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Metabolism of Thienamycin and Related Carbapenem Antibiotics by the Renal Dipeptidase, Dehydropeptidase-I

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Thienamycin (THM), the *N*-formimidoyl thienamycin derivative MK0787, and related carbapenem antibiotics were metabolized extensively in mice, rats, rabbits, dogs, rhesus monkeys, and chimpanzees. Urinary recovery of THM ranged from a low of 5% in dogs to 58% in rhesus monkeys. Renal clearance rates in dogs and chimpanzees were unusually low, less than glomerular filtration rates. The reduction in clearance of THM and MK0787 from plasma of rats and rabbits after ligation of renal arteries indicate that the kidneys are responsible for 35 and 92%, respectively, of metabolic drug clearance. Degradation was detected only in kidney homogenates. The enzyme activity was membrane bound and sensitive to inhibitors of Zn-metalloenzymes such as EDTA. A renal dipeptidase, dehydropeptidase-I (DHP-I), EC 3.4.13.11, was found to be responsible for the metabolism of the THM-class antibiotics, which exhibit a structural homology to dehydropeptides. A parallel increase in specific activity against THM and the substrate of DHP-I, glycyldehydrophenylalanine, was observed during solubilization and purification of the enzyme from porcine and human renal cortex. DHP-I was found to catalyze the hydrolysis of the beta-lactam ring in THM and MK0787. The products of the enzyme reaction were identical by high-powered liquid chromatography to their respective metabolites found in the urine. Nonbasic *N*-acylated THM and natural *N*-acylated carbapenems (epithienamycins and olivanic acids) were degraded 4- to 50-fold faster than THM when exposed to the enzymatic hydrolysis of DHP-I. Good correlations were obtained between the increased susceptibility of the carbapenem antibiotics to DHP-I as measured in the in vitro enzyme assay and the generally lower recoveries of active antibiotic in the urine of test animals. Despite this unusual degree of metabolism localized in the kidney, the plasma half-life of MK0787 and its efficacy against experimental systemic infections in animals remain satisfactory.

Thienamycin (THM) and the *N*-formimidoyl thienamycin derivative MK0787 are members of a new class of structurally novel beta-lactam antibiotics, the carbapenems. Both are known for their high orders of activity against a broad spectrum of bacteria (11, 18, 19). Their breadth of activity is in part attributable to resistance to attack by bacterial beta-lactamases (17).

The low recovery of unaltered THM and MK0787 in the urine of laboratory animals suggested that these antibiotics are extensively metabolized. Incomplete urinary recoveries of cephalosporins such as cephalothin and cefotaxime have been ascribed to enzymatic (hepatic) cleavage of their acetylated side chains. In both instances, desacetyl metabolites with reduced antimicrobial activity and an intact beta-lactam are recovered in the urine of laboratory animals and in humans (6, 7, 9, 20).

Biochemical studies presented in this report show that low urinary recoveries of THM and MK0787 result from the hydrolysis of the beta-lactam ring by the renal dipeptidase, dehydropeptidase-I (DHP-I), EC 3.4.13.11. A structural similarity exists between this new class of beta-lactam antibiotics and dehydropeptides as illustrated by the assay substrate, glycyldehydrophenylalanine (Gly-dh-Phe), shown in Fig. 1. This peculiar likeness of structures may be the principle basis for the unexpected enzymatic attack on this new class of antibiotics.

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MATERIALS AND METHODS

Antibiotics. The concentration of THM, MK0787, and other carbapenems, in prepared solutions was measured by a differential spectrophotometric assay procedure (10), adopting $E^{1\%}_{297} = 274$ for THM, $E^{1\%}_{299} = 305$ for MK0787, and values of 274 to 305 for the other carbapenems at their respective spectral maxima. Solutions of THM or MK0787 were prepared in either 10

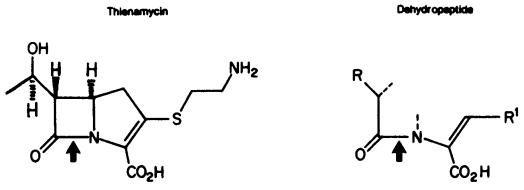


FIG. 1. Structural homology postulated between THM and dehydropeptide substrates of DHP-I. The site of hydrolysis is indicated by an arrow. For Gly-dh-Phe, R = NH_2, R^1 = Phenyl.

mM MOPS [3-(*N*-morpholino)propanesulfonic acid; pH 7.1; Calbiochem] or in 10 mM sodium phosphate buffer (pH 7.1). To avoid the instability of THM in concentrated solutions, concentrations were limited to 2.0 mg/ml. Epithienamycins, provided by P. Cassidy (5) of our laboratories, were evaluated under the same conditions described for THM, as was PS-5 (15), which was kindly provided by Sanraku-Ocean Co. Ltd, Fujisawa, Japan. PS-5 was 95% pure.

