II β -lactamase in Figs. 5C and 5D, by the -45° -rotated version of the Eadie-Hofstee plot using the constant-difference method (at a constant difference of 1). In general, each progress curve is divided into 5 stages of substrate inhibition (SI), initial Michaelian phase (IM), transition (TR), secondary Michaelian phase (SM) and unsaturated enzyme (UE). Of course, not all the five stages are always observed in every progress curve, depending on the assay conditions. It is possible that the transition stage be also regarded to be Michaelian, but the present authors prefer to think it non-Michaelian, based on the observation that some assays showed sudden fluctuations in absorbance change specifically only at this stage.

There remains much to be studied about the reaction patterns of DHP-I and *B. cereus* type II β -lactamase, particularly with reference to reaction conditions and the function of the zinc cofactor, because both enzymes are crucially important in metabolism and resistance expression of β -lactam compounds including carbapenems.

Relative Stabilities of Carbapenem Compounds to DHP-I's
Under the circumstances described above, the Michaelian analysis is meaningless to express the DHP-I stability of a carbapenem compound in an objective term such as K_m and K_i . For convenience of relative comparison in number, the DHP-I stability of a carbapenem derivative is expressed tentatively in relation to that of PS-5 as 1.0. The results of assays by mouse, dog and human fresh DHP-I homogenates are contained in Table I. The PS-5 derivatives were substantially inferior to THM and IPM in stability to mouse DHP-I; but the advantage of THM and IPM over PS-5 disappeared in stability to human DHP-I. Among the tested C-3 side chains, aminoethylthio (derivative **5**; NS-5) and 6-aminopenicillanyl (derivative **25**) were comparable to

Fig. 6. Comparative Stabilities of PS-5 ($\circ$), OA-6129A ($\blacktriangle$), OA-6129B₂ ($\triangle$) and OA-6129C ($\bullet$) to Dog DHP-I in $1/100\text{ M}$ PBS, pH 7.0

OA-6129 carbapenems			
X	C ₅ -C ₆	C ₆	compound
H	trans	R	OA-6129A
OH	trans	S	OA-6129B ₂
OSO ₃ H	cis	R	OA-6129C

THM and IPM in *in vitro* stability to human DHP-I. D-Cysteiny side chain (**11**) was more stable to DHP-I than the L-cysteiny side chain (**10**). As explained above, however, it is improbable that the *in vitro* DHP-I stability data of carbapenem derivatives can be extrapolated to predict their *in vivo* stabilities and clinical efficacy. Derivatives **11** and

TABLE I. Structures and Relative Stabilities of 35 *S*-Substituted Carbapenem Derivatives to Mouse, Dog, and Man DHP-I's Homogenates

<div> </div> 1—9					<div> </div> 10—19, 27—35					<div> </div> 20—26				
No.	R	Mouse	Dog	Man	No.	R	Mouse	Dog	Man					
1	-NHCOCH ₃ (=PS-5)	1.0	1.0	1.0	10	<div><div>(R)</div><div>-CH₂-CH-COOH</div><div>NH₂</div></div>	4.5	1.4	1.7					
2	-CH ₂ -CH ₃	—	—	—	11	<div><div>(S)</div><div>-CH₂-CH-COOH</div><div>NH₂</div></div>	7.7	1.7	2.3					
3	-OH	0.0	—	—	12	<div><div>(S)</div><div>-CH₂-CH-COOH</div><div>NH₂</div></div>	2.7	2.5	1.8					
4	-COOH	0.7	0.8	0.4	13	<div><div>(S)</div><div>-CH₂-CH-CONH₂</div><div>NH₂</div></div>	4.1	1.0	1.4					
5	-NH ₂	13.4	5.3	4.5	14	<div><div>(S)</div><div>-CH₂-CH-CONH-</div><div>NH₂</div><div> </div></div>	0.8	0.3	0.7					
6	-N(CH ₃) ₂	0.0	—	—	15	<div><div>(S)</div><div>-CH₂-CH-COOH</div><div>N(CH₃)₂</div></div>	2.7	2.3	2.7					
7	-NHCO-CH ₂ -	1.7	—	—	16	<div><div>(S)</div><div>-CH₂-CH-COOH</div><div>NHCOCH₃</div></div>	0.0	0.0	1.6					
8	-NHPO(OH) ₂	13.8	5.3	2.3	17	<div><div>(S)</div><div>-CH₂-CH-COOH</div><div>NHCO-</div><div> </div></div>	0.0	0.0	1.1					
9		0.6	—	—	18	<div><div>(S)</div><div>-CH₂-CH-CONH-</div><div>NH₂</div><div> </div></div>	2.4	3.4	2.2					
88617		25.9	4.6	4.2	19	<div><div>(S)</div><div>-C(CH₃)-CH-COOH</div><div>NH₂</div></div>	5.6	3.6	2.9					
THM		24.8	2.6	4.1	27		2.1	—	—					
IPM		44.0	5.5	4.8	28		1.1	—	—					
20		1.3	—	—	29		21.2	—	0.8					
21		0.0	—	—	30		21.6	—	1.3					
22		—	—	—	31		0.8	—	—					
23	<div><div>C(CH₃)₂</div><div>-CONH-C-COOH</div></div>	0.0	0.8	1.3	32		0.8	—	2.5					
24	<div><div> </div><div>-CONH-</div></div>	0.4	0.5	2.6	33		—	—	—					
25	<div><div> </div><div>-CONH-</div></div>	37.0	—	4.9	34		1.1	—	—					
26	<div><div> </div><div>-CONH-</div></div>	1.0	2.0	2.0	35		4.4	—	—					

