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Thienamycin: development of imipenem-cilastatin

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Thienamycin, a natural product produced by *Streptomyces cattleya* is the first representative of a unique class of β-lactam antibiotics, the carbapenems. Despite its outstanding potency and antibacterial spectrum, thienamycin was itself unsuited for further development because of its chemical instability in concentrated solution and in the solid state. Synthesis of the amidine derivative, *N*-formimidoyl thienamycin (imipenem, MK0787) resulted in a crystalline product with much improved stability and with antibacterial properties significantly superior to thienamycin. Imipenem has an unusually broad antimicrobial spectrum. A high order of bactericidal activity is found against *Pseudomonas aeruginosa, Serratia, Bacteroides fragilis*, enterococci and numerous other species intrinsically resistant to other antibiotics. Imipenem is refractory to hydrolysis by all important classes of bacterial β-lactamases and thus exhibits no cross-resistance with penicillins or cephalosporins. Imipenem is distinguished from the new generation of extended-spectrum cephems by its unusually high potency against Gram-positive as well as Gram-negative organisms. Offsetting these excellent antimicrobial properties was an unusual susceptibility exhibited by imipenem to renal metabolism in animal species and in man. Very low urinary recoveries resulted without, however, any significant reduction in the serum half-life of imipenem. A brush-border dipeptidase, dehydropeptidase-I, was shown to be responsible for renal metabolism. Metabolism has been countered with the development of cilastatin (MK0791), a substituted amino-propenoate inhibitor of dehydropeptidase which is specific, potent and well matched in its pharmacokinetic properties for co-administration with imipenem. With the imipenem/cilastatin combination, uniformly high urinary concentrations and recovery are obtained regardless of the varying but often extensive metabolism suffered by imipenem in human populations. An additional benefit conferred by cilastatin results from its ability to exclude imipenem competitively from entry into and subsequent metabolism within the proximal tubular epithelium of the kidney. The tubular necrosis induced by imipenem alone when it is administered at very high doses to susceptible mammalian species is thereby eliminated. Thus the imipenem/cilastatin combination affords reliability and enhanced safety in the application of the antibiotic's unusual antibacterial potential in the treatment of difficult infections regardless of the site of disease.

Introduction

The subject of this review is a new combination antimicrobial imipenem/cilastatin. It comprises the novel β-lactam antibiotic imipenem (MK0787) and an enzyme inhibitor, cilastatin (MK0791)—itself devoid of antibacterial activity—which was specifically designed to block the extensive renal metabolism which imipenem undergoes in man. An account will be given in this introduction of the sequence of development of this

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combination. We will then review the many independent laboratory studies published in the last 3 years on the very promising antibacterial activity of imipenem. In a second section, studies from this laboratory, which elucidated the location and enzymic basis of imipenem metabolism, will be related, followed by new laboratory evidence on the effectiveness of cilastatin in controlling metabolism and allied problems encountered by imipenem in the mammalian kidney.

(i) *From thienamycin to imipenem*

Imipenem is a derivative of thienamycin, a unique β-lactam antibiotic discovered in the course of screening soil micro-organisms for the production of inhibitors of peptidoglycan synthesis (Kahan *et al.*, 1979). Thienamycin was produced by a hitherto undescribed *Streptomyces* species which was given the name *Streptomyces cattleya* after the resemblance of pigmentation in the sporulated aerial mycelium to the colour of the classic Cattleya orchid (Plate 1). The structure of thienamycin determined by Albers-Schonberg *et al.* (1978) is shown in Figure 1. It is remarkable not only for its nucleus but for the nature and conformation of its sidechain. Thienamycin was the first representative of a now diverse family of naturally-occurring and synthetic antibiotics that share its carbapenem nucleus (Ratcliffe & Albers-Schonberg, 1982). The term carbapenem denotes similarity with the 4 : 5 fused ring lactam of penicillins, the substitution of carbon for sulphur and the presence of unsaturation in the 5-member ring. The hydroxyethyl sidechain of thienamycin is a radical departure from the design of conventional penicillins and cephalosporins, all of which have an acylamino substituent on the β-lactam ring. Thienamycin and imipenem owe their resistance to β-lactamases to the unusual *trans*-conformation of this sidechain, as shown by the lactamase sensitivity of subsequently discovered carbapenems, the epithienamycins, several of which share with conventional β-lactams the *cis*-conformation (Cassidy, 1981*a*). On the 5-membered ring, the basic alkylthio sidechain in thienamycin and imipenem is the source of their valuable activity against *Pseudomonas* (Christensen, 1981) and as will be demonstrated below, is a critical determinant of their susceptibility to metabolism. It was from the attachment of the exocyclic sulphur to the ene-lactam system in this molecule that thienamycin derived its name (Kahan *et al.*, 1979).

Although thienamycin possessed high potency against an unusually broad spectrum of bacteria both *in vitro* and *in vivo* (Kropp *et al.*, 1976), its further development was prevented by the instability it exhibited in concentrated solutions and in the solid state (Kahan *et al.*, 1979). Synthesis of the *N*-formimidoyl derivative (Leanza *et al.*, 1979) provided control over solution instability and with the subsequent crystallization of this derivative, a stable pharmaceutical preparation was achieved. *N*-Formimidoyl-

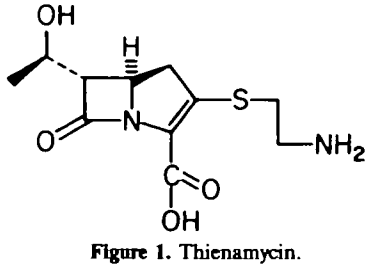

Figure 1. Thienamycin.

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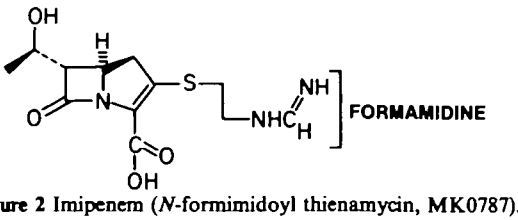

Figure 2 Imipenem (*N*-formimidoyl thienamycin, MK0787).

thienamycin (Figure 2) was originally designated MK0787. Much of the existing literature on imipenem uses either of those names. The generic name, imipemide, was assigned for a brief period and has been used in several U.S. publications during 1983, but has now been superseded by the USAN and WHO approved name, imipenem.

(ii) *From imipenem to imipenem/cilastatin*

The susceptibility of carbapenem antibiotics to extensive metabolic inactivation in mammals was dramatically evident from the outset in laboratory studies with this family of antibiotics. Thienamycin and imipenem were metabolically quite stable compared with the carbapenems subsequently encountered in nature. The latter were so rapidly metabolized in the mouse that protective efficacy was rarely demonstrable against experimental infections despite their high order of *in-vitro* activity. This was a consequence of the non-basic acylaminoalkyl-thio sidechain invariably present in the natural carbapenems other than thienamycin, and held true for semisynthetic *N*-acyl derivatives of thienamycin as well (Kropp *et al.*, 1982). Metabolism of imipenem had its chief impact upon urinary recoveries, which were in the adult chimpanzee, the closest approximation we had of man, as low as 10% of the administered dose. However, acceptable plasma half-lives were observed, suggesting that metabolic inactivation was localized in the kidney of larger mammals. The enzyme responsible for carbapenem inactivation was shown to be a brush-border dipeptidase, dehydropeptidase-I. A small programme was started to search for inhibitors of this enzyme in the event that metabolism of imipenem in man was comparable to that observed in the chimpanzee.

Our worst fears were confirmed when imipenem was administered for the first time to man. In a study of its disposition in 12 volunteers conducted in the U.K. at East Birmingham Hospital by A. M. Geddes, A. P. Ball and I. D. Farrell (reported in Follath *et al.*, 1981), urinary recoveries ranged from 30% down to 6%. Yet plasma half-lives (1 h) were again satisfactory. These results were typical of those to be found when imipenem was administered alone to volunteers in subsequent studies.

Systematic modification of dehydropeptidase inhibitors had by then yielded highly potent and specific compounds which were effective in restoring imipenem urinary recovery in experimental animals (Ashton *et al.*, 1980). Their structures had been modified until optimal pharmacokinetic matching with imipenem had been achieved in the chimpanzee and urinary recoveries in excess of 70% obtained (Kropp *et al.*, 1980*b*). The first candidate inhibitor from this programme was MK0789, whose efficacy in man was reported by Norrby *et al.* (1981*a*). Because of local irritation encountered in animals receiving high concentrations of MK0789 in the course of toxicity studies at very high doses, the present dehydropeptidase inhibitor MK0791

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(Figure 3) was substituted in place of MK0789. The efficacy of MK0791 in restoring urinary recovery in man was established by Norrby *et al.* (1981*b*, 1983*b*), and led to a decision that this inhibitor would be combined with imipenem in all of its therapeutic applications. The generic name adopted for MK0791 is cilastatin.

Imipenem/cilastatin has had a rather long gestation period, if conception is dated from the discovery of thienamycin. The major limiting factor by far, has been past difficulties in meeting the needs of laboratory and clinical studies for imipenem. That imipenem is at present a reality is in large measure due to the success of a bold total chemical synthesis, notable for its elegance and efficiency (Pines, 1981). It is the first example of an industrial-scale process yielding fused ring β-lactams of the complexity of imipenem, that starts from non β-lactam precursors. An important dividend of this venture has been the provision of routes to future generations of carbapenem antibiotics which could not readily be synthesized from thienamycin itself.

I. Review of laboratory studies of the antibacterial activity of imipenem *in vitro* and *in vivo*

In their initial description of the antibacterial properties of imipenem, Kropp *et al.* (1980*a*) compared this derivative with thienamycin, and found that the broad antibacterial spectrum of the parent antibiotic had been preserved and in important instances, such as antipseudomonal activity, significantly improved. Imipenem was also found consistently superior to thienamycin in treating experimental infections, a consequence of the longer half-life exhibited by imipenem in a variety of animal species. Comparative studies showed imipenem to have, with few exceptions, more potent, bactericidal activity *in vitro* and greater protective efficacy *in vivo* against all organisms tested than any of the established or experimental cephalosporins and penicillins. In an accompanying study from the Merck laboratories in Japan, Kesado, Hashizume & Asahi (1980) showed MIC distributions for a very large number of isolates and strains, which further established the comprehensive scope of activity of imipenem. A review is now presented of the numerous independent studies of the antibacterial properties of imipenem published since these initial findings.

(i) *Antibacterial spectrum and potency*

A summary of the many susceptibility surveys published for imipenem is presented in Tables I, II and III to show its spectrum of activity and potency against the major

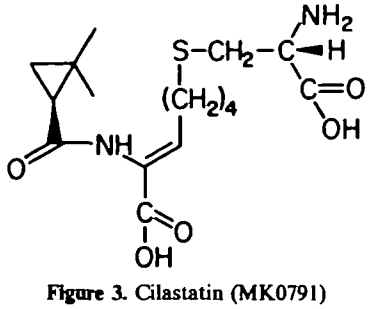

Figure 3. Cilastatin (MK0791)

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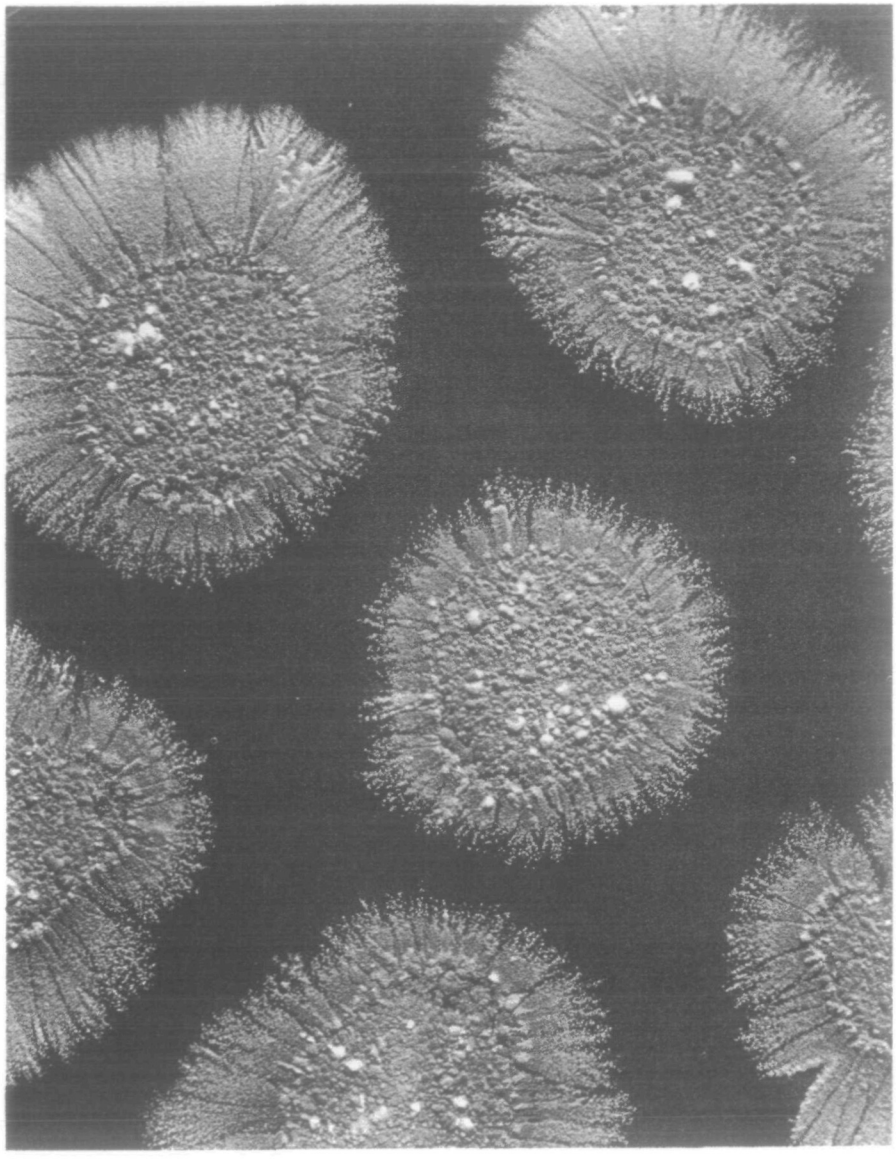

Plate 1. *Streptomyces cattleya.* The producing organism for thienamycin, showing the characteristic orchid-pigmentation of the sporulated aerial mycelium after which the species name was derived. (Photograph by J. Kath, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey, U.S.A.)

