Ocular Penetration of N-Formimidoyl Thienamycin (MK-787) and Potentiation by Dipeptidase Inhibitor (MK-791)

Kelvin R. Finlay, Catherine L. Carlson, and Anthony W. Chow

N-formimidoyl thienamycin (MK-787) is a new β-lactam with potent activity against both aerobic and anaerobic gram-positive and gram-negative bacteria. Its spectrum and activity suggest it may be useful in treatment of complicated intraocular infections. Its ocular penetration was studied in New Zealand white rabbits immediately before and after the third dose of 40 mg/kg administered intravenously at q6h intervals. Plasma, aqueous humor, and vitreous humor were obtained by direct aspiration, and antibiotic levels were asayed using an agar well diffusion method. MK-787 penetrated uninflamed intraocular fluids, including vitreous humor, although vitreous concentrations achieved (0.1–0.2 μg/ml) were significantly lower than the mean peak plasma (15 μg/ml) and aqueous concentrations (7 μg/ml). Nevertheless, the intraocular levels attained approached or exceeded the MIC_{90} for most sensitive organisms including some gram-negative bacilli important in bacterial endophthalmitis. When administered in combination with the renal enzyme inhibitor MK-791, plasma and aqueous concentrations of MK-787 were markedly potentiated, although vitreous concentrations were minimally affected. The potential usefulness of MK-787 in conjunction with MK-791 in the infected eye should be examined further in an animal model of bacterial endophthalmitis. Invest Ophthalmol Vis Sci 24:1147–1149, 1983

N-formimidoyl thienamycin (MK-787) is a structurally novel β-lactam with extremely high potency and markedly enhanced spectrum of activity against both gram-positive and gram-negative bacteria.[1,2] It is a stabilized derivative of thienamycin, a naturally occurring β-lactam belonging to a new class of antibiotics known as the carbapanems. As with other β-lactams, its mechanism of action is through inactivation of one of the transpeptidases important in bacterial cell wall synthesis. Its broad spectrum of activity is in part attributable to its stability against a wide variety of bacterial β-lactamases. Its extremely high potency against a variety of gram-positive and gram-negative bacteria suggests that MK-787 might be particularly useful for the management of bacterial endophthalmitis. Most antibiotics, with the exception of relatively lipid-soluble agents such as chloramphenicol, minocycline, and doxycycline, have poor ocular penetrations.[3] Due to its potency, we reasoned that even if the ocular penetration of this new drug is equivalent to other β-lactams, the antibiotic concentrations achieved may still be clinically effective in the treatment of bacterial endophthalmitis. We therefore studied its ocular penetration, especially into vitreous humor, in a rabbit model following intravenous administration. Furthermore, MK-787 is unique among β-lactam antibiotics in that it is metabolized and inactivated in the brush border of the renal tubules by the renal dipeptidase enzyme, dehydropeptidase-I, which hydrolyses the β-lactam ring of MK-787.[4] The degradation of MK-787 in vivo can be halted, and its urinary and plasma concentrations potentiated by the co-administration of a dipeptidase inhibitor (MK-791, monosodium-Z-S-[6-carboxy-6-{[(2,2-dimethyl-(S)-cyclopropyl) carbonyl]amino}5-hexenyl]-L-cysteine) in chimpanzees and man.[5] Accordingly, we also examined the effect of MK-791 on ocular penetration of MK-787 in the rabbit.

Materials and Methods. *Study animals:* Groups of New Zealand white female rabbits weighing 2–3 kg were studied. Each rabbit was adapted in individual cages for 48 hrs prior to experimentation. Anesthesia was attained by intramuscular injection of ketamine hydrochloride (100 mg/ml) admixed with acepromazine maleate (25 mg/ml) in a proportion of 10 ml to 1 ml, each animal requiring approximately 1 ml per injection. Animals were administered either MK-787 alone or in combination with the enzyme inhibitor MK-791 at 6-hr intervals by intravenous bolus infusion through the anterior marginal ear veins. Immediately before, and at 0.5, 1, 2, 4, and 6 h after the third dose of antibiotic, samples were obtained by direct aspiration from the anterior chamber, vitreous cavity, and the anterior marginal ear veins. Approximately 2.5 ml of plasma, 0.2 ml of aqueous humor, and 0.5–0.8 ml of vitreous humor could be obtained per animal at each sampling. Aqueous and vitreous humor were aspirated only once per eye for each animal. No bloody samples were accepted for analysis. All samples were immediately stabilized in a 50% (vol/vol) ethylene glycol: 1 M morpholinoethane-sulfonate buffer, pH 6.0 (MES) in equal proportions (vol/vol), quick frozen in dry ice and acetone, and stored at −80° C until ready for assay. Animals were killed by intracardiac injection of sodium pentobarbital.