—: Not tested.

Fig. 7. Comparative Stabilities of 88617 (—○—) and Imipenem (—●—) to the Partially Purified Dog DHP-I Preparation in 1/10 M MOPS, pH 7.0

30 which seemed promising from the viewpoint of antimicrobial activity²⁾ were clearly concluded to be unsatisfactorily stable to DHP-I.

Figure 6 illustrates the effects of the C-6 side chains on DHP-I stability, revealing that, when the C-3 side chain was limited to pantetheinyl, sulfonylethyl (OA-6129C) was the most stable to DHP-I, and ethyl (OA-6129A) the least. Contrary to the authors' expectation, hydroxyethyl (OA-6129B₂) was significantly better than ethyl (OA-6129A). The importance of the C-6 side chain on the DHP-I stability is also demonstrated in Fig. 7, where the C-6 fluoroethyl group (carbapenem 88617) is compared with the C-6 hydroxyethyl group (IPM) by dog DHP-I, indicating that the stereospecific introduction of fluorine at C-8 was very effective to stabilize carbapenem to DHP-I, although they differed slightly in the C-3 side chain.

Plasma Levels and Urinary Recoveries of Carbapenem Derivatives in Mice The pharmacokinetic parameters and urinary recoveries of carbapenem derivatives which were determined in mice after subcutaneous or intravenous administration are summarized in Table II. Some of the C-3 side chain-modified PS-5 derivatives were not tested on account of no sample supply. Mice have very strong DHP-I activity and seem to significantly differ from human in the activity and substrate profile of the enzyme. Thus it seems difficult to reach by conjecture the therapeutic effects of carbapenem derivatives only from the results in Table II, but, in general, the PS-5 derivatives had shorter half lives than IPM after intravenous administration. IPM combined with cilastatin, a DHP-I inhibitor, is the sole carbapenem drug available on the market. It is probable, however, that carbapenem derivatives which are clinically useful must have better DHP-I stabilities and urinary recoveries than THM and IPM. The *in vivo* results in Table II, and the

TABLE II. Plasma Levels and Urinary Recoveries of PS-5 and Related Derivatives after Single s.c. or i.v. Administration in Mice (*n*=5)

No.	Dose (mg/kg)	Route: s.c.		Route: i.v.		
		<i>C</i> _{max} (μg/ml)	U.R. (%)	<i>C</i> _{max} (μg/ml)	<i>T</i> _{1/2} (min)	U.R. (%)
1 (=PS-5)	50	18.7	0.07			
	100	40.2	0.10	70.9	1.3	0.09
	200	56.6	0.10	149	1.5	0
3	200	73.8	0.04			
5	100	23.2	0	101.2	1.5	0.48
6	200	72.4	0.63			
7	100	2.8	0			
8	100	41.6	1.65	108	1.5	6.0
9	100	16.8	0.04			
11	100	21.6	0.6	62.4	1.0	3.6
12	100			263	1.5	1.25
13	100			56.9	1.0	0
14	100			90.8	1.2	0.11
15	100			45.9	1.2	0.2
16	200			69.4	1.0	0.13
17	200			282	1.8	0.2
21	100	42.0	0.02			
25	100			84.2	4.0	6.8
27	100	8.2	0.09			
28	100	11.3	0.2			
29	100	48.4	0.06	51.4	7.7	0.49
30	100	70.7	1.6			
32	100	23.6	0.2			
33	100	28.2	1.45			
34	100	0	0			
35	100	32.3	1.2			
THM	50	36.8	5.8			
IPM	20	19.6	N.T.	31.7	7.0	N.T.

*C*_{max}: maximum plasma level; *T*_{1/2}: plasma half life; U.R.: urinary recovery N.T.: not tested.

antibacterial evaluation²⁾ yield a conclusion that the 34 PS-5 derivatives having modified C-3 side chains are not clinically useful.

References and Notes

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