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Table I. Susceptibility of Gram-negative aerobes to imipenem

Pathogen	No. of isolates	MIC_{50} (mg/l)	MIC_{90}
E. coli	1122	0·14	0·26
Enterobacter spp.	1276	0·34	1·30
Klebsiella spp.	952	0·18	0·41
Serratia spp.	805	0·76	1·93
Proteus spp. (indole +ve & −ve)	1655	1.3	2·90
Ps. aeruginosa	2278	1·57	3·54
carbenicillin-resistant	142	1·70	3·70
aminocyclitol-resistant	317	2·10	3·80
Acinetobacter spp.	436	0·22	0·47
Alcaligenes spp.	86	0·97	2·0
Br. melitensis	98	1·0	2·0
Eikenella corrodens	56	0·15	0·22
H. influenzae	302	0·95	1·82
Moraxella spp.	37	0·08	0·37
N. gonorrhoeae	387	0·10	0·30
N. meningitidis	266	0·05	0·11
Shigella spp.	33	0·17	0·27
Y. enterocolitica	234	0·23	0·44
Citrobacter spp.	370	0·30	0·62

divisions of infectious bacteria. This compilation includes all species whose MIC_{90} was found less than the provisional 8 mg/l breakpoint currently used to define susceptibility to imipenem. Only three species were disqualified by this criterion: *Pseudomonas maltophilia* (which is uniformly resistant to imipenem), *Pseudomonas cepacia*, and *Streptococcus faecium*. No other antibiotic has so few deficiencies in its spectrum of activity. Moreover, the minimal spread between MIC_{50} and MIC_{90} seen with few exceptions throughout the spectrum, testifies to an unprecedented consistency of activity and lack of cross-resistance.

The following additional features of the spectrum are particularly noteworthy.

Imipenem distinguishes itself from recent extended-spectrum β-lactams by exhibiting a degree of activity against all isolates of Gram-positive cocci rivalling that of penicillins against susceptible isolates. As shown in Table II, imipenem shows no trace of cross-resistance with penicillin-resistant staphylococci. The intrinsic activity of

Table II. Susceptibility of Gram-positive aerobes to imipenem

Pathogen	No. of isolates	MIC_{50} (mg/l)	MIC_{90}
Staph. aureus	1290	⩽0·07	⩽0·13
penicillin-resistant	110	0·06	0·08
methicillin-resistant	161	0·72	4·70
Staph. epidermidis	509	0·09	1·33
Streptococcus spp. (Groups A, B, C, G)	1293	⩽0·03	⩽0·05
Strep. faecalis	795	0·93	1·58
List. monocytogenes	36	0·08	0·11
Noc. asteroides	45	1·15	2·66

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Table III. Susceptibility of anaerobes to imipenem

Pathogen	No. of isolates	MIC_{50} (mg/l)	MIC_{90}
Gram-negative			
Bact. fragilis	99	⩽0·08	⩽0·33
Fusobacterium spp.	57	0·03	0·50
Viellonella spp.	23	0·05	0·25
Campylobacter fetus	92	0·07	0·07
Gram-positive			
Cl. perfringens	31	0·06	0·54
Cl. difficile	80	4·06	6·05
Clostridium spp.	133	0·09	0·28
Eubacterium spp.	6	0·06	2·0
Peptococcus spp.	55	0·03	0·07
Peptostreptococcus spp.	32	0·01	0·05

Values presented are the geometric means of broth- or agar-dilution susceptibility distributions measured at inoculum densities, *c.* 10^5 cfu. Represented in this compilation are the findings in 72 studies published from 1980 to 1982.

imipenem toward enterococci is also noteable, considering the virtual absence of such activity in the second and third generation cephems.

Against *Pseudomonas aeruginosa* imipenem again shows a compact MIC_{90} : MIC_{50} ratio with no detectable cross-resistance to the subset of carbenicillin or aminocyclitol resistant strains.

Against anaerobes (Table III) imipenem has an order of activity against *Bacteroides fragilis* that is unequalled by any β-lactam antibiotic and is equivalent or superior to metronidazole, clindamycin or chloramphenicol.

Imipenem exhibits excellent activity against infrequent yet generally antibiotic-insensitive species, such as *Serratia, Acinetobacter* and *Nocardia asteroides.*

(ii) *Bactericidal activity*

As expected for an inhibitor of cell wall biosynthesis, imipenem is bactericidal in its action. Kropp *et al.* (1980) showed that imipenem had MBCs equal to or very similar to the MICs against several genera of organisms. The equivalence of MIC and MBC was maintained over an inoculum range of 10^3 to 10^7 cfu with but few exceptions. Similar results have been found by a number of investigators studying specific groups of pathogens. Borobio *et al.* (1981) found that the MIC/MBC ratio against *Bacteroides* strains and Gram-positive anaerobes in general was unity. The ratio was independent of increasing inoculum levels (10^3 to 10^7 cfu) and was unaffected by the medium used or its pH. Similar results with anaerobes were obtained by Owens & Finegold (1983) and by Spelhaug *et al.* (1981) for several strains of *Campylobacter.* In a study by Howard & Hence (1982), using ampicillin-resistant *Haemophilus influenzae*, the MIC_{90} was 1 mg/l with MBCs often being equal to the MICs regardless of inoculum size or resistance pattern.

Wise, Andrews & Patel (1981) found the MBC exceeded the MIC only in a minority of species. They also demonstrated that added serum, up to 75% of the medium, did not increase either MIC or MBC. They related this finding to the slight binding, 20%, of imipenem to serum protein. Similarly, Neu & Labthavikul (1982), studying a large

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variety of clinical isolates, showed that there was little effect of inoculum levels on inhibitory concentrations and that MBCs were no greater than two- to fourfold the MIC.

(iii) *Resistance to β-lactamase*

Thienamycin was originally inferred to be resistant to hydrolysis by β-lactamases from its lack of cross-resistance with penicillins and cephalosporins. Equivalent activity was found on pairs of isogenic strains one of which contained plasmid-mediated resistance to all other β-lactam antibiotics available at that time (Kropp *et al.*, 1976). A similar pattern of activity on resistant strains was demonstrated for imipenem (Kropp *et al.*, 1980). Direct evidence of resistance to hydrolysis by β-lactamases was obtained by Richmond (1981) who evaluated the stability of imipenem to β-lactamases from *Staphylococcus aureus* and *Bacteroides fragilis* as well as Types Ia, IIIa and IVc enzymes. In all cases, the antibiotic was very stable. Imipenem also proved to be a potent inhibitor of the Type Ia and *Bact. fragilis* enzymes. Studies on the rates of hydrolysis by the Type IIIa enzyme showed a relative V_{max} for imipenem of only 0·01, compared with rates of 1014 for cephaloridine and 2·28 for cefotaxime. Neu & Labthavikul (1982) tested cell lysates of strains exhibiting a variety of β-lactamases and could not detect any measurable hydrolysis of imipenem, even though several preparations slowly hydrolysed relatively resistant cephem antibiotics, such as cefoxitin, cefotaxime and moxalactam. Hanslo *et al.* (1981) tested 28 enzymes of which only three preparations showed detectable hydrolysis of imipenem equal to or less than 1·0% of the cephaloridine control. Vuye (1982) first characterized the susceptibility of 21 Gram-negative isolates selected for their β-lactamase production to imipenem and other β-lactams. In every case, imipenem was highly active and showed only minor increases in MIC when the inoculum level was increased from 10^4 to 10^6 cfu. In contrast, the MIC of cefotaxime and cefamandole increased by as much as 100-fold at increased inoculum levels. The hydrolysis rates of imipenem and five different cephem antibiotics were then tested in lysates of each of these strains. No hydrolysis of imipenem and cefoxitin could be detected regardless of the type of enzyme.

The above studies prove that imipenem is almost totally resistant to β-lactamase of all recognized types from aerobes and anaerobes, regardless of their substrate preference and regardless of their genomic origin.

Only one exception has appeared to date, and in the one species that is uniformly resistant to imipenem (as well as most other β-lactam antibiotics), *Ps. maltophilia*. Saino *et al.* (1982) characterized an inducible penicillinase, that could be elicited in seven isolates of this species, as an unusual zinc metalloenzyme which had significant activity on imipenem but had a low affinity. They were unable to conclude whether the kinetic parameters of this enzyme against various β-lactams could fully account for the resistance patterns they observed.

(iv) *Susceptibility of antibiotic-resistant strains to imipenem*

Numerous studies attest to the full antibacterial activity retained by imipenem against strains resistant to one or more β-lactam antibiotics by virtue of the β-lactamases they produce (Calderwood *et al.*, 1982; Chabbert & Jaffe, 1982; Cullmann *et al.*, 1982a; Dudek *et al.*, 1982; Vuye, 1982). This is the expected consequence of imipenem's stability to hydrolysis by β-lactamases as reviewed above. Specific examples of

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imipenem's activity against strains exhibiting resistance to multiple antibiotics are in the following survey studies.

Bartmann & Tarbuc (1982) studied the susceptibility of Gram-negative strains and found that imipenem was very active against the most frequently encountered resistant organisms responsible for nosocomial infections. MICs ranged from 0·25 to 1 mg/l for multi-resistant *Enterobacteriaceae*, and from 0·5 to 4 mg/l for multi-resistant *Ps. aeruginosa*. Similarly, Sanders & Sanders (1982) found that imipenem was active against all *Enterobacteriaceae* resistant to cephalosporins and cephamycins. Two enterobacteria, resistant to cephalothin, cefamandole, cefoxitin and moxalactam were highly susceptible to imipenem with no increase in MIC or MBC as compared to non-resistant strains. In another survey, Hanslo *et al.* (1982) showed that imipenem was very active against ampicillin- and cephaloridine-resistant enterobacteria. Many strains of Gram-negative organisms multiresistant to mezlocillin, cefoperazone, cefotaxime and moxalactam are still susceptible to imipenem (Cullman *et al.*, 1982*b*). In this study, good activity of imipenem was observed also against oxacillin-resistant *Streptococcus faecalis*. Verbist & Verhaegen (1981) have also reported good potency of imipenem against oxacillin-resistant *Staph. aureus*. Fainstein *et al.* (1982) reported similar results with strains of *Enterobacter, Klebsiella, Pseudomonas, Serratia* and *Achromobacter*. More than 90% remained sensitive to imipenem regardless of their resistance pattern.

Imipenem's outstanding activity on *Ps. aeruginosa* has prompted many studies of its activity on strains with acquired resistance to carbenicillin, the newer ureido-penicillins and aminoglycoside antibiotics. Alford (1983) found that gentamicin-resistant *Ps. aeruginosa* are just as susceptible to imipenem as the gentamicin-sensitive strains. Lang & Durack (1980) found that all carbenicillin-resistant *Ps. aeruginosa* that they surveyed were still susceptible to imipenem. Multiple-resistant strains of *Ps. aeruginosa* isolated from cystic fibrosis patients were found by Prince & Neu (1981) to be susceptible to imipenem and ceftazidime. Many of these isolates showed high resistance to carbenicillin, ureidopenicillins and all aminocyclitol antibiotics that were tested. Matzkowitz *et al.* (1982) showed that *Ps. aeruginosa* resistant to amikacin were still sensitive to imipenem.

Strausbaugh & Laun (1983) studied the susceptibility of *Ps. aeruginosa* clinical isolates to imipenem, moxalactam and azthreonam. Imipenem had the most potent inhibitory activity and was >10-fold and >25-fold more active than azthreonam and moxalactam, respectively. Against gentamicin-resistant strains, imipenem's activity was not diminished. In a broad survey of activity of 13 β-lactam antibiotics, Muytjens & Van Der Ros-Van De Repe (1982) tested a series of gentamicin-resistant *Ps. aeruginosa*. Imipenem had an MIC_{90} of 4 mg/l and was the most active agent by a considerable margin. The second most potent agent was ceftazidime with an MIC_{90} of 32 mg/l. Similar results were obtained with gentamicin-resistant Enterobacteriaceae.

Stability toward β-lactamases is not the sole basis for imipenem's lack of cross-resistance with other classes of β-lactam antibiotics. Many resistant strains originate in a clinical setting by the selection of heterotypic variants exhibiting stepwise resistance. The structural similarity of the sidechains that have been used to confer anti-pseudomonal activity on cephalosporins and penicillins make them liable to cross-resistance with each other when heterotypic resistance arises. Imipenem's structure differs so radically from these other agents that cross-resistance might not be expected, and indeed, Kropp *et al.* (1980) showed thienamycin and imipenem to be fully active

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against carbenicillin-resistant variants they selected in the laboratory. An important study by Livermore, Williams & Williams (1981) has shown the laboratory finding to apply to the more relevant population of carbenicillin resistant organisms of clinical origin. Using a panel of isolates whose resistance was shown *not* to result from carbenicillinase, they demonstrated that isolates with increased MIC for carbenicillin showed a correlated increase in resistance to cefotaxime, moxalactam, cefoperazone, cefsulodin, ceftriaxone and ceftazidime. By contrast, the susceptibility to imipenem remained constant for isolates exhibiting 20-fold differences in MIC to carbenicillin.

These studies establish clearly that imipenem's potency and lack of cross-resistance with penicillins, cephalosporins, and aminocyclitols, forecast an excellent potential in the treatment of infections due to *Ps. aeruginosa* and other multi-resistant Gram-negative bacilli.

(v) *Activity of imipenem on Gram-positive isolates exhibiting atypical resistance to β-lactams or tolerance*

In common with all other β-lactam agents, imipenem shows a major reduction in activity against isolates whose acquired resistance is mediated by mechanisms other than β-lactam hydrolysis. Landesman *et al.* (1981) reported that MICs for imipenem, against penicillin-resistant variants of *Str. pneumoniae* were 0·125 to 2 mg/l, compared with the usual sensitive range of 0·0078 to 0·03 mg/l. Similar studies (Ward & Moellering, 1981; Cherubin *et al.*, 1981) also showed that penicillin-resistant pneumococci have an MIC_{90} of 1 mg/l for imipenem in comparison to a number of cephalosporins and penicillins which were several-fold less active.