Antibiotic preparations: N-formimidoyl thienamycin (MK-787) powder (Merck Sharp & Dohme Research Laboratories, Rahway, NJ) was dissolved in sterile normal saline and injected via an anterior marginal ear vein at the dosage of 40 mg/kg by bolus infusion. Antibiotic solutions containing either MK-

0146-0404/83/0800/1147/$0.95

Table 1. Concentrations (mean ± SE, μg/ml) of N-formimidoyl thienamycin (MK-787) after third q6h dosage at 40 mg/kg administered intravenously without renal enzyme inhibitor (MK-791)

	No. of samples	Time (hrs)				
		Pre	0.5	1	2	4
Plasma	5	0	15.12 ± 1.31	1.98 ± 0.14	0.28 ± 0.04	0
Aqueous	5	0	3.10 ± 0.34	6.96 ± 1.70	0.25 ± 0.01	0
Vitreous	5	0	0.17 ± 0.03	0.15 ± 0.06	0.06 ± 0.03	0

787 alone or combinations of MK-787 with the enzyme inhibitor MK-791 (Merck Sharp & Dohme Research Laboratories, Rahway, NJ), were prepared by admixing stock solutions in a water bath maintained at 80 C. The completely dissolved MK-787/MK-791 solution was similarly injected at the final dosage of 40 mg/kg MK-787 and 40 mg/kg MK-791.

Antibiotic assay: Concentrations of MK-787 in plasma, aqueous humor, and vitreous humor were assayed by a standard agar-diffusion bioassay technique using *Bacillus subtilis* ATCC 12432 (American Type Culture Collection, Rockville, MD) as the test organism.[6] Preliminary studies in our laboratory indicated that this bioassay technique is highly reproducible with the lowest detectable concentration of 0.02 μg/ml. Furthermore, studies with standards of ocular fluids, plasma, or MES buffer (50% ethylene glycol: 1 M morpholino-ethane-sulfonate, pH 6.0), spiked with known concentrations of either MK-787 alone or with MK-787/MK-791 yielded identical results, indicating that neither aqueous humor, vitreous humor, plasma nor MK-791, interfered with the bioassay of MK-787. Therefore, standards for both ocular fluids and plasma were prepared in MES buffer. Repeated measurements using spiked samples of either aqueous humor, vitreous humor, or plasma indicated that the standard error of this bioassay technique varied from 0 to 5%.

Pharmacokinetic determinations: The concentrations of MK-787 in plasma and ocular fluids measured after the third intravenous dose were fitted to a regression line by the method of least mean squares. The half-life ($t_{1/2}$) of this agent in plasma or ocular fluids was calculated by dividing ln2 by the elimination constant k, where k = 2.3 × the slope of the regression line. The area-under-the concentration vs time curve (AUC) for plasma and aqueous as well as vitreous humor were obtained by successive trapezoidal approximation for time = 0 to time = ∞.[7]

Results. The intraocular and plasma concentrations of MK-787 attained without and with co-administration of the renal enzyme inhibitor MK-791 are shown in Tables 1 and Table 2, respectively. A mean peak plasma concentration of 15.12 μg/ml without, and 20.4 μg/ml with, co-administration of MK-791 were observed at 30 min after infusion of the third dose. Without MK-791, the plasma half-life was 0.27 hr, and the AUC value was 9.45 μg hr/ml. With co-administration of MK-791, the plasma half-life was 0.45 hr, and the AUC value was 14.2 μg hr/ml. Mean plasma concentrations of MK-787 administered with MK-791 were significantly higher than corresponding concentrations without MK-791 at 0.5 and 1 hr after infusion ($P < 0.05$, Student's t-test) (Table 2).

The mean peak aqueous concentrations of MK-787 in these animals were 6.96 μg/ml without co-administration of MK-791, and 8.66 μg/ml with co-administration of Mk-791, and in both instances, mean peak aqueous concentrations were delayed until 1 hr after drug administration, confirming a barrier in intraocular penetration. Mean aqueous concentrations of MK-787 co-administered with MK-791 were significantly higher than corresponding concentrations without MK-791 at 0.5 and 2 hrs after infusion ($P < 0.05$) (Table 2). Based on the ratio of aqueous AUC to serum AUC (×100), the penetration of MK-787 into aqueous humor was estimated at 76%, both when administered alone, and when co-administered with MK-791.

Vitreous penetration by MK-787 in the normal rabbit was relatively poor and was not significantly

Table 2. Concentrations (mean ± SE, μg/ml) of N-formimidoyl thienamycin (MK-787) after third q6h dosage at 40 mg/kg co-administered intravenously with renal enzyme inhibitor (MK-791)

	No. of samples	Time (hrs)					
		Pre	0.5	1	2	4	6
Plasma	5	0	20.40 ± 1.90*	3.66 ± 0.43*	0.76 ± 0.24	0.06 ± 0.05	0
Aqueous	5	0	4.82 ± 0.38*	8.66 ± 2.01	1.22 ± 0.43*	0.05 ± 0.03	0
Vitreous	5	0	0.30 ± 0.13	0.13 ± 0.03	0.05 ± 0.03	0	0

* Significantly higher than levels achieved without renal enzyme inhibitor ($P < 0.05$, Student's t-test).

affected by co-administration with MK-791 (2.9% and 2.3%, respectively). Nevertheless, mean concentrations of 0.1–0.3 μg/ml was attained. MK-787 was cleared from all compartments at 4 hrs postinfusion without the enzyme inhibitor and at 6 hrs postinfusion when co-administered with MK-791.