^{14}C-labeled MK0787, used for high-powered liquid chromatographic (HPLC) analysis of metabolites, was labeled in the formate carbon of the formimidoyl side chain of MK0787 (prepared by H. Mertel of our laboratories). ^{3}H-labeled MK0787 was prepared from ^{3}H-labeled THM, randomly labeled by incorporation of [^{3}H]acetate during fermentation of the producing organism *Streptomyces cattleya* (10).

Tissue homogenates. Fresh kidney cortex and liver tissues were collected from mice, rats, and pigs. A fresh human liver specimen and a frozen human kidney were received from the organ transplant department of a local hospital. All tissues were freed of extraneous fat and homogenized with the use of a Potter-Elvehjem tissue grinder in an equal volume of 75 mM phosphate buffer (pH 7.1) or 50 mM MOPS buffer (pH 7.1). The homogenates were centrifuged at 1,200 × *g*, and the supernatant and loosely packed debris were saved for drug degradation studies. Reaction mixtures contained THM, 1.0 to 100 μg/ml, in 75 mM phosphate buffer (pH 7.5) and tissue homogenate supernatant, with a final protein concentration of 5.0 mg/ml.

Tissue homogenate assay. After a 30-min incubation period at 37°C, the reaction mixture was deproteinized by centrifugal ultrafiltration (Amicon Corp., Centriflo membrane cones, 224-CF-50), and bioassays were conducted by the disk-diffusion method (tester strain, *Bacillus subtilis* ATCC 12432) or by direct measurement of UV extinction at 297 nm after addition of hydroxylamine to the residual THM concentration in the filtrate of the reaction mixture.

Renal dipeptidase assays. The substrate for DHP-I, Gly-dh-Phe, was synthesized by E. Walton of our laboratories by methods previously described (4). Solubilized peptidase fractions were assayed by measuring the decline in absorbance at 275 nm for Gly-dh-Phe at a substrate concentration of 50 μM and the decline at 297 to 299 nm for THM and MK0787, respectively, at concentrations of 100 μM. Upon incubation at 37°C, the rate of reaction was obtained from the slope of log absorbance versus time. Units of enzyme activity are expressed as micromoles per minute for Gly-dh-Phe. Protein concentrations were determined by the method of Lowry et al. (12).

The DHP-I-catalyzed hydrolysis of the beta-lactam in the THM was determined by measuring the loss in specific UV absorbance at 297 nm and by the simultaneous production of a titratable acid function, which was measured by the addition of alkali to restore the pH to 7.0. The initial reaction mixture consisted of 7.1 μmol of THM and 3.3 U of DHP-I in 2.0 ml of 10 mM MOPS buffer (pH 7.0) at 37°C.

The relative susceptibility to DHP-I of THM derivatives and naturally occurring carbapenem antibiotics was measured in reaction mixtures containing 100 μM antibiotic in 50 mM MOPS buffer and 0.23 (Gly-dh-Phe) U of DHP-I, sufficient to reduce the absorbance of THM by 2%/min. Reaction rates for other carbapenems are normalized to the rate observed with THM.

Partial purification of DHP-I. Enzyme activity was partially purified from swine kidneys to the 50 to 75% ammonium sulfate fraction described by Campbell (3). This method was modified only by substitution of Tris-hydrochloride buffer with MOPS buffer and the improved procedure by Armstrong et al. (1) for using an Amicon TCF-10 ultrafiltration apparatus equipped with an XM-50 membrane to concentrate dilute protein solutions.

Further purification of DHP-I, using immobilized enzyme inhibitor. Purification of DHP-I to apparent homogeneity as judged by gel electrophoresis and by using system no. 1 as described by Maurer [13]) was achieved by use of columns containing immobilized DHP-I inhibitors linked to Sepharose CL-6B resin (Pharmacia Fine Chemicals). The action of DHP-I inhibitors was described by Kropp et al. (Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 20th, New Orleans, La., 1980 abstr. no. 270). A gently stirred suspension of Sepharose CL-6B (25 ml of packed resin) in 150 ml of 2.0 M potassium phosphate (pH 12.0) was activated with 25 g of cyanogen bromide (added as a stock solution of 1.0 g of cyanogen bromide per ml of DMF) for 3 min in an ice bath followed by washing of the resin with 1.0 liter of distilled water. The activated resin was then immediately added to the coupling solution consisting of 100 ml of a selective inhibitor of DHP-I (5.0 mg/ml), the *Z*-*S*-[6-carboxy-6-([(2,2-dimethyl-(*S*)-cyclopropyl)carboxyl]-amino)5-hexenyl]-L-cysteine MK0791 (described by D. Graham et al., 20th ICAAC, abstr. no. 271), in 0.2 M sodium bicarbonate buffer (pH 10.0) and gently stirred for 30 min at room temperature. Excess active groups were blocked by the addition of 10 ml of a 1.0 M ethanolamine-hydrochloride solution (pH 8.0), with continuous stirring for 1 h. The resin was then washed as before and stored in 0.5 M MOPS buffer stock (pH 7.3) at 4°C.