As shown in Table II, imipenem has relatively substantial activity against a majority of methicillin-resistant *Staph. aureus*, although it is affected by the underlying general mechanism of resistance to all β-lactam agents. There are major differences among the published studies on the distribution of susceptibilities to imipenem. Cherubin *et al.* (1981) studied a group of methicillin-resistant *Staph. aureus* and found that the MIC_{50}, 0·03 mg/l, falls within the range of MICs of methicillin-sensitive *Staph. aureus*. The MIC_{90} for these strains was, however, relatively high, 4 mg/l. Thompson, Fisher & Wenzel (1982) also found that a significant proportion of methicillin-resistant *Staph. aureus* are very sensitive to imipenem. Witte, Sapico & Canawati (1982) found that although methicillin-resistant *Staph. aureus* is sensitive to imipenem by MIC determination, a significant portion of the strains showed tolerance (MBC $\geqslant$32-fold the MIC). Recently, a group of methicillin-resistant *Staph. aureus* with multiple resistance patterns to other non-β-lactam antibiotics as well, was studied by Markowitz *et al.* (1983) and found to have an $MIC_{90} = 0{\cdot}8$ mg/l when incubated under standard conditions (18 h at 37°C). However, when incubated at 30°C for 48 h, the susceptibility distribution was shifted to above 25 mg/l. The relevance of these findings to the utility of imipenem against methicillin-resistant *Staph. aureus* will doubtless soon emerge from further laboratory and clinical testing.

An apparently rare form of imipenem resistance, unrelated to antibiotic degradation, has recently been reported for coagulase-negative *Staphylococci* by Blumenthal *et al.* (1983). Out of 25 strains surveyed, 8 were resistant to imipenem, but only 2 of these were isolated from patients treated with this antibiotic. The resistant strains were resistant to all other β-lactams tested including oxacillin. However, other β-lactam-resistant strains they tested were sensitive to imipenem. Resistance to

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imipenem was constitutive in 3 strains and inducible in the remainder. Induction occurred at growth-inhibitory concentrations and was correlated with the production of unusual double zones in disk-diffusion tests consisting of growth immediately around the disk followed by a hazy zone of inhibition. No correlation was found between resistance and plasmid pattern, nor could the resistance be eliminated by treatment with plasmid 'curing' agents. Imipenem resistance appears not to be a widespread phenomenon since numerous laboratories have reported that nearly all isolates of coagulase-negative *Staphylococci* are susceptible (Horadam *et al.*, 1980; Tutlane *et al.*, 1981; Neu & Labthavikul, 1982; Muytjens & Van Der Ros-Van De Repe, 1982).

(vi) *Combination of imipenem and aminocyclitols*

It has become the usual practice to treat certain organisms, particularly *Ps. aeruginosa* and tolerant Gram-positive cocci (e.g. *Str. faecalis*), with combinations of antibiotics. The interaction of imipenem with aminocyclitol antibiotics, has been studied by Kallick and associates (1982) with numerous strains of *Ps. aeruginosa* and *Staph. aureus* isolated from patients with endocarditis. The slowest rate of kill observed with imipenem alone was, nevertheless, more rapid than that exerted by either carbenicillin or tobramycin. Imipenem in combination with tobramycin was rapidly bactericidal (e.g. a 1000-fold reduction in 4 h), more so than carbenicillin plus tobramycin for all strains of *Ps. aeruginosa* tested. For *Staph. aureus*, the killing rates for imipenem plus tobramycin were significantly enhanced compared to those of the individual antibiotics. The investigators conclude that imipenem in combination with tobramycin is potentially effective for treating endocarditis caused by *Ps. aeruginosa* and *Staph. aureus*. Measurement of bactericidal kinetics appears to be the only reliable (and therapeutically relevant) means of estimating whether this beneficial interaction occurs for, in conventional 18 h MIC determinations, imipenem shows little synergy in combination with aminocyclitols against *Ps. aeruginosa*. Most of the effects are additive or indifferent (Diez Enciso *et al.*, 1982; Neu & Labthavikul, 1982).

Strains of enterococci (*Str. faecalis*) are known to be tolerant to β-lactam antibiotics. They exhibit a large difference between MIC and MBC. Imipenem appears likewise affected. Such findings are in keeping with the work of Krogstad & Parquette (1980) who showed that enterococci are tolerant to most inhibitors of bacterial cell-wall biosynthesis. They suggest that a defective autolytic enzyme system in these cells prevents death of the organism even when new cell-wall synthesis has been stopped by effective levels of antibiotics. For example, Eliopoulos & Moellering (1981) and Auckenthaler *et al.* (1982) showed that many strains of enterococci judged highly sensitive by their MIC have MBCs >128 mg/l. These isolates also showed lack of killing by penicillin. A contributing factor to the finding by Auckenthaler *et al.* (1982) of apparent tolerance was the severe criterion set for MBC by the macrodilution technique they employed. A 99·9% kill was required, as opposed to the 99% kill implicit in the conventional microdilution MBC. Eliopoulos & Moellering (1981) also examined the effect on tolerance of combining imipenem with aminocyclitol antibiotics. The killing rate of imipenem against enterococci was enhanced dramatically by combination with gentamicin. The activity against *Str. faecium* was also enhanced by the combination, but to a lesser extent than for *Str. faecalis*. In a separate study, more than 95% of the enterococci surveyed were killed much more

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rapidly by a combination of imipenem with either gentamicin or tobramycin (Watanakunakorn & Tisone, 1982). The cell population was reduced from 10^5 to $< 10^2$ cfu/ml in 6 h and further reduced to less than 10 cfu/ml at 24 h.

Gombert, Berkowitz & Cummings (1983) evaluated the synergistic interaction of imipenem with amikacin and gentamicin against *Str. faecalis*. Sixty per cent of the synergistic combinations were totally bactericidal (produced sterile cultures) at 24 h. A more rapid bactericidal effect was observed at all times when the imipenem-aminocyclitol combination was used in comparison to either agent when used alone.

(vii) *Efficacy of imipenem against experimental infections*

When evaluated for protective efficacy in the mouse against acute intraperitoneal infections, imipenem shows the same comprehensive spectrum of activity and unusual potency observed *in vitro* (Table IV). Comparative efficacy studies show the very low dose rates of imipenem active against Gram-positive infection, and its advantages over established therapeutics throughout the Gram-negative spectrum. Imipenem is particularly impressive in its efficacy against *Ps. aeruginosa*, especially in comparison with other available β-lactam agents with anti-pseudomonal activity (Table V).

The *in-vivo* activity of imipenem has been evaluated in a variety of additional animal models of acute infections. Pennington & Johnson (1982) studied imipenem in a guinea pig model of a pneumonia induced by *Ps. aeruginosa*. At the dose rates chosen, imipenem was equal in its efficacy to tobramycin and superior to ticarcillin in preventing mortality and in achieving clearance of viable organisms from the lung. In a model of peritonitis in rats induced by injection of human faecal suspensions, imipenem was found to be more effective in curative activity than either cefoxitin or the combination of gentamicin plus clindamycin (Hau, Phuangsab & Nishikawa, 1982). Lahnborg Hedstrom & Nord (1982) established an experimental model of intra-abdominal infections in rats which simulates intra-abdominal sepsis in patients. In this model, about 75% of the untreated animals died by 4 days after infection. Cefoxitin and imipenem were the only antibiotics that gave almost complete cures as single agents. Of the two, imipenem was more potent than cefoxitin. A combination of tinidazole plus netilmicin gave excellent protection, but the individual components as single agents were significantly less effective. These results indicate that imipenem, due to its comprehensive anti-aerobe spectrum and unusually high activity against anaerobes, should be efficacious in treatment of intra-abdominal infections caused by polymicrobic infections, without the need to co-administer another kind of antibiotic.

Johnson *et al.* (1982) studied a *Ps. aeruginosa* bacteraemia in rats made neutropenic by cyclophosphamide treatment. Mortality was prevented in 60% of the animals by imipenem treatment even upon challenge with 250 LD_{50}s. This activity was significantly superior to that of amikacin which gave only 10% survival. When imipenem was combined with amikacin, 95% of the neutropenic rats survived. This study provided laboratory evidence that imipenem may be particularly useful in treatment of immuno-compromised patients.

Ritzerfeld (1983) studied imipenem in comparison to cefotaxime in an acute pyelonephritis model using rats. Even at low doses, a clear antibacterial effect of imipenem superior to that of cefotaxime, was observed for both *Escherichia coli* and *Ps. aeruginosa* pyelonephritis.

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Table IV. Comparative efficacy of imipenem against experimental infections in mice

Infecting organism	ED_{50} (mg/kg per dose) Imipenem	Cefazolin	Ampicillin	Cefoxitin	Cefotaxime
Staph. aureus 2985[a]	0·06	0·6	0·4	—	7·8
Strep. pyogenes 2874[a]	0·04	0·8	0·3	—	0·031
Strep. pneumoniae 212[a]	0·03	0·25	0·4	—	> 1·0
E. coli 2884[a]	2·5	6·4	25·0	6·6	—
E. coli 2891	0·65	37·7	334·0	31·2	3·1
K. pneumoniae 2888	0·64	> 500·0	> 400·0	250·0	1·2
E. cloacae 2646	0·65	> 500·0	> 500·0	94·0	85·0
Pr. mirabilis 3125	1·0	15·1	6·0	13·0	0·02
M. morganii 2833	0·94	82·8	> 500·0	6·3	0·6
Ser. marcescens 3548	3·8	—	—	61·3	61·3

Comparative efficacy data were reproduced from Kropp *et al.* (1980) by permission and supplemented with additional endpoints for cefoxitin and cefotaxime.

In all cases, challenge inoculum of 3 to 100 LD_{50}s was given intraperitoneally followed immediately by treatment by the subcutaneous route. Except for those strains noted otherwise, two additional treatments were administered at 2 and 4 hours after the challenge. ED_{50} is the calculated dose per treatment providing 50% survival on the seventh day after treatment.

[a]One additional treatment was given 6 h after challenge.

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Table V. Comparative efficacy of imipenem against experimental infections in mice with selected clinical strains of *Ps. aeruginosa*

	ED_{50} (mg/kg)						
Ps. aeruginosa	Imipenem	Gentamicin	Amikacin	Moxalactam	Cefotaxime	Carbenicillin	Piperacillin
40	0·74	85·0	15·0	13·2	118·0	133·0	29·4
3286	0·95	10·3	10·3	50·0	> 500·0	334·0	245·0
2835	1·2	9·4	10·0	> 100·0	> 500·0	> 500·0	> 500·0
4293	0·78	50·0	50·0	> 100·0	—	—	29·0
4294	1·9	50·0	50·0	> 100·0	—	—	> 500·0
3350*	1·2	> 200·0	25·0	25·0	—	—	250·0

Comparative efficacy data were adapted from Kropp *et al.* (1980*a*) by permission and supplemented with additional endpoints for several of the comparative agents. A three-dose regimen with treatment at 0, 2 and 4 h after challenge was applied.

*Resistant to gentamicin and carbenicillin

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The effect of imipenem on an *E. coli*-induced meningitis in rabbits was studied by Patamasucon & McCracken (1982). These investigators observed good penetration of imipenem into the cerebrospinal fluid (CSF) of animals with inflamed meninges (CSF/plasma = 31%), whereas the penetration of drug into the CSF of uninfected animals was <1%. Using a continuous infusion of imipenem, a 10^4 reduction in *E. coli* counts was observed at the end of 9 h with most of the animals having sterile CSF. With a single dose of imipenem, there was a 100-fold drop by 2 h, without any further decrease in colony-forming units of *E. coli*. The mean CSF level of imipenem was 8·3 mg/l, well above the MIC (and many MBCs) of the organisms which most frequently cause infections of the central nervous system.

A similar study was performed by Funk, Laun & Strausbaugh (1982) who used a pneumococcal meningitis model in rabbits. In this case, a 10% penetration into the CSF was observed. Much lower penetration was observed in the uninfected rabbit. Washburn, Perfect & Durack (1983) also studied the pharmacokinetics of imipenem in an experimental rabbit meningitis model induced by *H. influenzae*. These workers observed 18–23% penetration of the antibiotic measured at 1 hour post-administration with a much lesser amount of the drug penetrating the CSF of non-infected animals.

Only a few studies in the literature have reported on the activity of imipenem in endocarditis. Auckenthaler *et al.* (1982) reported that imipenem was equal to penicillin G in efficacy against enterococcal endocarditis in rabbits. They observed, however, that imipenem was not as bactericidal as the combination of penicillin plus gentamicin, presumbably due to the tolerance these organisms show towards all β-lactam antibiotics. Regrettably, these investigators did not include the combination of imipenem plus gentamicin in their protocols. This combination has been found to exhibit a synergistic bactericidal effect with enterococci in some studies (Eliopoulos & Moellering, 1981; Watanakunakorn & Tisone, 1982; Gombert *et al.*, 1983).

In a survey of *Staph. aureus* isolates from endocarditis patients, Scheld & Keeley (1982) found for all 32 strains an MBC ⩽ 0·06 mg/l. These investigators, therefore, studied imipenem, in comparison with nafcillin, in a rabbit endocarditis model with *Staph. aureus*. The dose administered was designed to produce approximately the blood levels achieved in man. In this study, imipenem reduced the *Staph. aureus* counts by 10^6 relative to untreated controls. Nafcillin in the same study reduced counts by only 10^5. Further, more than 50% of the imipenem-treated animals had sterile aortic valves. It was concluded that imipenem was significantly more bactericidal than nafcillin.

Imipenem thus exhibits in many infectious disease models the same broad spectrum and potency that it exhibits *in vitro*. The activity of imipenem is superior to that of many agents proved effective clinically. It is, therefore, expected that imipenem will provide new opportunities for the treatment of difficult clinical infections.

(viii) *Mode of action of imipenem*

Thienamycin was shown in this laboratory (Kahan *et al.*, 1976) to induce the biosynthetic consequences expected of a β-lactam antibiotic. Cell-wall synthesis was arrested, as measured by labelled diaminopimelic acid incorporation into *E. coli*, without interference with protein synthesis. The morphological consequences of inhibition were, however, unusual; Gram-negative rods were converted directly to

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small spheres or ellipsoids without the intervention of elongated filaments as was common for classical penicillins and cephalosporins.

These observations suggested that thienamycin and, by analogy, imipenem interact at a different target in the cross-linking and/or extension of murein. In addition, imipenem has been shown to penetrate the outer membrane barriers more readily than other β-lactam antibiotics. Vuye (1982), using inhibition of intracellular β-lactamase as a measure of levels, showed that imipenem permeates unusually rapidly into *Enterobacter cloacae*.