Discussion. Although the intraocular penetration of MK-787 is comparable to other β-lactams[3] and is relatively poor in vitreous humor even after co-administration with MK-791, the concentrations obtained in the uninflamed eye, coupled with the exquisite activity of this antibiotic, make its use in the management of endophthalmitis potentially important. In the present study, mean vitreous concentrations attained (0.1–0.3 μg/ml) exceeded or approached the MIC_{90} (minimal antibiotic concentration inhibiting 90% of strains) for most sensitive bacteria including *Staphylococcus aureus* (0.1 μg/ml), *Staphylococcus epidermidis* (0.2 μg/ml), *Streptococcus pyogenes* (0.1 μg/ml), *Streptococcus pneumoniae* (0.01 μg/ml), *Hemophilus influenzae* (0.1 μg/ml), and *Escherichia coli* (0.1 μg/ml).[1] Other gram-negative bacilli potentially important in bacterial endophthalmitis such as *Pseudomonas aeruginosa* (MIC_{90} 12.5 μg/ml), *Serratia marcescens* (6.3 μg/ml), and *Proteus mirabilis* (1.6 μg/ml) are much less susceptible.[1] It is conceivable, however, that intraocular penetration of MK-787 in the inflamed eye may be considerably higher. As shown by Barza,[3] inflammatory mediators in the inflamed eye may lower the blood retinal barrier and allow for greater antibiotic penetration.

Co-administration of the renal enzyme inhibitor MK-791, known to have no antibiotic effect by itself, resulted in higher and more prolonged plasma and aqueous levels of MK-787. This most likely reflects the greater systemic bioavailability, as evidenced by the increased plasma half-life, of MK-787 due to inhibition of renal metabolism and inactivation of the drug by MK-791. Our data do not suggest presence of the inactivating dipeptidase enzyme within intraocular compartments, but do suggest that the renal metabolism of MK-787 in the rabbit may be similar to that reported in chimpanzees and man.[5]

The observation that MK-787 penetrates uninflamed intraocular fluids at levels above the MIC_{90} for most sensitive bacteria, and that its intraocular concentrations may be further augmented by the co-administration of MK-791, strongly suggest its clinical potential for the treatment of bacterial endophthalmitis. Further studies of the clinical efficacy of this new β-lactam in experimental infective endophthalmitis are clearly indicated.

Key words: endophthalmitis, ocular pharmacokinetics, N-formimidoyl thienamycin, dipeptidase inhibitor, MK-787, MK-791, vitreous humor, aqueous humor

Acknowledgment. The authors thank Karen H. Bartlett for technical assistance, and Kathy Holding for preparation of this manuscript.

From the Division of Infectious Disease, Department of Medicine, and the Department of Ophthalmology, University of British Columbia and Vancouver General Hospital, Vancouver, BC, Canada. Supported in part by a Medical Research Council of Canada Development Grant (DG-189). Submitted for publication: November 18, 1983. Reprint requests: Anthony W. Chow, MD., Division of Infectious Disease, GF Strong Research Laboratories, Vancouver General Hospital, 700 West 10th Avenue, Vancouver, BC, Canada V5Z 1M9.

References

1. Neu HC and Labthavikul P: Comparative in vitro activity of N-formimidoyl thienamycin against gram-positive and gram-negative aerobic and anaerobic species and its β-lactamase stability. Antimicrob Agents Chemother 21:180, 1982.
2. Tutlane VA, McCloskey RV, and Trent JA: In vitro comparison of N-formimidoyl thienamycin, piperacillin, cefotaxime and cefoperazone. Antimicrob Agents Chemother 20:140, 1981.
3. Barza M: Factors affecting the intraocular penetration of antibiotics. The influence of route, inflammation, animal species and tissue pigmentation. Scand J Infect Dis Suppl 14:151, 1978.
4. Kropp H, Sundelof JG, Hajdu R, and Kahan FM: Metabolism of thienamycin and related carbapenem antibiotics by the renal dipeptidase, dehydropeptidase-I. Antimicrob Agents Chemother 22:62, 1982.
5. Norrby SR, Alestig K, and Ferber F: Enhanced urinary recovery of N-formimidoyl thienamycin (MK 0787) on administering an inhibitor of the renal dipeptidase responsible for antibiotic metabolism. *In* Current Chemotherapy and Immunotherapy; Proceedings of the 12th International Congress of Chemotherapy, Periti P, Grassi GG (eds.) Washington, DC, American Society for Microbiology, 1982, pp. 743–745.
6. Kropp H, Sundelof JG, Kahan JS, Kahan FM, and Birnbaum J: MK0787 (N-formimidoyl thienamycin): Evaluation of in vitro and in vivo activities. Antimicrob Agents Chemother 17:993, 1980.
7. Rowland M and Tozer TN: Clinical Pharmacokinetics; Concepts and Applications. Philadelphia, Lea and Fibiger, 1980, pp. 288–291.