Dialyzed DHP-I enzyme extract, partially purified up to and including the *n*-butanol step by the method of Campbell (3), was adjusted (with 0.5 M MOPS and dry NaCl) to a final 0.05 M MOPS–0.1 M NaCl concentration. The extract was passed over a small column (5% of extract volume) of carboxymethyl-Sepharose to remove strongly basic proteins. Enzyme was absorbed on further passage through a column (2% of extract volume) of immobilized inhibitor. The resin was washed with 10-column volumes of 0.5 M NaCl in MOPS buffer. Upon elution with 0.5 M

sodium cyanide in MOPS, DHP-I was found in the first column volume. Cyanide was removed from enzyme-rich fractions by dialysis at 4°C against 200 volumes of 50 mM MOPS (two changes), 5 mM MOPS (two changes), and against 5 mM ammonium acetate buffer before lyophilization.

Disposition studies in laboratory animals. Recovery of THM and MK0787 in urine was determined in the following laboratory animals: Mice (CD/1 Swiss albino, females, body weight, 20 g, $n = 50$ for each agent); rats (CDF, females, body weight, 150 g, $n = 20$ for each agent); rabbits (New Zealand white males, body weight, 3.0 to 3.5 kg, $n = 10$); dogs (beagles, males, body weight, 8 to 10 kg, $n = 3$ for each agent) and rhesus monkeys (*Macaca mulatta*, males, body weight, 5.0 to 8.0 kg, $n = 3$ for each agent). Antibiotic was administered in all studies as an intravenous bolus containing 5.0 mg/kg of body weight. Antibiotic activity was measured by the bioassay method of urine collected at 1-hour intervals over a 3-h period.

The pharmacokinetic parameters of THM and MK0787 were defined in a dog and a chimpanzee. At 40 min before drug administration, a male dog (body weight, 14.0 kg) received a 2.0-g bolus dose of creatinine subcutaneously. A constant infusion of dextrose, 5% in 0.9% saline, was administered at a rate of ~2.0 ml min^{-1} to promote urine flow for the duration of the 3-h study. The chimpanzee, a male weighing 60 kg, received, 2 h before drug administration, a loading dose of inulin (50 mg/kg) and a constant intravenous infusion of 5% mannitol in 0.45% saline supplemented with 22 mg of inulin per ml and maintained at 3.0 ml min^{-1} from −1.5 to +3 h. Antibiotic was administered as an intravenous bolus containing 5.0 mg/kg of body weight. Each subject was catheterized for urine recovery at successive 20-min intervals. Renal clearance rates of the antibiotics were calculated from the equation: renal clearance (milliliters per minute per kilogram) = urinary recovery (micrograms per kilogram of body weight)/area under plasma curve (micrograms per ml × minute). The antibiotic activity in plasma and urine specimens was determined by the bioassay method.

The effects of bilateral ligation of renal arteries on antibiotic plasma clearance rates were explored in two rats and two rabbits. Both test and control animals were laparotomized and held under ether (rats) or methoxyflurane (rabbits) anesthesia for the duration of the 3- to 4-h study. Drug (4.0 mg/kg) was administered subcutaneously to the rats (CDF males, body weight, 200 g) and as an intravenous bolus dose (5.0 mg/kg) to the rabbits (New Zealand white males, body weight, 3.0 to 3.5 kg).

Pharmacokinetic analysis. Parameters (see Table 2) for the dog and chimpanzee were derived by fitting plasma levels to a two-compartment open model, using the nonlinear regression program (COMPT) developed by Pfeffer (16).