Bactericidal events appear to follow rapidly upon exposure to imipenem. In studies with clinical isolates of *Serratia marcescens*, Miller, Le Frock & Verder (1981) observed marked morphological changes, including mid-cell defects and spheroplast formation with imipenem at two- to eightfold less than the MIC. Shah *et al.* (1982) showed that imipenem causes rapid killing at the MIC and at 1/4 the MIC with little lag in the onset of cell death for *E. coli, Ser. marcescens, Ps. aeruginosa, Klebsiella pneumoniae, Staph. aureus* and *Str. faecalis*. A similar study by Yourassowsky *et al.* (1982) compared bactericidal kinetics of imipenem and cefsulodin on *Ps. aeruginosa*. Upon addition of the antibiotics, at various multiples of their MIC, imipenem caused an immediate decrease in turbidity, whereas cefsulodin-treated cultures continued to increase in optical density until extensive filament formation was observed. Similarly, Semenitz, Gstraunthaler & Pfaller (1982) reported dramatic changes in microscopic morphology in *E. coli, Pseudomonas* and *Proteus* upon addition of imipenem even at a fraction of the MIC values. The cells were swollen, and the cell wall was separated from the protoplast. The periplasmic space was remarkably widened.

McDonald *et al.* (1982) observed a post-antibiotic effect in *Staph. aureus* and *E. coli* with imipenem that was similar to that observed with ampicillin. In addition, a small post-antibiotic effect was observed with imipenem for *Ps. aeruginosa*, but not with other *Pseudomonas*-active β-lactams. The post-antibiotic effect is believed to have therapeutic relevance since bacteria that are in the recovery phase after the antibiotic has been removed are more susceptible to phagocytosis and intracellular killing (Pruul, Witheral & McDonald, 1981).

The interaction of thienamycin with penicillin-binding proteins was investigated by Spratt, Tobanputra & Zimmerman (1977). Thienamycin bound to all the PBPs of *E. coli*, showing the greatest affinity for PBP-2 and the lowest for PBP-3. The preferential binding to PBP-2 explains the distinctive morphological effects of imipenem reported for this antibiotic. It had been established previously that preferential binding of β-lactams to PBP-2 results in production of round cells. Those agents that do not bind to PBP-2 but have a high affinity for PBP-3, induce elongated filaments (Spratt, 1977). The relevance of binding to PBP-2 to the mode of action of thienamycin was established by the study of a mutant of *E. coli* with an altered PBP-2 protein. In this mutant, the susceptibility to thienamycin decreased significantly and the activity of cephaloridine, which has no affinity for PBP-2, remained unchanged (Spratt, 1978).

Thienamycin binds avidly to PBPs other than PBP-2. For example, it binds to PBP-1 of *E. coli* with higher affinity than most other β-lactams (Spratt, *et al.*, 1977). In *E. coli*, a component of PBP-1 has the properties which are expected of the enzyme peptidoglycan transpeptidase (Spratt, 1977). Moore, Jevons & Brammer (1979) established directly that thienamycin is an exceedingly potent inhibitor of the enzyme —about eightfold more potent than ampicillin. The increased potency of

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thienamycin was correlated with a 10-fold lower MIC than ampicillin for the organism from which the transpeptidase was tested.

Imipenem was studied for inhibition of transpeptidase activities and D-alanine carboxypeptidase I activities of purified PBPs of *E. coli* (Matsuhashi *et al.*, 1981). The antibiotic was more potent than penicillin G as an inhibitor for PBP-1A, PBP-1B, PBP 4 and PBP-5, confirming and extending the work of Moore *et al.* (1979). In a separate study, Ishino and Matsuhashi (1981) tested purified PBP-3 and, in this case, imipenem was many fold less active in keeping with the binding data.

Imipenem thus interacts with several target sites in the cell responsible for the synthesis and cross linking of murein. Its preferential action on PBP-2 prevents filament formation and leads to dramatic morphological and surface disturbances of the cell followed by death and lysis. The exceptional potency of imipenem appears to be correlated with superior penetration into cells and high affinity for an array of PBPs.

II. Metabolism of imipenem in animals and in man

Thienamycin and related carbapenem antibiotics undergo extensive metabolism which varies both in its pattern and extent among different species. Urinary recoveries were unusually low. Yet, in the case of thienamycin and imipenem, their persistence in the circulation, as judged by efficacy against experimental infections or serum half-lives did not appear reduced to the extent often found with highly metabolized agents. This disparity suggested early on that metabolism might be localized in the kidney and biochemical studies to be summarized below have proved that a renal dipeptidase, dehydropeptidase-I, is responsible for the metabolism of thienamycin, imipenem and other carbapenem and penem antibiotics (Kropp *et al.*, 1982).

In Table VI, the pattern of metabolism for thienamycin and imipenem is shown in three animal species. Included is a comparison of the three thienamycin-like antibiotics whose disposition has been evaluated in man: imipenem, MM13902, an intensely metabolized natural carbapenem (Basker *et al.*, 1983, also referred to as epithienamycin F in the independent discovery by Cassidy *et al.* (1981*b*)), and SCH 29482, an orally absorbed [thia] penem antibiotic (Ganguly *et al.*, 1982). The related structures of these antibiotics are shown in Figure 4 which also lists their susceptibilities to hydrolytic inactivation by dehydropeptidase-I.

In the rabbit, the contribution of renal and extrarenal pathways for imipenem elimination were estimated from the reduction in plasma clearance following bilateral ligation of the renal arteries. Non-renal metabolism accounts for only 5% of total clearance, insufficient to explain the 60% deficit in urinary recovery in this species and thus implicating the kidney as the site of major metabolism. In the dog, chimpanzee and in man, localization of metabolism in the kidney can be directly inferred from the finding that renal clearance rates are only a fraction of the glomerular filtration rate. Urinary recoveries of MM13902 in man, were 10-fold lower than imipenem. The increased metabolism of MM13902 occurs in the kidney, since its renal clearance rate was proportionately reduced, sevenfold below that of imipenem, but the plasma clearance rate was only doubled. The renal clearance rates observed with SCH 29482 were the lowest of the three agents compared, in part due to the extensive serum protein binding of this agent.

Low renal clearance rates might be postulated to result from tubular resorption of the antibiotic, followed by metabolism outside the kidney (despite the above evidence

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Table VI. Disposition of imipenem and related antibiotics in laboratory species and in man

Species	Antibiotic[a]	$T_{\frac{1}{2}}$[b] (min)	Urinary recovery (%)	*Clearance rates* (ml/min/kg) Plasma	Renal	Glomerular filtration rate[g]
Rabbit	IPM[b]	16·0	44·0	33·0	14·0	(2·7)
Anephric	IPM[c]	86·0	—	1·4	—	—
Dog	IPM	31·0	8·0	6·2	0·5	4·9
	THM	17·0	5·0	8·6	0·4	—
Chimpanzee	IPM	45·0	14·0	4·4	0·6	2·0
	THM	36·0	12·3	8·4	1·0	—
Man	IPM[d]					
	High Metabolism	55·0	14·0	2·8	0·39	(1·8)
	Low Metabolism	—	30·0	3·2	0·96	—
	MM13902[e]	45·0	1·8	7·4	0·13	—
	Range		(0·1–5·3)			
	SCH 29482[f]	78·0	2·0	(2)	(0·04)	—

[a]Abbreviations: IPM – imipenem (MK0787); THM – thienamycin; MM13902 – N-acetyl-10,11-dehydro-6R,8S-thienamycin-8-sulphate; SCH 29482 – 2-ethyl-thio-6S,8R-hydroxyethyl-penem. (Structures shown in Figure 4(a).

[b]Kropp *et al.*, 1982. Dose rates were 5·0 mg/kg and administered as an intravenous bolus.

[c]Rabbit was rendered anephric by bilateral ligation of renal arteries.

[d]Data from Norrby *et al.* (1983b) for two panels of eight subjects each, grouped according to urinary recovery of imipenem. Individuals with high metabolism showed a range of recoveries from 7 to 17%. Individuals with low metabolism had urinary recoveries from 20 to 37%. Imipenem was administered intravenously at a dose of 500 or 1000 mg.

[e]Based on serum levels reported by Basker *et al.* (1983) following 250 mg intramuscular dose.

[f]Half-life and urinary recoveries reported by Gural *et al.* (1982) following oral administration. Clearance rates computed from reported serum AUC after compensating for estimated absorption of 50% of the dose, the bioavailability reported for various animal species by Loebenberg *et al.* (1982).

[g]Inulin clearance rates; values in parenthesis from handbook references to that species.

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Substrates	R_1	X	R_2	Relative rate of hydrolysis by DHP
Gly-dehydro-Phe	$CHNH_2$	–	C_6H_5	100
thienamycin	*(8R, 6S)* CH_3CHOH	C	$(CH_2)_2NH_2$	1·0
imipenem	*(8R, 6S)* CH_3CHOH	C	$(CH_2)_2NHCH{=}NH$	0·85
MM 13902	*(6S, 8R)* CH_3CHOSO_3H	C	$CH{=}CH{-}NHAc$	30
SCH 29482	*(8R, 6S)* CH_3CHOH	S	CH_2CH_3	0·6

Inhibitors				I_{50} vs DHP, μM porcine	human
benzoyl-NH_2-acrylate	C_6H_5	–	CH_3	30	45
hexenoate	$(CH_3)_2$ (S)	–	CH_2CH_3	0·11	—
MK0789	"	–	$(CH_2)_4CH_3$	0·08	0·08
MK0791 (cilastatin)	"		$(CH_2)_4SCH_2CH(NH_2)COOH$ (L)	0·11	0·13

Figure 4. Kinetic parameters for substrates and inhibitors of renal Dehydropeptidase-I: Relative rates of hydrolysis (Gly-dehydro-Phe = 100) were determined at 0·1 mM of the indicated substrates employing dehydropeptidase-I purified 5100-fold from hog kidney employing, as the final step, absorption to and elution from a sepharose column bearing immobilized MK0791 (Kropp *et al.*, 1982). Inhibitory constants (I_{50}) are the concentrations of the indicated inhibitors which reduced by 50% the rate of hydrolysis of 0·05 mM Gly-dehydro-Phe. Human dehydropeptidase was solubilized and purified 162-fold from extracts of surplus donor kidneys from transplant centres.

for minimal extrarenal metabolism in the rabbit). A test of this alternate hypothesis is afforded by the wide range of urinary recoveries of imipenem that has been observed between individual subjects. Each individual's extent of metabolism has been shown to be a repeatable trait on successive exposures to imipenem (Norrby *et al.*, 1983*a*). Significantly, plasma clearance rates differed little between subjects with the lowest urinary recoveries and those with the highest in these studies. This finding excludes tubular reabsorption as a possible basis for the low renal clearance rates, for had recycling of antibiotic to the circulation been present, plasma clearance in the group with the lower urinary recoveries would have been proportionally reduced and half-life increased. Since for imipenem in the chimpanzee and in man at least one-half of plasma clearance must be allocated to glomerular filtration, metabolism of this fraction of antibiotic must occur during its passage through the lumen of the nephron. We have used the term 'post-excretory metabolism' to describe the extra-systemic location of this portion of imipenem inactivation.

(i) in-vitro *evidence that renal dehydropeptidase-I is responsible for imipenem inactivation*

In their initial studies, Kropp *et al.* (1982), surveyed homogenates of different animal organs for enzymic inactivation of thienamycin. Significant rates of degradation were found only in homogenates of the kidney, a finding consistent with the conclusions of disposition studies. Loss of antibiotic activity in extracts was accompanied by extinction of the ultraviolet-absorbing chromophore of the antibiotic, a hallmark of scission of the β-lactam bond. The observation that the degrading enzyme was membrane-associated and zinc-dependent led to a review of the distribution and properties of known renal metallopeptidases. Most similar was the renal dipeptidase

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which had originally been designated dehydropeptidase-I by its discoverer Greenstein (1948) in view of its broad substrate specificity which included unnatural LD and L-dehydro dipeptides, as well as the natural LL-dipeptides. The structural homology between the susceptible bond in thienamycin and dehydropeptides, (shown in Figure 4) prompted purification of the enzyme from hog kidney by published procedures (Campbell, 1970). The specific activity for hydrolysis of thienamycin was found to increase in parallel with activity on the nominal substrate, glycyl-dehydrophenylalanine (Gly-dehydro-Phe), over the course of a 162-fold purification. Solubilized and purified preparations of porcine renal DHP have a maximum catalytic rate with imipenem which is 10-fold less than that with conventional dipeptide substrates. A 10-fold lower affinity for thienamycin (K_m = 5·7 mM versus 0·6 mM for Gly-dehydro-Phe) results in a net 100-fold lesser activity when tested at equal concentrations (Figure 4). The product of enzyme-catalysed inactivation was proved identical by NMR and mass spectroscopy with that generated by controlled acid hydrolysis of the β-lactam. Dehydropeptidase-I thus acts as an unprecedented mammalian β-lactamase upon thienamycin antibiotics, all the more remarkable considering the stability of thienamycin and imipenem toward classical bacterial β-lactamases. Conversely, the classical penicillins and cephalosporins, despite their sensitivity to bacterial β-lactamases show little or no detectable susceptibility to hydrolysis by dehydropeptidase-I.

(ii) In-vivo *evidence that metabolism of imipenem is mediated by dehydropeptidase-I*

Following administration of radiolabelled imipenem to the rat, rabbit and more recently in man, at least 90% of the administered radioactivity was found in the urine either as intact antibiotic or a fraction identical in its chromatographic mobility to the product of dehydropeptidase-I catalysed hydrolysis. Dehydropeptidase-I's role in metabolism readily explains the very low urinary recovery of *N*-acylated carbapenems of natural origin, of which MM13902 shown above is an example, since these agents are uniformly much more susceptible *in vitro* to dehydropeptidase-I (Kropp *et al.*, 1982). Accounting nicely for the phenomenon of post-excretory metabolism, as defined above, is the subcellular localization of dehydropeptidase-I which has been shown to be associated with the brush-border microvilli of the proximal renal tubule (Welch & Campbell, 1978; Park, 1982). There it has access to antibiotic in the glomerular ultrafiltrate, and as will be shown below, can also degrade antibiotic that enters the tubular epithelium from the circulation in the course of tubular secretion. Perhaps the most satisfying and practical validation of the role of dehydropeptidase-I, is the successful restoration of urinary recovery of imipenem upon coadministration of a class of specific potent inhibitors of this enzyme. The development of these inhibitors, resulting in the choice of cilastatin (MK0791) for combination with imipenem will now be described.

III. Efficacy of cilastatin in controlling renal metabolism of imipenem

(i) *Development of inhibitors of dehydropeptidase-I*

It was a matter of concern that renal metabolism of imipenem in man might result in suboptimal levels of antibiotic in the urinary tract, even with dose regimens sufficient

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for the treatment of systemic infections. For example, in an individual with a 10% urinary recovery, concentrations of imipenem in the urine would fall below any desired level approximately 3 h earlier than those achieved by another non-metabolized antibiotic. (This follows from the fact that concentrations fall 10-fold in a 3 h period for agents with half-lives of 1 h.) Restated non-analytically, there is neither precedent nor a rational basis for expecting urinary tract infections to be reliably treated at dose rates less than one-tenth of those used for treating other infections. It was, therefore, decided to develop an inhibitor of dehydropeptidase-I suitable for co-administration with imipenem. We presumed that this enzyme could be temporarily inhibited without adverse consequences during the limited course of antibiotic therapy because of its extra-systemic location and the minor role it has been postulated to have in normal metabolism; i.e. as a salvage pathway for excreted LL-dipeptides (Welch & Campbell, 1980).

In a directed search for potential inhibitors, compounds resembling or containing the dehydropeptide bond were screened *in vitro* against dehydropeptidase-I. Benzoyl-2-amino-crotonate (Figure 4) was found to have moderate inhibitory activity. Systematic modification of this lead by Ashton *et al.* (1980) resulted in the discovery of a class of 2,2-(+) dimethylcyclopropylcarboxy-2-amino-3-alkyl-(Z)-propenoates with 300-fold greater inhibitory activity against both porcine and human renal dehydropeptidase-I. These inhibitors are competitive and reversible in their action and showed a high degree of specificity. For example, at concentrations up to 1 mM (10,000 times their K_i versus dehydropeptidase-I), little or no inhibition was detected with the following zinc-metallopeptidases: acylase-I (hog kidney), carboxypeptidase-A (bovine pancreas), carboxypeptidase-B (bovine pancreas) and angiotensin-converting enzyme (rat lung). These compounds are also devoid of antimicrobial activity when tested at the highest concentrations likely to be achieved in either blood or urine. By varying the length and polarity of the 3-alkyl sidechain, the half-life of these inhibitors could be matched to that of imipenem. Laboratory evaluation of the efficacy of these inhibitors relied upon the close similarity of every detail of imipenem's disposition in the chimpanzee to that found in man. Renal metabolism was inhibited for the longest duration by two analogues, the octenoate (MK0789) and the L-cysteinyl-thio-hexenoate (cilastatin, MK0791) (Figure 4).

(ii) *Efficacy of cilastatin in restoring urinary recovery of imipenem*

Figure 5(a) and (b) show the effect of co-administered cilastatin on the plasma profile and time course of renal clearance of imipenem in the chimpanzee as measured in a series of cross-over studies with one individual. Imipenem, alone or in combination, was administered as an intravenous bolus in one comparison. In the second, imipenem was administered as an intramuscular suspension with or without cilastatin, the latter being in solution as the suspending vehicle. In the intramuscular case, prolonged blood levels of imipenem were achieved by virtue of the limited solubility of imipenem, *c.* 10 mg/ml, which establishes a slow release of antibiotic from the site of injection (Kropp *et al.*, 1983). Note that the dose rate of each agent in the intramuscular study was about three times greater than that used in the intravenous bolus. As shown in Table VII, overall urinary recoveries of imipenem were increased from six- to eight-fold by co-administered cilastatin.

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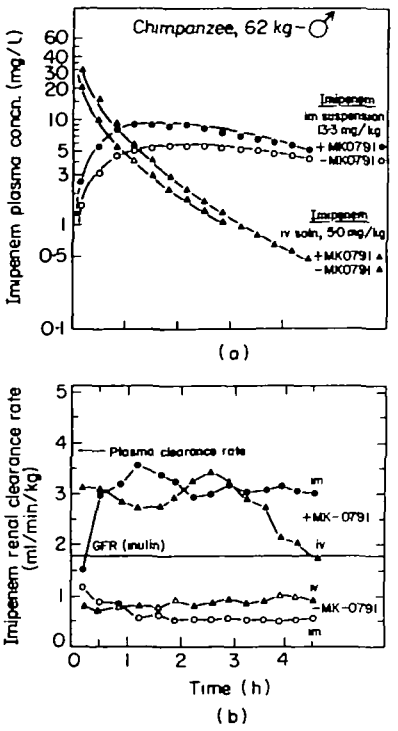

Figure 5. **(a)** Single-subject comparison of disposition in an anaesthetized chimpanzee of imipenem injected as an intramuscular suspension or an intravenous bolus in each case with or without an equal weight of the renal dehydropeptidase inhibitor, cilastatin. Intramuscular injections were 3·3 to 3·5 ml of a 250 mg/ml suspension of MK0787 in water or MK0791 solution. Intravenous injections were 15·5 ml of 20 mg/ml supersaturated solutions injected over 1 to 2 mins. The chimpanzee 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 4·7 h. Each subject was catheterized for urine recovery at successive 20-min intervals.

(b) Incremental clearance rates measured in successive 20-min urine collections for the single-subject comparison of the effect of cilastatin on the disposition of an intravenous or intramuscular dose of imipenem [details under 5(a)]. Indicated to scale are the mean plasma clearance rates for imipenem determined from the intravenous study of imipenem plus cilastatin and the mean value for Glomerular filtration rate determined in four chimpanzees utilizing continuously infused inulin [see Figure 6(a)].

Restoration of high urinary concentrations over a duration comparable with that obtained with non-metabolized antibiotics was our stated objective. Hence, the time-course of metabolic blockade was considered a more relevant criterion of an inhibitor's efficacy than total urinary recovery, most of which occurs within the first 90 min for antibiotics with half-lives of 1 h. A quantitative measure of the span of effective inhibition is obtained from measurements of incremental renal clearance (RCl_i) computed from successive 20-min collections of urine and the corresponding segment of area under the plasma curve. RCl_i reached a maximum value which was approximately 75% of the plasma clearance rate and 50% higher than glomerular filtration rate, giving clear evidence of a hitherto masked fraction of the renal clearance of imipenem that must be mediated by secretion. At 4 mg/kg of cilastatin, RCl_i declined to glomerular filtration rate by 4·5 h. With the 13 mg/kg dose, RCl_i was

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Table VII. Pharmacokinetic parameters for imipenem and cilastatin administered separately or in combination to a chimpanzee

Component and route	Dose (mg/kg) imipenem	Dose (mg/kg) cilastatin	Plasma C_{max} mg/l	Plasma $T_{\frac{1}{2}\beta}$ h	Plasma AUC (mg/l *h) 0–4·5	Plasma AUC (mg/l *h) 0–∞	Urinary recovery (%) 0–4·5	Urinary recovery (%) 0–∞	Clearance rates (ml/min/kg) Cl_p	Clearance rates (ml/min/kg) Cl_r
Imipenem										
IV	5	0	20·0	0·70	18·5	19·0	12·2	13·0	4·75	0·6
IV	5	4	30·0	0·73	21·4	22·0	75·8	77·8	3·8	2·9
IM	13	0	6·0	2·3	20·6	36·3	4·6	8·1	—	—
IM	13	13	9·0	2·4	28·6	44·8	42·5	63·3	—	—
Cilastatin										
IV	5	4	21·0	0·95	13·27	14·26	117·0	126·0	4·67	5·5
IM	13	13	12·6	1·2	25·9	26·0	97·4	⩾98·0	—	—

*Cilastatin levels were determined by measurement of inhibitory titre against porcine renal dehydropeptidase-I. This assay responds to both parent cilastatin and its *N*-acetyl metabolite. The latter has twice the inhibitory activity of cilastatin

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maintained at the higher value, 75% of plasma clearance, throughout the study except for reduced rates during the first hour after injection, the significance of which will be discussed below. In studies with normal volunteers (Norrby *et al.*, 1983*b*), both MK0789 and cilastatin restored urinary recovery of imipenem to a highly consistent value (around 70%) regardless of the subject's baseline urinary recovery, which ranged from 6% to 40%. RCl_i was maintained above or at glomerular filtration rate for up to 7 h when 500 mg cilastatin was combined in a 1 : 1 ratio with imipenem.

In addition to the intended enhancement of urinary recovery, an elevation of imipenem plasma levels and plasma AUC was observed in the chimpanzee which was more prominent at the higher dose used in the intramuscular study. Similarly, in man, a 20% elevation of AUC is observed when as little as 250 mg cilastatin is combined with imipenem. Plasma clearance tends to decline further in man when the cilastatin dose rate reaches 1000 mg (Norrby *et al.*, 1983*b*). In both man and in these chimpanzee studies, the mechanism by which cilastatin reduces plasma clearance rates of imipenem did not persist throughout the study period, since terminal elimination half-lives were not increased.

(iii) *Disposition of cilastatin*

Pharmacokinetic parameters for cilastatin co-administered with imipenem, listed in Table VII, were based on assays of the inhibitory titre of plasma and urine samples when tested against purified renal dehydropeptidase-I acting on Gly-dehydro-Phe as substrate. Half-life is almost identical with that of imipenem, and increases to 70 min upon intramuscular injection. Urinary recoveries equivalent to *c.* 120% of the administered dose were consistently observed with the chimpanzee and the Rhesus monkey. An acidic metabolite with inhibitory activity on dehydropeptidase-I was subsequently isolated from urine and shown to be cilastatin acetylated on the cysteinyl amino group. Synthetic *N*-acetyl cilastatin had an I_{50} of 0·06 μM; i.e. twice the potency of cilastatin accounting thereby for the initial observation of excess urinary recovery of inhibitory equivalents. Fractionation of the urine, showed that 70% of the administered dose was recovered as parent cilastatin, the remainder being recovered as the *N*-acetyl metabolite. *N*-Acetyl cilastatin was shown to be as effective as cilastatin in restoring overall urinary recovery of imipenem in the chimpanzee. However, its relatively short half-life, 0·5 h, precluded its use in place of cilastatin in combination with imipenem. The renal clearance rate for cilastatin itself, (3·3 ml/min/mg) is in excess of the inulin clearance rate in the chimpanzee (1·8 to 2·2 ml/min/kg), showing it to be subject to tubular secretion. Surprisingly, levels of *N*-acetyl-cilastatin measured directly in solvent extracts of plasma were either below detection levels or reached no more than 5% of concurrent plasma levels. Yet the proportion of cilastatin recovered in the urine as the *N*-acetyl derivative held constant at 30% for at least 2 h following drug administration. This finding could be explained if acetylation occurred exclusively in the kidney and if the *N*-acetyl-cilastatin was transferred directly to the urine, with minimal reflux to the circulation. In man, cilastatin undergoes biotransformation to a comparable extent. Furthermore, the half-life of cilastatin is increased well beyond that of imipenem in subjects with severe renal insufficiency (Verpooten *et al.*, 1983). These findings are consistent with localization in the kidney of the bulk of cilastatin metabolism.

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(iv) *Pathway of renal clearance of imipenem*

Prior to the availability of dehydropeptidase inhibitors, details of the tubular handling of imipenem were obscured by massive renal metabolism. For example, when imipenem was combined with probenecid to evaluate the presence of anionic secretion, plasma levels were not significantly elevated and the renal clearance rate (Cl_r) of surviving antibiotic underwent only a partial reduction (Figure 6). Similar negative effects were reported with man (Norrby *et al.*, 1983*a*). Yet the achievement of renal clearance rates exceeding glomerular filtration rate, as shown above, in the presence of cilastatin can only be explained by the presence of a competent tubular secretion pathway for imipenem. In a study to demonstrate whether this unmasked pathway is sensitive to probenecid, the hexenoate homologue shown in Figure 4 was employed.

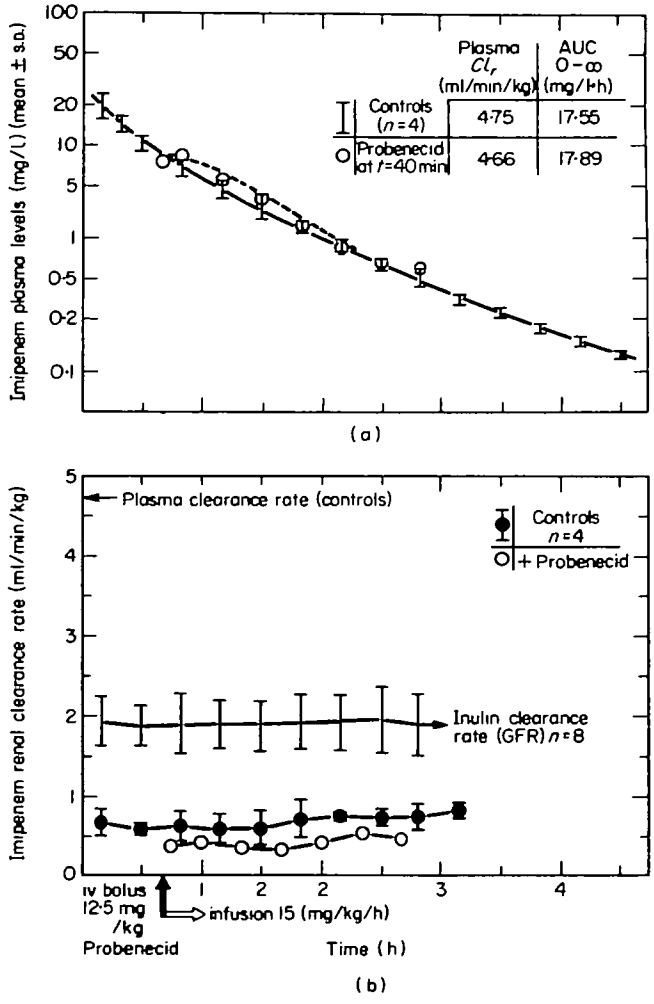

Figure 6. (a) and (b) Effect of an intercurrent probenecid administration on the plasma profile (a) and incremental renal clearance rate (b) of imipenem in the chimpanzee. Imipenem (5 mg/kg) was injected as an intravenous bolus followed at 40 min by probenecid. The latter was given as an intravenous bolus loading dose (12·5 mg/kg) and a maintenance infusion (15 mg/kg/h). The average disposition of imipenem in four subjects (males, 60 ± 4 kg) is shown for comparison.

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This inhibitor has a short half-life, 24 min, which is manifested in the time course of RCl_i (Figure 7). After reaching a peak which is 76% of the plasma clearance rate (Cl_p), it declines rapidly to values approaching baseline Cl_r for imipenem. (This exemplifies *in vivo* the reversible nature of inhibition of dehydropeptidase-I by this class of compounds.) Upon further combination with probenecid, imipenem plasma levels and AUC were elevated; the terminal elimination half-life was increased to 0·94 h and RCl_i was suppressed to a value equivalent with the inulin clearance rate in the chimpanzee [Figure 7(b)]. Imipenem was thus proven to undergo net tubular secretion provided that it was protected from destruction in transit through the cell. Dehydropeptidase must, therefore, have access both to the transtubular flux of antibiotic as well as the flux in the lumen that originates from filtration.