Bioassay for plasma and urinary recovery measurements. Measurements of antibiotic, THM, MK0787, and epithienamycins in plasma and urine were conducted on the same day that the specimens were collected by the following disk-diffusion bioassay, using *Bacillus subtilis* ML-32 (ATCC 12437) as the test organism. Plates were prepared by inoculation with 2.0 ml of 7.8×10^7 colony-forming units of MB-32 spores per ml to 100 ml of autoclaved brain heart infusion or Mueller-Hinton medium supplemented with 1.5% agar and cooled to 60°C. Ten milliliters of inoculated medium was then transferred into a petri dish (100 by 15 mm) on a level surface, distributed evenly, and allowed to harden. Plates were refrigerated until used (up to 24 h after preparation). Measured samples 20 μl of standard antibiotic solutions diluted in 50 mM MOPS buffer (pH 7.1) at concentrations of 0.25, 0.5, 1.0, and 2.0 μg/ml, were deposited onto paper disks (6.3-mm diameter). These standard disks were then placed on seeded agar plates (in triplicates) adjacent to disks bearing test samples appropriately diluted in 50 mM MOPS. The plates were incubated at 35°C overnight, and the zone diameters were measured and averaged for preparation of a standard curve; potencies of the test samples were calculated from the standard curve by conventional methods.

Elution profiles of products from labeled MK0787 digested with DHP-I or by controlled acid hydrolysis were determined by HPLC (see Fig. 4A).

Sample preparation and HPLC conditions. (i) Enzyme digest. ^{3}H-labeled MK0787 was prepared from ^{3}H-labeled THM, which was randomly labeled by incorporation of [^{3}H]acetate during fermentation. Antibiotic (1.2 μmol) was exposed to 4 μg of purified hog renal DHP-I (0.16 IU [versus Gly-dh-Phe]) in 0.1 ml for 15 min at 37°C.

(ii) Acid hydrolyzate. *N*-^{14}C-labeled MK0787, (16 mM) was reacted in 0.1 N H_2SO_4 at 20°C for 10 min. Products were absorbed on Dowex-50×2, H+ and eluted by suspension of resin in water and titration with NaOH to pH 7.3.

(iii) HPLC. Ten microliters of a mixture of the above digests was injected on a Waters Bondapak C_{18} column. The solvent was tetrabutylammonium-PO_4 (5 mM), pH 7.1, pumped at 1.5 ml/min. Fractions of ~0.5 ml (8 drops) were collected and counted.

An HPLC elution profile of labeled components in urine from a rabbit which received ^{14}C-labeled MK0787 was determined. The urine sample was subsequently spiked with DHP-I digest of ^{3}H-labeled MK0787 (see Fig. 4B).

(iv) Urine. Pooled urine samples were collected over a 30-min period after rabbits were administered ^{14}C-labeled MK0787 intravenously (5 mg/kg).

(v) HPLC conditions. Ten microliters of urine spiked with enzyme digest was injected on a Waters Bondapak C_{18} column. The solvent was 0.1 M $(NH_4)H_2PO_4$, pumped at 1.5 ml/min. The component was tracked by using absorbance values at 313 nm and identified as MK0787, a mixture of two rotamers undergoing slow interconversion at pH 4.2.

RESULTS

Evidence for metabolism of THM. THM and its *N*-formimidoyl derivative, MK0787, underwent substantial species-variable metabolism as measured by the low urinary recovery of antibiotic (Table 1). Pharmacokinetic parameters for THM and MK0787 in the dog and the chimpanzee are shown in Table 2 and Fig. 2. Antibiotic renal clearance rates were unusually low and represented only a fraction of the glomerular filtration rate measured using creatine or inulin (Table 2). The relative contribution of renal and extrarenal

TABLE 1. Urinary recovery of THM and MK0787 in laboratory animals

Species	No.	Antibiotic	Range of urinary recovery, 0 to 3 h (% of dose)
Mice	50	MK0787	26–40
	50	THM	20–28
Rats	20	MK0787	20–25
	20	THM	17–23
Rabbits	10	MK0787	37–51
Dogs	3	MK0787	8–32
	3	THM	5–13
Rhesus monkeys	3	MK0787	41–57
	3	THM	28–58

processes to the clearance of antibiotic from the blood was estimated from the effect on plasma clearance rates of ligating the renal arteries in rats and rabbits (Fig. 3). In rats, plasma clearance rates were reduced from 11.7 in a sham-operated control to 6.1 (ml/min per kg) in the ligated subject. Thus, the kidney is responsible for clearing one-half of the administered dose, yet urinary recoveries are only 20% in this species. More strikingly, in rabbits the plasma clearance rate was reduced from 33 in controls to 1.44 after ligation, indicating that the kidney clears 95% of the antibiotic from the circulation; yet only 37 to 50% of the administered dose was recovered in the urine. Stated alternatively, in rats, 35% of total antibiotic loss due to metabolism occured in the kidney. In rabbits, this portion amounted to 92%. The remainder was extrarenal metabolism measurable directly as the plasma clearance rate in animals with ligated renal arteries. Local renal metabolism of antibiotic during the course of excretion must be postulated to account for the above discrepancies.