A model is proposed in Figure 8 to account for the fate of imipenem in the absence and presence of dehydropeptidase inhibitors. Since maximal RCl_i in the presence of inhibitor was three-quarters of Cl_p and since the maximum urinary recovery observed in man at 1000 mg cilastatin was 75% (as in the chimpanzee), about one-quarter of the

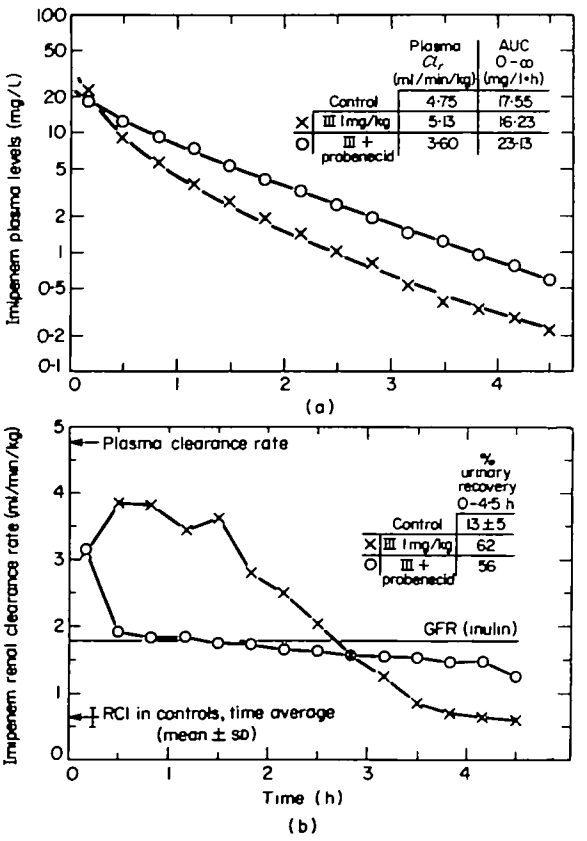

Figure 7. (a) and (b) Effect of probenecid infusion on the plasma profile (a) and incremental renal clearance rate (b) of imipenem co-administered with a renal dehydropeptidase inhibitor in a male chimpanzee. In both studies, imipenem (5 mg/kg) was co-administered as an intravenous bolus with 1 mg/kg of the hexenoate homologue (structure in Figure 4) indicated as 'III' in these figures. Probenecid was administered starting with a loading dose of 12·5 mg/kg 10 min prior to the imipenem/inhibitor injection and was maintained by infusion at 15 mg/kg throughout the study. Control values for imipenem administered alone and for glomerular filtration rate were determined on four male chimpanzees.

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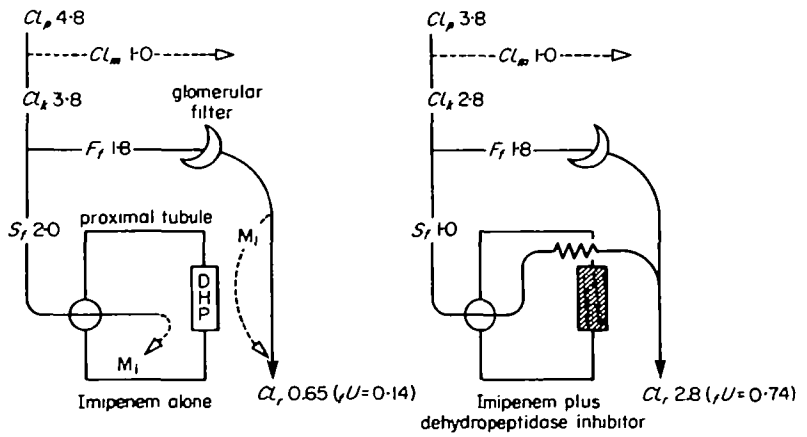

Figure 8. Model of renal clearance pathways accounting for the disposition of imipenem in the absence and presence of inhibitors of renal Dehydropeptidase-I: The proximal tubule is shown with dehydropeptidase-I imbedded on the lumenal surface to the right. Designated clearance rates (ml/min/kg) were determined in the chimpanzee and apply, in the case of co-administered dehydropeptidase-I inhibitors, to the period of maximal inhibition. Metabolic pathways are indicated by open arrows. Symbols used are: Cl_p, plasma clearance rate; Cl_m, non-renal metabolism; Cl_k, input renal clearance F_f, filtered fraction; S_f, secreted fraction; M_l, lumenal metabolism; M_i, intratubular metabolism; Cl_r, urinary clearance of intact antibiotic; fU, fraction of the dose recovered in urine. Entry of both imipenem and dehydropeptidase inhibitors is mediated by a probenecid sensitive anionic transport site shown at the contraluminal surface. The reduced secreted fraction resulting from inhibition of dehydropeptidase-I is suggested to result from impedance to net trans-tubular when imipenem accumulates to intratubular levels exceeding those in the circulation.

observed plasma clearance rate must be attributable to a non-inhibitable pathway of degradation. This pathway is probably extrarenal, since imipenem has been found in human volunteers with increasing degrees of pre-existent renal insufficiency to reach a limiting half-life of approximately 3 h (Verpooten, Verbist & De Broe, 1983). Of the input flux of imipenem undergoing clearance by the kidney, approximately two-thirds can be assigned to glomerular filtration; the remainder enters the tubular epithelium. In the absence of inhibitor, the surviving concentration of imipenem at this level of the tubule must be below that present in the circulation. Dehydropeptidase-mediated degradation thus provides a metabolic sink to which imipenem can flow passively. We presume that the minimal effect of probenecid on the disposition of imipenem alone reflects this absence of active transport. The net secretion of imipenem observed in the presence of dehydropeptidase inhibitors does, however, require active transport. Under such circumstances, probenecid can exert its expected effect.

To account for the reduced plasma clearance rates and elevated early plasma levels resulting from combination of dehydropeptidase inhibitors, the fraction of kidney clearance entering the tubule (the secreted fraction) must be presumed to decrease two-fold relative to that entering and undergoing degradation in the absence of inhibitors. The reduction in transtubular flux is attributed to impedance generated by the intratubular accumulation of impenem to a level exceeding that in circulation. As plasma levels of inhibitor decline, a point is reached where intratubular degradation can no longer be suppressed. Intratubular accumulation of imipenem and net secretion then ceases, the impedance disappears and the plasma clearance rate of imipenem increases to the baseline value for imipenem alone. This sequence readily explains the

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findings in both these studies and in man, that AUC can be elevated by co-administered cilastatin without increasing the terminal elimination half-life. A measure of the intensity of intratubular metabolism is provided by the finding that plasma levels of cilastatin at the third hour in the intravenous study shown in Figure 5(a) were 1 μM, corresponding to 10 times the I_{50} of dehydropeptidase-I *in vitro*. At that time, the secretory contribution to RCl_t became diminished. This implies that dehydropeptidase-I levels directed toward intratubular imipenem are well in excess of the minimum required to destroy antibiotic during its residence time within the cell, thereby establishing the aforementioned metabolic sink that drives passive entry of imipenem when administered alone. Conversely, the fact that any portion of the filtered fraction of imipenem survives when it is administered alone, implies that dehydropeptidase is rate limiting for that phase of metabolism. Thus the low levels of cilastatin present late after administration can readily preserve the entire filtered fraction against inactivation.

(v) *Protection afforded by cilastatin against the nephrotoxic potential of imipenem in laboratory animals*

When administered to the rabbit at dose rates in excess of 100 mg/kg, imipenem induces acute proximal tubular necrosis. Cilastatin when co-administered with imipenem at a ratio of 1 : 1 prevents the occurrence of necrosis at the highest dose rate tested, 360 mg/kg of imipenem +360 mg/kg of cilastatin. The dehydropeptidase-I inhibitor, MK0789, was also effective in preventing nephrotoxicity. Studies with the rabbit in the Merck laboratories (MacDonald *et al.*, in preparation) support the hypothesis that protection results from competition between cilastatin and imipenem for a common pathway of tubular entry. The fraction of imipenem excluded from the tubule is eliminated by filtration. The proposed mechanism of protection is analagous with that proposed by Tune (1972) to explain the reversal of nephrotoxicity of cephaloridine in the rabbit by co-administered probenecid, para-aminohippurate or benzylpenicillin. Pharmacokinetic evidence that cilastatin can suppress the tubular secretion of imipenem at therapeutically relevant dose rates, which are less than one-tenth of those used in toxicological experiments in the rabbit, can be inferred from the chimpanzee study employing 13 mg/kg cilastatin in combination with 13 mg/kg imipenem administered as an intramuscular suspension [see Figure 5(a) and (b)]. Incremental renal clearance rates were initially suppressed to glomerular filtration rate, and reached their maximum sustained rate only after 90 min. During this same interval, plasma levels of imipenem were conspicuously elevated relative to the treatment with imipenem alone. These joint observations establish that secretion of imipenem was suppressed at least transiently, much as is achieved on a continuous basis when probenecid and a dehydropeptidase-I inhibitor are both administered with imipenem as shown in Figure 7(b).

The identity of the agent responsible for the nephrotoxicity of imipenem when it is administered alone has yet to be proven. By analogy with the findings for cephaloridine in the rabbit (Tune, 1972), the immediate nephrotoxic principle might be expected to accumulate in the tubular epithelium and persist there well after elimination of imipenem from the circulation. Accumulation of imipenem *per se* seemed unlikely, since extensive intratubular metabolism had, in the case of the chimpanzee and in man, been implicated to account for pharmacokinetic findings.

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Furthermore, intratubular imipenem that survived dehydropeptidase-I should not accumulate since imipenem (unlike cephaloridine) possesses a competent pathway for egress from the tubule. Accumulation of imipenem-related metabolites to a level comparable with that found by Tune for intact cephaloridine has, however, been demonstrated in the renal cortex of rabbits receiving [^{14}C]-imipenem at a dose known to cause proximal tubular degeneration. As shown in Table VIII, two radiolabelled metabolites of imipenem together account for 8% of the administered dose. A majority of radioactivity was found as hydrolysed imipenem (imipenem-OH), the product of dehydropeptidase-I-mediated metabolism. A second metabolite is unique to the kidney, being undetectable in either plasma or urine. R. Hajdu in this laboratory has shown this metabolite (imipenem-cysteine) to be chromatographically identical with the initial and spontaneously rearranged products formed by reacting imipenem with cysteine. They are presumed to be, respectively, the thiol-ester and the peptide formed when cysteine reacts with imipenem's lactam carbonyl.* Intact imipenem was present at low levels, even after *ex vivo* degradation has been minimized by homogenization of the excised cortex in buffers containing high concentrations of cilastatin.

When the dehydropeptidase-I inhibitor, MK0789, was co-administered at a dose ratio of imipenem that eliminates nephrotoxicity, cortical levels of both imipenem-OH and the already low levels of imipenem were both reduced seven-fold. Imipenem-cysteine was reduced only 1·7-fold, suggesting that neither this metabolite nor any alteration of intracellular glutathione pools resulting from its formation, were implicated in causation of nephrotoxicity. Accompanying pharmacokinetic findings shown in Table VIII attest to the blockade of secretion imposed by MK0789. The Cl_p for imipenem in the absence of MK0789 was already only one-third of the rate found at low dose rates suggesting thereby that tubular transport was substantially saturated. Of the observed 11·8 ml/min/kg, approximately 4 ml/min/kg was attributable to the sum of filtration plus extrarenal metabolism (see Table VIII). The 5·5 ml/min/kg reduction imposed by MK0789 thus represents a 70% blockade of the secreted fraction.

(vi) *Rationale for combining cilastatin with imipenem in all therapeutic applications*

At first sight, the fixed-ratio combination of imipenem with cilastatin invites comparison of its underlying rationale with that of the two already established fixed-ratio combination antimicrobials, trimethoprim/sulphamethoxazole and amoxicillin/clavulanate. In the latter two cases, both components are directed against bacterial metabolic processes. Incremental benefit is achieved by mutual potentiation of antibacterial activity against that fraction of organisms insensitive to either component or by restoration of activity against a fraction of strains exhibiting pre-existent resistance to one of the components. In the present case, however, imipenem is the sole antimicrobial principle. Its breadth of spectrum, potency and lack of cross-resistance with other antibiotics leaves little that could be improved upon by a co-administered antimicrobial partner. Cilastatin is instead directed to the control of antibiotic inactivation exhibited, in varying degrees, in all individuals so far examined. This role of cilastatin is closely analogous to that of carbidopa in the carbidopa/levodopa

*Imipenem has been shown to react rapidly with β-aminothiols, such as cysteine, but much more slowly with glutathione (Kahan *et al.*, 1979). Potential non-enzymic pathways for the formation of imipenem-cysteine include reaction either with free cysteine or with an *N*-terminal cysteine intermediate (e.g. cysteine-glycine) formed in the glutathione cycle. (Meister, 1983).

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Table VIII. Effect of co-administered Dehydropeptidase inhibitor MK0789 on distribution and clearance of [^{14}C]imipenem[a] and imipenem-metabolites in the rabbit

Dose imipenem	142·0	128·0
mg/kg MK0789	0·0	90·0
Imipenem in plasma		
Concn (mg/l)		
30 min	140·0	230·0
60 min	10·0	20·0
AUC, 0–120 (mg/l *h)	201·0	342·0
Cl_p (ml/min/kg)	11·8	6·3
Kidney cortex		
at 120 min (μg/g)		
Imipenem	60·0	8·0
Imipenem-OH[b]	1200·0	160·0
Imipenem-Cys[c]	1060·0	570·0
Urinary recovery		
(0–120 min)		
Imipenem – % of dose	29·0	58·0
Imipenem-OH	58·0	17·0

Indicated dose rates of [^{14}C]imipenem were administered in super-saturated solution, 100 mg/ml, with or without MK0789 (structure shown in Figure 4). Rabbits were male with body weights of 2·5 kg. Urine was collected by catherization. At 120 min, kidney was removed, cortex excised on ice and homogenized in 1 vol. of 0·1 M MK0789, pH 7·0. metabolites in cortex homogenate and urine were separated by HPLC using methods described for imipenem-OH by Kropp *et al.* (1982). Imipenem-Cys was in two peaks with retention times immediately preceding and coinciding with imipenem. The levels of imipenem were determined by A_{310nm} during HPLC and confirmed by bioassay in collected fractions.

[a]Labelled in formate carbon of formimidoyl sidechain.

[b]Hydrolysed imipenem.

[c]7-Cysteinyl conjugate of imipenem

combination. In both cases, the 'active' ingredient is spared from universally present and undesirable metabolism and can achieve efficacy at a lower dose with attendant economic and safety benefits.

The benefit of restoring effective urinary concentrations of imipenem by co-administered cilastatin can be argued to extend beyond the explicit treatment of urinary tract infections. The treatment of Gram-negative septicaemias that have ascended from the urinary tract provides one obvious example. Even where the urinary tract is not the originating focus for infection, theoretical concern is warranted over the potential sanctuary for bacterial persistence the kidney or urinary tract might provide if local metabolism were allowed to reduce imipenem levels below effective levels for substantial intervals throughout therapy. A final justification for universal combination of cilastatin with imipenem is the additional margin of safety provided. To the extent that the nephrotoxic potential exhibited by high doses of imipenem administered alone to the rabbit raise concern over its safety in man, the absence of renal damage in that species when much higher doses of imipenem/cilastatin were

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administered should, *pari passu*, provide confidence that this combination is likely to exhibit the tolerability that is expected of β-lactam antibiotics in man.

References

Albers-Schonberg, G., Arison, B. H., Hensens, O. D., Hirshfield, J., Hoogsteen, K., Kaczka, E. A., Rhodes, R. E., Kahan, J. S., Kahan, F. M., Ratcliffe, R. W., Walton, E., Ruswinkle, L. J., Morin, R. B. & Christensen, B. G. (1978). Structure and absolute configuration of thienamycin. *Journal of the American Chemical Society* **100**, 6491–9.

Alford, R. H. (1983). Comparison of ceftazidime and *N*-formimidoyl thienamycin against gentamicin-susceptible and resistant *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy* **11**, 599–601.

Ashton, W. T., Barash, L., Brown, J. E., Brown, R. D., Canning, L. F., Chen, A., Graham. D. W., Kahan, F. M., Kropp, H., Sundelof, J. G. & Rogers, E. F. (1980). Z-2-Acylamino-3-substituted propenoates, inhibitors of the renal dipeptidase (dehydropeptidase-I) responsible for thienamycin metabolism. *20th Interscience Conference on Antimicrobial Agents and Chemotherapy*, New Orleans, Louisiana, Abstract 271.

Auckenthaler, R., Wilson, W. R., Wright, A. J., Washington II, J. A., Durack, D. T. & Geraci, J. E. (1982). Lack of *in vivo* and *in vitro* bactericidal activity of *N*-formimidoyl thienamycin against enterococci. *Antimicrobial Agents and Chemotherapy* **22**, 448–52.

Bartmann, K. & Tarbuc, R. (1982). *N*-Formimidoyl thienamycin—*in vitro* activity against bacteria resistant to beta-lactam antibiotics or gentamicin. *Infection* **10**, 361–70.

Basker, M. J., Boon, R. J., Box, S. J., Prestige, E. A., Smith, G. M. & Spear, S. R (1983). Properties of MM 13902 and other carbapenem antibiotics *in vitro* and *in vivo*. *Journal of Antibiotics* **36**, 416–22.

Blumenthal, R. M., Raeder, R., Takemoto, C. D. & Freimer, E. H. (1983). Occurrence and expression of imipemide (*N*-formimidoyl thienamycin) resistance in clinical isolates of coagulase-negative *Staphylococci*. *Antimicrobial Agents and Chemotherapy* **24**, 61–9.

Borobio, M. V., Nogales, M. C., Pascual, A. & Perea, E. J. (1981). *N*-Formimidoyl thienamycin activity against anaerobes: effect of the inoculum, pH and culture media. *Journal of Antimicrobial Chemotherapy* **8**, 213–8.

Calderwood, S. B., Gardella, A., Philippon, A. M., Jacoby, G. A. & Moellering, R. C., Jr. (1982). Effects of azlocillin in combination with clavulanic acid, sulbactam and *N*-formimidoyl thienamycin against beta-lactamase-producing carbenicillin-resistant *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **22**, 266–71.

Campbell, B. J. (1970). Renal dipeptidase. In *Methods in Enzymology* (Colowick, S. P. & Kaplan, N. O., Eds.), vol 19, pp. 722–9. Academic Press, New York.

Cassidy, P. J. (1981*a*). Novel naturally occurring beta-lactam antibiotics—a review. *Developments in Industrial Microbiology* **22**, 181–209.

Cassidy, P. J., Albers-Schonberg, G., Goegelman, R. T., Miller, T., Arison, B., Stapley, E. O. & Birnbaum, J. (1981*b*). Epithienamycins. II. Isolation and Structure Assignment. *Journal of Antibiotics* **34**, 637–48.

Chabbert, Y. A. & Jaffe, A. (1982). Sch 29482: Activity against susceptible and beta-lactam resistant variants of Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy* **9**, 203–12.

Cherubin, C. E., Corrado, M. L., Sierra, M. F., Gombert, M. E. & Schulman, M. (1981). Susceptibility of Gram-positive cocci to various antibiotics, including cefotaxime, moxalactam, and *N*-formimidoyl thienamycin. *Antimicrobial Agents and Chemotherapy* **20**, 553–5.

Christensen, B. G. (1981). Structure activity relationship in beta-lactam antibiotics. In *Beta-Lactam Antibiotics. Mode of Action, New Developments, and Future Prospects* (Salton, M. E. J. & Schockman, G. D., Eds.), pp. 101–125. Academic Press, London.

Cullmann, W., Stieglitz, M., Werkmeister, V. & Opferkuch, W. (1982*a*). Susceptibility of ampicillin-resistant strains of enterobacteria and *Pseudomonas* to thienamycin. *Current Chemotherapy and Immunotherapy vol 1*, pp. 714–6. Proceedings of the 12th International Congress of Chemotherapy.

Cullmann, W., Opferkuch, W., Stieglitz, M. & Werkmeister, U. (1982*b*). A comparison of the antibacterial activities of *N*-formimidoyl thienamycin (MK0787) with those of other

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recently developed beta-lactam derivatives. *Antimicrobial Agents and Chemotherapy* **22,** 302–7.

Diez Enciso, M., Mateos Lindemann, M. L. & Gutierrez Altes, A. (1982). *In vitro* evaluation of *N*-formimidoyl thienamycin (MK-0787) combined with amikacin against Gram-negative bacilli and *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **22,** 1064–6.

Dudek, E. J., Stephenson, J. D., Bohnhoff, M. & Lerner, S. A. (1982). Susceptibility of *Neisseria meningitidis* and *Neisseria gonorrhoeae* isolates to *N*-formimidoyl thienamycin. *Antimicrobial Agents and Chemotherapy* **22,** 926–9.

Eliopoulos, G. M. & Moellering, R. C., Jr. (1981). Susceptibility of enterococcus and *Listeria monocytogenes to N*-formimidoyl thienamycin alone and in combination with an aminoglycoside. *Antimicrobial Agents and Chemotherapy* **19,** 789–93.

Fainstein, V., Le Blanc, B., Weaver, S. & Bodey, G. P. (1982) A comparative *in vitro* study of thienamycin. *Infection* **10,** 50–2.

Follath, F., Geddes, A. M., Spring, P., Ball, A. P., Jones, K. H., Ferber, F., Kahan, J. S. & Kahan, F. M. (1981). Tolerability and Pharmacokinetics of Single Doses of *N*-formimidoyl thienamycin. *21st Interscience Conference on Antimicrobial Agents and Chemotherapy,* Chicago, Illinois, Abstract 590.

Funk, E. A., Laun, P. R. & Strausbaugh, L. J. (1982). Penetration of *N*-formimidoyl thienamycin (NFT) into cerebrospinal fluid (CSF) in rabbits. *22nd Interscience Conference on Antimicrobial Agents and Chemotherapy,* Miami Beach, Florida, Abstract 233.

Gombert, M. E., Berkowitz, L. B. & Cummings, M. C. (1983). Synergistic effect of *N*-formimidoyl thienamycin with gentamicin and amikacin against *Streptococcus faecalis*. *Antimicrobial Agents and Chemotherapy* **23,** 245–7.

Ganguly, A. K., Girijavallabhan, V. M., McCombie, S., Pinto, P., Rizvi, R., Jeffrey, P. D. & Lin, S. (1982). Synthesis of Sch 29482—a novel penem antibiotic. *Journal of Antimicrobial Chemotherapy* **9,** 1–5.

Greenstein, J. P. (1948). *Advances in Enzymology and Related Subjects of Biochemistry* (Nord, F. F., Ed.), pp. 117–69. Interscience Publish., Inc., New York.

Gural, R., Lin, C., Chung, M., Oden, E. & Digiore, C. (1982). Oral absorption and tolerance in man, of a new penem antibiotic, Sch 29482. *Journal of Antimicrobial Chemotherapy* **9,** 239–43.

Hanslo, A. K., King, A., Shannon, K., Warren, C. & Phillips, I. (1981). *N*-formimidoyl thienamycin (MK0787): *In vitro* antibacterial activity and susceptibility to beta-lactamases compared with that of cefotaxime, moxalactam and other beta-lactam antibiotics. *Journal of Antimicrobial Chemotherapy* **7,** 607–17.

Hanslo, D., Phillips, I., Shannon, K., King, A. & Warren, C. (1982). Activity of *N*-formimidoyl thienamycin (MK0787) against beta-lactam resistant enterobacteria and *Pseudomonads* and its sensitivity to beta-lactamases. *Current Chemotherapy and Immunotherapy vol. 1,* pp. 710–2. Proceedings of the 12th International Congress of Chemotherapy.

Hau, T., Phuangsab, A. & Nishikawa, R. A. (1982). MK0787 in the treatment of intraperitoneal polymicrobial infections. *Annual Meeting of the American Society of Microbiology,* Dallas, Texas, Abstract A61.

Horadam, V. W., Smilack, J. D., Montgomery, C. L. & Werringloer, J. (1980). *In vitro* activity of *N*-formimidoyl thienamycin (MK0787), a crystalline derivative of thienamycin. *Antimicrobial Agents and Chemotherapy* **18,** 557–61.

Howard, A. J. & Hince, C. E. (1982). The activity of *N*-formimidoyl thienamycin (MK0787) against *Haemophilus influenzae* and *Streptococcus pneumoniae*. *Journal of Antimicrobial Chemotherapy* **10,** 383–90.

Ishino, F. & Matsuhashi, M. (1981). Peptidoglycan synthetic enzyme activities of highly purified penicillin-binding protein 3 in *Escherichia coli*: a septum-forming reaction sequence. *Biochemical and Biophysical Research Communications* **101,** 905–11.

Johnson, D. E., Calia, F. M., Snyder, M. J., Warren, J. W. & Schimpff, S. C. (1982). *N*-formimidoyl thienamycin (MK0787) therapy of *Pseudomonas aeruginosa* bacteremia in neutropenic rats. *22nd Interscience Conference on Antimicrobial Agents and Chemotherapy,* Miami Beach, Florida, Abstract 811.

Kahan, J. S., Kahan, F. M., Goegelman, R., Currie, S. A., Jackson, M., Stapley, E. O., Miller, T. W., Miller, A. K., Hendlin, D., Mochales, S., Hernandez, S. & Woodruff, H. B. (1976). Thienamycin. A new beta-lactam antibiotic. I. Discovery and isolation. *16th Interscience*

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Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, Abstract 227.

Kahan, J. S., Kahan, F. M., Goegelman, R., Currie, S. A., Jackson, M., Stapley, E. O., Miller, T. W., Miller, A. K., Hendlin, D., Mochales, S., Hernandez, S., Woodruff, H. B. & Birnbaum, J. (1979). Thienamycin, a new beta-lactam antibiotic: I. Discovery, taxonomy, isolation and physical properties. *Journal of Antibiotics* **32,** 1–12.

Kallick, C., Rice, T., Norsen, J., Rajashekaraiah, K., Marsh, D., McCulley, D. & Thadhani, K. (1982). *In vitro* activity of N-formimidoyl thienamycin against *Pseudomonas* and Staphylococci associated with endocarditis. *Current Chemotherapy and Immunotherapy,* **1,** 725–6. Proceedings of the 12th International Congress of Chemotherapy

Kesado, T., Hashizume, T. & Asahi, Y. (1980). Antibacterial activities of a new stabilized thienamycin, *N*-formimidoyl thienamycin in comparison with other antibiotics. *Antimicrobial Agents and Chemotherapy* **17,** 912–7.

Kim, H. S. P. (1982). Cellular orientation of renal dipeptidase in the microvilli and its catalytic activity on a novel beta-lactam antibiotic, N-formimidoyl thienamycin. Dissertation for Ph.D., University of Missouri, Columbia, University Microfilms International (DA 8226184).

Krogstad, D. J. & Parquette, A. R. (1980). Defective killing of enterococci: A common property of antimicrobial agents acting on the cell wall. *Antimicrobial Agents and Chemotherapy* **17,** 965–8.

Kropp, H., Kahan, J. S., Kahan, F. M., Sundelof, J., Darland, G. & Birnbaum, J. (1976). Thienamycin: *In vitro* and *in vivo* evaluation. *15th Interscience Conference on Antimicrobial Agents and Chemotherapy,* Chicago, Illinois, Abstract 228.

Kropp, H., Sundelof, J. G., Kahan, J. S., Kahan, F. M. & Birnbaum, J. (1979). MK0787 (N-formimidoyl thienamycin): Evaluation of *in vitro* and *in vivo* activities. *19th Interscience Conference on Antimicrobial Agents and Chemotherapy,* Boston, Massachusetts, Abstract 231.

Kropp, H., Sundelof, J. G., Kahan, J. S., Kahan, F. M. & Birnbaum, J. (1980*a*). MK0787 (*N*-formimidoyl thienamycin): Evaluation of *in vitro* and *in vivo* activities. *Antimicrobial Agents and Chemotherapy* **17,** 993–1000.

Kropp, H., Sundelof, J. G., Bohn, D. L. & Kahan, F. M. (1980*b*). Improved urinary tract bioavailability of MK0787 (*N*-formimidoyl thienamycin) when co-administered with inhibitors of renal Dehydropeptidase-I. *20th Interscience Conference on Antimicrobial Agents and Chemotherapy,* New Orleans, Louisiana, Abstract 270.

Kropp, H., Sundelof, J. G. Hajdu, R. & Kahan, F. M. (1980*c*). Metabolism of thienamycin & related carbapenem antibiotics by the renal dipeptidase: Dehydropeptidase-I. *20th Interscience Conference on Antimicrobial Agents and Chemotherapy,* New Orleans, Louisiana, Abstract 272.

Kropp, H., Sundelof, J. G., Hajdu, R. & Kahan, F. M. (1982). Metabolism of thienamycin and related carbapenem antibiotics by the renal dipeptidase: Dehydropeptidase-I. *Antimicrobial Agents and Chemotherapy* **22,** 62–70.