Isolation of THM degrading enzyme from the kidney. Kidney and liver homogenates of several species (including humans) were examined for their ability to degrade THM (Table 3). Activity was detected only in the kidney extracts, confirming the conclusions reached from the renal ligation experiment described above. The loss of antibiotic activity was accompanied by a parallel decline in specific absorbance of THM measured at 297 nm. Degradation was abolished by prior heat inactivation of the tissue homogenate or by treatment with EDTA. Activity of the kidney homogenates was retained after extensive dialysis, and activity showed no requirement for O_2, suggesting a hydrolytic process. Since the activity of EDTA-inactivated preparations was restored by Zn^{2+} (but not by Mg^{2+} or by Co^{2+}), involvement of a Zn-metallopeptidase appeared likely. The activity in kidney homogenates was associated with the particulate fraction sedimentable at 30,000 × g. The following soluble Zn-metalloenzymes: carboxypeptidase-A, carboxypeptidase-B, leucine-aminopeptidase, and acylase-I, were found not to degrade the antibiotic. The need for solubilization, the requirement for Zn^{2+}, and a structural homology between THM and dehydropeptides (e.g., Gly-dh-Phe) suggested that DHP-I, the enzyme described earlier by Greenstein (8), might be responsible for a hydrolytic inactivation of THM, and attempts were made to purify the enzyme responsible for THM degradation.

When DHP-I was partially purified (162-fold, based on Gly-dh-Phe substrate activity) from swine kidney, its specific activity was proportionately increased for THM (Table 4). A further 32-fold purification was accomplished by using a column of DHP-I inhibitor immobilized on Seph-

TABLE 2. Pharmacokinetic parameters of THM and MK0787 in a dog and a chimpanzee after a 5.0-mg/kg intravenous bolus dose

Parameter	Dog		Chimpanzee	
	THM	MK0787	THM	MK0787
Plasma half-life (β, h)[a]	0.28	0.51	0.6	0.69
AUC (μg/ml × h)[b]	9.69	13.38	9.95	18.96
Urinary recovery (% of dose; 0 to 3 h)	4.80	7.80	12.30	13.70
Plasma clearance rate (ml/min per kg)	8.60	6.23	8.38	4.39
Renal clearance rate (ml/min per kg)	0.41	0.49	1.03	0.60
Glomerular filtration rate (GFR) (ml/min per kg)				
$GFR_{creatinine}$ (mean ± SD)	4.9 ± 0.8			
GFR_{inulin} (mean ± SD)			2.14 ± 0.07	

[a] Terminal elimination (β) phase of antibiotic disposition.
[b] AUC, Area under the curve.

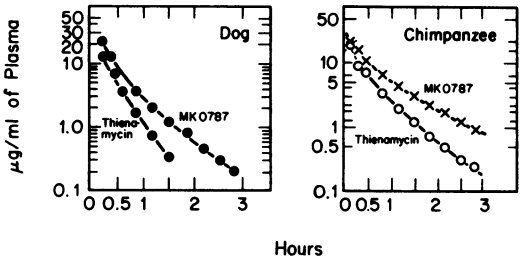


FIG. 2. Plasma concentrations of MK0787 and THM in a dog and a chimpanzee.

arose, resulting in enzyme protein which was judged to be homogeneous by gel electrophoresis. DHP-I purified by the inhibitor column procedure was used in all subsequent experiments described in this report. The following kinetic parameters were determined with purified enzyme for the two substrates THM(K_m = 5.7 mM; V_{max} = 99 μmol/min per mg) and Gly-dh-Phe(K_m = 0.6 mM, V_{max} = 900 μmol/min per mg).

Evidence that DHP-I catalyzes hydrolysis of the beta-lactam in THM. During the time course of inactivation of THM by DHP-I, UV absorbance was lost and a titratable acid function was formed in equimolar proportions (Table 5). After the complete reaction of THM, no UV absorbance peaks were found in reaction mixtures other than end absorbtion at 230 nm. The spectrum resembled that found when the lactam of THM is reacted to completion with 10 mM hydroxylamine. This finding strongly suggested that DHP-I hydrolyzed the beta-lactam of THM. Structural studies in these laboratories (O. Hensens, and J. Liesch, unpublished data) have shown that MK0787 and THM degraded by DHP-I have a nuclear magnetic resonance spectrum identical to that of antibiotic hydrolyzed with dilute acid. The spectra showed no evidence for any concurrent loss of side chains from the carbapenem nucleus. The identity of enzyme-degraded material with acid-hydrolyzed MK0787 was confirmed by HPLC (Fig. 4A), using a ^{3}H-labeled (generally) sample of MK0787 for the DHP-I reaction and a ^{14}C (side chain)-labeled preparation for acid hydrolysis. We conclude that DHP-I catalyzes beta-lactam hydrolysis by acting as a mammalian beta-lactamase on THM-type compounds.

Proof that DHP-I was responsible for the major fraction of antibiotic metabolism was provided by the chromatographic identity of an in vivo metabolite with DHP-I-hydrolyzed MK0787. Urine samples from rabbits receiving ^{14}C-labeled MK0787 (labeled in the formate carbon of the formimidoyl group) were found to contain a single peak, in addition to intact MK0787, by HPLC (Figure 4B). It was shown that the in vivo metabolite coeluted with a ^{3}H-labeled digest of MK0787 that had been treated previously with purified DHP-I. In rabbit urine collected over a 120-min period after intravenous administration, this metabolite, plus intact MK0787, accounted for 90 to 95% of total administered radioactivity.

Relative susceptibility of other carbapenems to DHP-I. All of the natural carbapenem antibiotics tested by us, as well as most semisynthetic derivatives of thienamycin, were metabolized more extensively than was either THM or MK0787 (Table 6). These compounds were also more susceptible to hydrolysis in vitro by DHP-I. There was good correlation between low urinary recovery and the extent of degradation of the carbapenem antibiotics by DHP-I.

DISCUSSION

The generally low urinary recoveries of THM and MK0787 observed in various laboratory species and in chimpanzees attest to the variable and sometimes extensive metabolic inactivation; whereas antibiotic in systemic circulation is spared from the rapid elimination that might be expected to result from metabolism of this magnitude. For example, the protective efficacy of these agents against experimental systemic infections in mice consistently exceeds that of nonmetabolized beta-lactam antibiotics and is in proportion to their relative in vitro antibacterial activities (11). Furthermore, plasma half-lives of MK0787, both in chimpanzees and in humans (F. Follath, A. M. Geddes, P. Spring, G. D. Ball, K. H. Jones, F. Ferber, J. S. Kahan, and F. M. Kahan, Program Abstr. 21st Intersci. Conf. Antimicrob. Agents Chemother. Chicago, Ill., abstr. no. 590, 1981), approach 60 min, which is equivalent to or longer than half-lives of several nonmetabolized penicillins and cephalosporins. In humans, plasma half-lives of MK0787 are reported to be constant between subjects, even though urinary recoveries be-

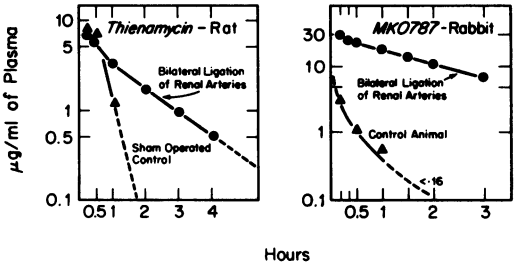


FIG. 3. Effect of bilateral ligation of renal arteries on antibiotic plasma levels in rats and rabbits.

TABLE 3. Degradation of THM in tissue homogenates

Species	Reaction condition			Assay[a]	Inactivation (%)
	Tissue	Substrate (μg/ml)	Pretreatment		
Mice	Liver	1		Bio	<5
	Kidney	1		Bio	30
	Kidney	1	Heat inactivated 30 min at 70°C	Bio	<5
Rats	Liver	2		Bio	<5
	Kidney	2		Bio	50
	Kidney	2	7 mM EDTA for 20 min	Bio	<5
Pigs	Kidney	100		UV	50
	Kidney	100	35 mM EDTA for 20 min	UV	0
Humans	Liver	100		UV	0
	Kidney	100		UV	22

[a] Bio, Bioassay; UV, hydrolysis measured by loss of UV absorbance at 297 nm.

tween those subjects varied from 6 to 40% (F. Ferber, K. Alestig, F. Kahan, K. Jones, J. Kahan, M. Meisinger, J. Rogers, and R. Norrby, 21st ICAAC, abst no. 591). This further illustrates that the systemic persistence of the antibiotic is insulated from the active renal metabolism of MK0787.

Localization of metabolism in the kidney is strongly suggested by the finding that renal clearance rates in dogs and chimpanzees are only a fraction of the minimum value set by the glomerular filtration rates measured in those species. Although tubular reabsorption of antibiotic might be proposed to account for low renal clearance rates, this alternative would require that a proportionately larger fraction of antibiotic undergoes extrarenal metabolism. The magnitude by which plasma clearance rates were reduced upon ligation of renal arteries in rats and rabbits limits the fraction of extrarenal metabolism to 50 and 5%, respectively. This amount of extrarenal metabolism is insufficient to account for the low levels of drug recovered in the urine of these species.