Kropp, H., Sundelof, J., Huber, J., Bohn, D., Portnoff, J. & Kahan, F. (1983). Prolonged blood levels achieved in primate species following intramuscular injection of suspensions of MK0787 (*N*-formimidoyl thienamycin). Proceedings of 13th International Congress of Chemotherapy.

Lahnborg, G., Hedstrom, K. G. & Nord, C. E. (1982). Efficacy of different antibiotics in the treatment of experimentally-induced intra-abdominal sepsis. *Journal of Antimicrobial Chemotherapy* **10,** 497–504.

Landesman, S. H., Cummings, M., Gruann, A. & Bernheimer, H. (1981). Susceptibility of multiple antibiotic-resistant pneumococci to the new beta-lactam drugs and rosaramicin. *Antimicrobial Agents and Chemotherapy* **19,** 675–7.

Lang, S. D. R. & Durack, D. T. (1980). *In vitro* efficacy of cefoperazone compared with cefotaxime, LYi27935, and thienamycin. *Clinical Therapeutics* **3,** 112–6.

Leanza, W. J., Windonger, K. J. Miller, T. W. & Christensen, B. G. (1979). *N*-acetimidoyl- and *N*-formimidoyl thienamycin derivatives: Antipseudomonal beta-lactam antibiotics. *Journal of Medicinal Chemistry* **22,** 1435–6.

Livermore, D. M., Williams. R. J. & Williams, J. D. (1981). *In vitro* activity of MK0787 (*N*-formimidoyl thienamycin) against *Pseudomonas aeruginosa* and other Gram-negative organisms and its stability to their beta-lactamases. *Journal of Antimicrobial Chemotherapy*

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8, 355–62.

Loebenberg, D., Miller, G. H., Moss, E. L., Jr., Oden, E., Hare, R. S., Chung, M. & Waitz, J. A. (1982). Evaluation of the *in vivo* activity of Sch 29482. *Journal of Antimicrobial Chemotherapy* **9,** 221–31.

Markowitz, N., Pohlod, D. J., Saravolatz, L. D. & Quinn, E. L. (1983). *In vitro* susceptibility patterns of methicillin-resistant and -susceptible *Staphylococcus aureus* strains in a population of parenteral drug abusers from 1972 to 1981. *Antimicrobial Agents and Chemotherapy* **23,** 450–7.

Matsuhashi, M., Ishino, F., Nakagawa, J., Mitsui, K., Nakajima-Iijima, S., Tamaki, S. & Hashizume, T. (1981). Enzymatic activities of penicillin-binding proteins of *Escherichia coli* and their sensitivities to beta-lactam antibiotics. In *Beta-Lactam Antibiotics. Mode of Action, New Developments, and Future Prospects* (Salton, M. & Shockman, D., Eds), pp. 169–84. Academic Press, London.

Matzkowitz, A. J., Baltch, A. L., Smith, R. P., Sutphen, N. T., Hammer, M. C. & Conroy, J. V. (1982). *In vitro* comparison of *N*-formimidoyl thienamycin (MK0787) and azlocillin with three aminoglycosides and ticarcillin against *Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy* **21,** 685–7.

McDonald, P. J., Hakendorf, P. & Pruul, H. (1982). Recovery period of bacteria after brief exposure to *N*-formimidoyl thienamycin and other antibiotics. *Current Chemotherapy and Immunotherapy* (Proceedings of the 12th International Congress of Chemotherapy) **1,** 741–3.

Meister, A. (1983). Selective modification of glutathione metabolism. *Science* **220,** 472–7.

Miller, M. A., Le Frock, J. L. & Vercler, M. J. (1981). Comparative activity of *N*-formimidoyl thienamycin with third generation cephalosporins and ureido penicillins against multiple-resistant *Serratia marcescens. Microbiology and Immunology* **25,** 1119–27.

Moore, B. A., Jevons, S. & Brammer, K. W. (1979). Inhibition of transpeptidase activity in *Escherichia coli* by "Thienamycin." *Antimicrobial Agents and Chemotherapy* **15,** 831–3.

Muytjens, H. L. & Van Der Ros-Van De Repe, J. (1982). Comparative activities of 13 beta-lactam antibiotics. *Antimicrobial Agents and Chemotherapy* **21,** 925–34.

Neu, H. C. & Labthavikul, P. (1982). Comparative *in vitro* activity of *N*-formimidoyl thienamycin against Gram-positive and Gram-negative aerobic and anaerobic species and its beta-lactamase stability. *Antimicrobial Agents and Chemotherapy* **21,** 180–7.

Norrby, S. R., Alestig, K., Ferber, F., Kahan, F. M., Kahan, J. S., Kropp, H. & Meisinger, M. A. P. (1981a). Enhanced urinary recovery of *N*-formimidoyl thienamycin (MK0787) on administering an inhibitor of the renal dipeptidase responsible for antibiotic metabolism. *Current Chemotherapy and Immunotherapy* (Proceedings of the 12th International Congress of Chemotherapy) **1,** 743–5.

Norrby, S. R., Alestig, K., Bjornegard, B., Burman, L., Ferber, F., Kahan, F., Huber, J. & Jones, K. (1981b). Enhanced urinary recovery of *N*-formimidoyl thienamycin (MK0787) by co-administration of MK0791. *21st Interscience Conference on Antimicrobial Agents and Chemotherapy,* Chicago, Illinois, Abstract No. 592.

Norrby, S. R., Alestig, K., Ferber, F., Huber, J. L., Jones, K. H., Kahan, F. M., Meisinger, M. A. P. & Rogers, J. D. (1983a). Pharmacokinetics and tolerance of *N*-formimidoyl thienamycin (MK0787) in humans. *Antimicrobial Agents and Chemotherapy* **23,** 293–9.

Noorby, S. R., Alestig, K., Bjornegard, B., Burman, L. A., Ferber, F, Huber, J. L., Jones, K. H., Kahan, F. M., Kahan, J. S., Kropp, H., Meisinger, M. A. P. & Sundelof, J. G. (1983b). Urinary recovery of *N*-formimidoyl thienamycin (MK0787) as affected by co-administration of *N*-formimidoyl thienamycin dehydropeptidase inhibitors. *Antimicrobial Agents and Chemotherapy* **23,** 300–7.

Owens, W. E. & Finegold, S. M. (1983). Comparative *in vitro* susceptibilities of anaerobic bacteria to cefmenoxime, cefotetan, and *N*-formimidoyl thienamycin. *Antimicrobial Agents & Chemotherapy* **23,** 626–9.

Patamasucon, P. & McCracken, G. H. (1982). Pharmacokinetics and bacteriological efficacy of *N*-formimidoyl thienamycin in experimental *Escherichia coli* meningitis. *Antimicrobial Agents and Chemotherapy* **21,** 390–2.

Pennington, J. E. & Johnson, C. E. (1982). Comparative activities of *N*-formimidoyl thienamycin, ticarcillin and tobramycin against experimental *Pseudomonas aeruginosa* pneumonia. *Antimicrobial Agents and Chemotherapy* **22,** 406–8.

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Pines, S. H. (1981). A practicable synthesis of thienamycin. IUPAC Symposium Series, *3rd Symposium on Organic Synthesis* pp. 327–34. Madison, Wisconsin.

Prince, A. S. & Neu, H. C. (1981). Activities of new beta-lactam antibiotics against isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *Antimicrobial Agents and Chemotherapy* **20,** 545–6.

Pruul, H., Witherall, B. L. & McDonald, P. H. (1981). Enhanced susceptibility of *Escherichia coli* to intracellular killing by human polymorphonuclear linkocytes after *in vitro* incubation with chloramphenicol. *Antimicrobial Agents and Chemotherapy* **19,** 945–51.

Ratcliffe, R. W. & Albers-Schonberg, G. (1982). The chemistry of thienamycin and other carbapenem antibiotics. In *Chemistry and Biology of Beta-Lactam Antibiotics. Non-traditional Beta-lactam Antibiotics,* Vol. 2, (Morin, R. B. & Gorman, M., Eds), pp. 227–314. Academic Press, New York and London.

Richmond, M. H. (1981). The semi-synthetic thienamycin derivative MK0787 and its properties with respect to a range of beta-lactamases from clinically relevant bacterial species. *Journal of Antimicrobial Chemotherapy* **7,** 279–85.

Ritzerfeld, V. W. (1983). *N*-formimidoyl thienamycin in animal studies. *Arzneimittel Forschung/Drug Research* **33,** 470–2.

Rolfe, R. D. & Finegold, S. M. (1981). Comparative *in vitro* activity of new beta-lactam antibiotics against anaerobic bacteria. *Antimicrobial Agents and Chemotherapy* **20,** 600–9.

Saino, Y., Kobayashi, F., Inoue, M. & Mitsuhashi, S. (1982). Purification and properties of inducible penicillin beta-lactamase isolated from *Pseudomonas maltophilia. Antimicrobial Agents and Chemotherapy* **22,** 564–70.

Sanders, C. C. & Sanders, W. E., Jr. (1982). Sch 29482: Comparative activity against Enterobacteriaceae multiply-resistant to beta-lactam antibiotics and mycobacteria. *Journal of Antimicrobial Chemotherapy* **9,** 59–69.

Scheld, W. M. & Keeley, J. M. (1982). Thienamycin (MK0787) therapy of experimental *Staphylococcus aureus* endocarditis. *Annual Meeting of the American Society of Microbiology,* Dallas, Texas, Abstract A25.

Semenitz, E., Gstraunthaler, G. & Pfaller, W. (1982). Mode of action of MK0787 (*N*-formimidoyl thienamycin): Microcalorimetric and morphological study. *Current Chemotherapy and Immunotherapy* (Proceedings of the 12th International Congress of Chemotherapy) **1,** 708–10.

Shah, P. M., Just, G. & Stille, W. (1982). Antibacterial activity of thienamycin against clinical isolates. *Current Chemotherapy and Immunotherapy* (Proceedings of the 12th International Congress of Chemotherapy) **1,** 723–4.

Spelhaug, D. R., Gilchrist, J. & Washington II, J. A. (1981). Bactericidal activity of antibiotics against *Campylobacter fetus* subspecies *intestinalis. Journal of Infectious Disease* **143,** 500.

Spratt, B. G., Tobanputra, V. & Zimmerman, W. (1977*a*). Binding of thienamycin and clavulanic acid to the penicillin-binding proteins of *Escherichia coli* K-12. *Antimicrobial Agents and Chemotherapy* **12,** 406–9.

Spratt, B. G. (1977*b*). Properties of the penicillin-binding proteins of *Escherichia coli* K-12 *European Journal of Biochemist* **72,** 341–52.

Spratt, B. G. (1978). *Escherichia coli* resistance to beta-lactam antibiotics through a decrease in the affinity of a target for lethality. *Nature* **274,** 713–5.

Strausbaugh, L. J. & Laun, P. R. (1983). *In vitro* susceptibility of *Pseudomonas aeruginosa* to three new beta-lactam antibiotics. *Annual Meeting of the American Society of Microbiology,* Miami, Florida, Abstract A28.

Thompson, R. L., Fisher, K. A. & Wenzel, R. P. (1982). *In vitro* activity of N-formimidoyl thienamycin and other beta-lactam antibiotics against methicillin-resistant *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy* **21,** 341–3.

Tune, B. M. (1972). Effect of organic acid transport inhibitors on renal cortical uptake and proximal tubular toxicity of cephaloridine. *Journal of Pharmacology and Experimental Therapeutics* **181,** 250–6.

Tutlane, V. A., McCloskey, R. V. & Treat, J. A. (1981). *In vitro* comparison of *N*-formimidoyl thienamycin, piperacillin, cefotaxime and cefaperazone. *Antimicrobial Agents and Chemotherapy* **20,** 140–3.

Verbist, L. & Verhaegen, J. (1981). *In vitro* activity of *N*-formimidoyl thienamycin comparison with cefotaxime, moxalactam and ceftaxidime. *Antimicrobial Agents and Chemotherapy* **19,** 402–6.

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Verpooten, G. A., Verbist, L. & De Broe, M. E. (1983). The Pharmacokinetics of MK0787 and a renal dehydropeptidase inhibitor (D.P.I.) in normal volunteers and in patients with differing degrees of renal failure. *Proceedings of the 13th International Congress of Chemotherapy.*

Vuye, A. (1982). *In vitro* activity and beta-lactamase stability of *N*-formimidoyl thienamycin compared to that of second and third generation cephalosporins. *Chemotherapy* **28,** 267–75.

Ward, J. I. & Moellering, R. C., jr. (1981). Susceptibility of Pneumococci to 14 beta-lactam agents: Comparison of strains resistant, intermediate-resistant, and susceptible to penicillin. *Antimicrobial Agents and Chemotherapy* **20,** 204–7.

Washburn, D. E., Perfect, J. R. & Durack, D. T. (1983). Pharmacokinetics of *N*-formimidoyl thienamycin and influence of a renal dipeptidase inhibitor in experimental meningitis. *Journal of Antimicrobial Chemotherapy* **12,** 39–45.

Watanakunakorn, C. & Tisone, J. C. (1982). Synergism between *N*-formimidoyl thienamycin and gentamicin or tobramycin against enterococci. *Antimicrobial Agents and Chemotherapy* **22,** 1082–3.

Welch, C. L. & Campbell, B. J. (1978). Uptake of tritiated glycine from L-alanyl tritiated glycine into kidney cortex brush-border vesicles: Role of renal dipeptidase. *Federation Proceedings* **37,** 1533.

Welch, C. L. & Campbell, B. J. (1980). Uptake of tritiated glycine from L-alanyl glycine into renal brush-border vesicles. *Journal of Membrane Biology* **54,** 39.

Wise, R., Andrews, J. M. & Patel, N. (1981). *N*-Formimidoyl thienamycin, a novel beta-lactam: An *in vitro* comparison with other beta-lactam antibiotics. *Journal of Antimicrobial Chemotherapy* **7,** 521–9.

Witte, J. L., Sapico, F. L. & Canawati, H. N. (1982). *In vitro* susceptibility of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains to *N*-formimidoyl thienamycin. *Antimicrobial Agents and Chemotherapy* **22,** 906–8.

Yourassowsky, E., Van Der Linden, M. P. Lismont, M. J. & Crokaert, F. (1982). Early response of *Pseudomonas aeruginosa* growth curve to cefsulodin and *N*-formimidoyl thienamycin. *Current Chemotherapy and Immunotherapy* (Proceedings of the 12th International Congress of Chemotherapy) **1,** 514–6.

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