The biochemical studies which identified DHP-I as the enzyme responsible for THM inactivation were conducted primarily with porcine kidney. The applicability of this finding to those with other species is supported by the identification of a hydrolysis product derived from antibiotic inactivated by porcine enzyme and found by HPLC analyses to be identical to the primary metabolite recovered in rabbit urine. Furthermore, the susceptibility of related carbapenems to purified hog renal DHP-I correlated well with their extent of metabolism in mice. The THM-degrading activity of human kidney homogenates has been found to similarly copurify, with activity against Gly-dh-Phe during enzyme solubilization and partial purification (H. Kropp, unpublished data). It is relevant that DHP-I is reported to have a subcellular localization on the brush border (lumenal face) of the proximal tubular epithelium (C. L. Welch and B. J. Campbell, Fed. Proc. 37:1533, 1978). It thus has access to antibiotic already cleared from the circulation by glomerular filtration and secretion. Since metabolism occurs only after

TABLE 4. Relative activity of DPH-I against THM and Gly-dh-Phe during successive stages of enzyme purification

Step	Fraction	Sp act (nmol/min per mg)		Ratio of Gly-dh-Phe to THM
		Gly-dh-Phe	THM	
I	Homogenate	6.8	0.74	9.2
II	First pH 5 precipitate	5.3	0.80	6.6
III	Solubilized enzyme	472	19.5	24.2
IVa	Ammonium-SO_4 (50 to 75% precipitation)	1,100	47.5	23.2
IVb	Immobilized DHP-I inhibitor column-Sepharose CL-68)	34,700	600	57.8

TABLE 5. Stoichiometry between loss in specific UV absorbance and production of titratable acid functions during the course of DHP-I-catalyzed inactivation of THM

Reaction time (min)	Loss of absorbance (μmol)[a]	Alkali required to restore pH to 7.0 (μmol)
10	3.3	3.4
20	4.2	4.3
30	4.8	5.1
40	5.5	5.8

[a] At 297 nm, ϵ = 7,500.

antibiotic is in transit to the urine, the aforementioned insulation of antibiotic in the systemic circulation from the site of DHP-I in the tubular epithelium is readily explained.

The enzymic cleavage of the beta-lactam ring of THM, MK0787, and related carbapenems is a unique metabolic step in mammalian metabolism. All other naturally occurring carbapenems tested are much more susceptible to DHP-I than to THM. Their higher sensitivity is thought to be a consequence of their acetylated amino-alkylthio side chain. The 5,6-*cis*-substituted diastereomer of THM produced by enzymatic deacetylation of epithienamycin A was similar to THM in its sensitivity. By contrast, the *cis* configuration of the hydroxyethyl side chain in this family of structures influences their sensitivity to microbial beta-lactamases (2). We have also observed significant sensitivity to DHP-I (60% of that of THM) of a penem antibiotic structurally related to THM, the potassium 2-ethyl-thio-6 (1-hydroxyethyl)pen-2-em-3-carboxylate (V. M. Girijavalla-Bhan, A. K. Ganguly, S. W. McCombie, P. Pinto, and R. Rizvi, 21st ICAAC, abstr. no. 829). Carbapenems and penems have the ene-lactam structural feature, which is homologous with dehydropeptides. Although cephalosporins also contain an ene-lactam system, they are nonetheless insensitive to DHP-I attack. It is of interest that the chromogenic cephalosporin, nitrocefin, which is highly reactive with most microbial beta-lactamases and displays significant reactivity of its beta-lactam with serum (14), is also insensitive to DHP-I. A slight susceptibility, approximately 2% that of THM, has been detected for benzyl penicillin (P. Cassidy, unpublished data). It is ironic that those carbapenems, related to THM and its analogs, are afflicted with this sensitivity to a mammalian beta-lactamase in view of their resistance to most of the microbial beta-lactamases acting on conventional penicillins and cephalosporins.

It was a matter of some concern that the renal metabolism of MK0787 in humans may result in suboptimal levels of antibiotic in the urinary tract. In this regard, a series of potent, selective inhibitors of DHP-I have been developed in our laboratories for co-administration with MK0787 (W. T. Ashton, L. Barash, J. E. Brown, R. D. Brown, L. F. Canning, A. Chen, D. W. Graham, F. M. Kahan, H. Kropp, J. G. Sundelof, and E. F. Rogers, 20th ICAAC, abstr. no. 271). One of the inhibitors, MK0791, served in the present study as a reversible immobilized ligand for conveniently achieving a dramatic purification of DHP-I. In chimpanzees and in humans, MK0791 and a related inhibitor, MK0789, have

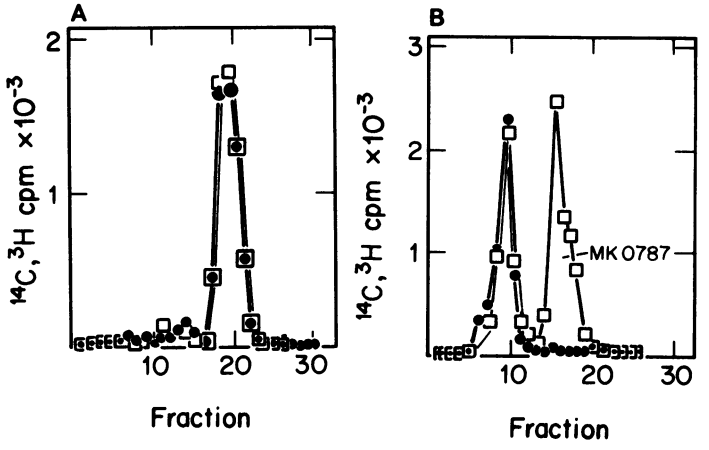
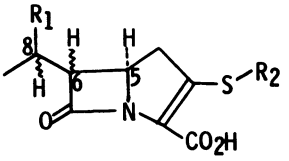


FIG. 4. (A) HPLC elution profile of products, using ^{3}H-labeled (●) MK0787 digested with DHP-I or ^{14}C-labeled MK0787 (□) for the controlled acid hydrolysis. (B) HPLC elution profile of labeled components in urine from rabbits receiving ^{14}C-labeled MK0787 (□). Sample was spiked with DHP-I digest of ^{3}H-labeled MK0787 (●).

TABLE 6. Relative susceptibility to DHP-I of THM derivatives and naturally occurring carbapenem antibiotics and its correlation with low urinary recovery

Antibiotic	 R_1	R_2	Configuration 5, 6	8	Ratio of Suscept. to DHP-I vs. THM (hog renal)	% Dose Recovered in Urine
Thienamycin	OH	$(CH_2)_2NH_2$	trans	R	1.0	25
N-Formimidoyl [MK-0787] thienamycin	OH	$(CH_2)_2NHCH{=}NH$	trans	R	0.9	28
N-Acetyl thienamycin	OH	$(CH_2)_2NHAc$	trans	R	4.2	11.4
N-Phenylacetyl thienamycin	OH	$(CH_2)_2NHAc\text{-}\phi$	trans	R	6.4	12.2
PS5	H	$(CH_2)_2NHAc$	trans	-	43.0	-
Epithienamycin A (MM22380)[a]	OH	$(CH_2)_2NHAc$	cis	S	12	1.0
Desacetyl-epi-THM A[b]	OH	$(CH_2)_2NH_2$	cis	S	1.3	9.7
Epithienamycin C (MM22381)	OH	$(CH_2)_2NHAc$	trans	S	20	-
Epithienamycin D (MM22383)	OH	$CH{=}CHNHAc$	trans	S	51	-
Epithienamycin E (MM13902)	OSO_3H	$CH{=}CHNHAc$	cis	S	30	< 1.0
Epithienamycin F (MM17880)	OSO_3H	$(CH_2)_2NHAc$ [Ac = $COCH_3$]	cis	S	8.3	-

[a] Alternate designations shown for epithienamycins are the serial numbers assigned at Beecham Pharmaceuticals Research Div., England, when independently discovered there as members of the olivanic acid series.
[b] Derived from epithienamycin A by enzymatic deacetylation (Cassidy et al. [5]).

been reported to be effective in reducing metabolism and thereby increasing urinary recoveries of MK0787 to 75%, a 10-fold improvement in those subjects showing the greatest renal metabolism of antibiotic (Kropp et al., 20th ICAAC, abstr. no. 270 and R. Norrby, K. Alestig, F. Kahan, J. Kahan, H. Kropp, F. Ferber, and M. Meisinger Prog. Abstr. 12th Intern. Congr. Chemother., Florence, Italy, 1981, abstr. no. 338; and R. Norrby, K. Alestig, B. Bjornegard, L. Burman, F. Ferber, F. Kahan, J. Huber, and K. Jones 21st ICAAC, abstr. no. 592). These findings are convincing proof that DHP-I plays a major role in the metabolism of MK0787 in humans.

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