Dose-Dependent Plasma Clearance of MK-826, a Carbapenem Antibiotic, Arising from Concentration-Dependent Plasma Protein Binding in Rats and Monkeys

BRADLEY K. WONG,* PATRICK J. BRUHIN, AND JIUNN H. LIN

Contribution from *Drug Metabolism, WP75-100, Merck Research Laboratories, West Point, Pennsylvania 19486.*

Received May 29, 1998. Final revised manuscript received September 22, 1998.
Accepted for publication October 2, 1998.

Abstract □ After intravenous administration of MK-826, a new carbapenem antibiotic, the compound exhibited nonlinear pharmacokinetics in rats and monkeys. In both species, time-averaged plasma clearance (based on total concentrations) increased about 5-fold over the 10- to 180-mg/kg dose range. MK-826 was extensively plasma protein bound in rat and monkey plasma, and the extent of binding was concentration dependent at plasma concentrations achieved after administration of these doses. Rosenthal analysis of the plasma protein binding indicated that there were two classes of binding sites. The binding capacity of the primary site was comparable to the plasma albumin concentration, which suggested that this primary site consisted of a single site on albumin. The extent of binding of MK-826 to rat albumin was similar to that in whole plasma. Clearance values based on unbound concentrations appeared independent of dose from 10 to 180 mg/kg, which is consistent with saturation of protein binding as the primary cause of the nonlinear pharmacokinetic behavior.

Introduction

MK-826 is a carbapenem antibiotic intended for the parenteral treatment of bacterial infections (see Figure 1).[1] Preliminary studies indicate that MK-826 is bound extensively to plasma proteins. Saturable plasma protein binding can lead to nonlinear pharmacokinetic behavior.[2,3] In the present studies, dose-dependent clearance of MK-826 was observed following intravenous (iv) administration to rats and monkeys. This clearance was explained by concentration-dependent protein binding to two different classes of plasma binding sites that probably reside on albumin.

Experimental Section

Synthesis—MK-826 (formula weight of monosodium salt, 497.4) was synthesized at Merck Research Laboratories (Rahway, NJ).

Animal Studies—Male Sprague–Dawley rats weighing 0.240 to 0.280 kg were purchased from Taconic Farms (Germantown, NY) and housed under standard conditions with free access to food and water. On the day prior to drug administration, a silicone rubber/polyethylene cannula was implanted in a jugular vein while the rats were under pentobarbital [40 mg/kg, intraperitoneally (ip)] anesthesia.[4]

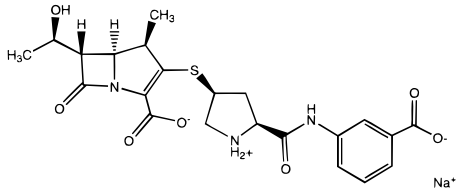

Figure 1—Chemical structure of MK-826.

Intravenous doses of MK-826 (monosodium salt dissolved in saline) of 10, 30, 60, and 180 mg/kg were administered by bolus injection into the jugular vein cannula to four rats per dose level. Male rhesus monkeys weighing 4.2 to 6 kg (Charles River, Key Lois, FL, $n = 4$) received the same dose levels as in the rat study, iv via a cephalic or saphenous vein. The rat study was of noncrossover design, but the monkeys received the MK-826 doses in a crossover fashion at least 1 week apart. Blood samples were drawn from rats before and 1, 3, 5, 15, and 30 min, and 1, 2, 3, and 4 h after the iv administration of MK-826. In the monkey studies, blood samples were obtained predose and at 5 and 30 min, and 1, 2, 4, 6, 12, 16, and 24 h post dose. Heparin was added to the blood samples to prevent clotting, and the resultant plasma was immediately frozen on dry ice. When stored at −70 °C, the compound was stable in plasma for at least 44 days; all assays were completed within that time.

Protein Binding—Protein binding of MK-826 was measured in triplicate in rat and monkey plasma by ultrafiltration over the 10- to 2000-μg/mL concentration range as previously described.[5] This range includes the concentrations observed in vivo; after the administration of the a 180-mg/kg dose to rats and monkeys, the concentration in plasma at the initial sampling time was about 1000 μg/mL. Briefly, stock solutions of MK-826 in water were added to freshly drawn plasma (pH 7.4) at 1% of total volume. After incubation at 37 °C for 10 min, aliquots of plasma were transferred to ultrafiltration devices (30 000 molecular weight cutoff; Centrifree, Amicon, Danvers, MA) and centrifuged at 1500 x g for 15 min. The binding of MK-826 to albumin was assessed similarly using solutions of rat (fraction V, fatty acid free) and rhesus (fraction V, not fatty acid free, Sigma) albumin (40 mg/mL). Concentrations of MK-826 in plasma and ultrafiltrate were determined by a liquid chromatographic method. A preliminary study showed that nonspecific binding of MK-826 to the ultrafiltration device was minor (<4%).

The binding capacity and association constants were estimated by fitting the protein binding data to the following equation that describes two classes of sites:

$$\frac{B}{U} = \frac{n_1 P_1 \times K_1}{1 + K_1 \times U} + \frac{n_2 P_2 \times K_2}{1 + K_2 \times U} \quad (1)$$

where B and U are the concentration of bound and unbound MK-826; n_1P_1 and n_2P_2 are the binding capacities or concentrations of

* Corresponding author. Telephone: 215-652-5644. Fax: 215-993-3533. E-mail: bradley_wong@merck.com.

10.1021/js980232k CCC: $18.00
Published on Web 12/11/1998

Table 1—Pharmacokinetic Parameters of MK-826 Based on Total Plasma Concentrations[a]

	rat			monkey		
dose, mg/kg	dose-normalized AUC, (μg x min/mL)/(mg/kg)	*CL*, mL/min/kg	terminal $t_{1/2}$, min	dose-normalized AUC, (μg x min/mL)/(mg/kg)	*CL*, mL/min/kg	terminal $t_{1/2}$, min
10	$203 \pm 50^{a,b,c}$	$5.17 \pm 1.30^{a,b,c}$	25 ± 9	$3458 \pm 696^{a,b,c}$	$0.299 \pm 0.061^{a,b,c}$	301 ± 24
30	$107 \pm 10^{c,d}$	$9.34 \pm 0.83^{c,d}$	34 ± 10	$1475 \pm 302^{b,e}$	$0.700 \pm 0.142^{b,e}$	290 ± 25
60	$97.1 \pm 11.8^{b,c}$	$10.4 \pm 1.3^{b,e}$	35 ± 5	$1276 \pm 181^{c,f}$	$0.795 \pm 0.102^{c,f}$	314 ± 38
180	$38.1 \pm 9.5^{a,d,e}$	$27.5 \pm 6.9^{a,d,e}$	48 ± 9	$594 \pm 53^{a,e,f}$	$1.70 \pm 0.15^{a,e,f}$	282 ± 13

[a] Shown as the mean (SD) of $n = 4$ per group. Dose-normalized AUC, *CL*, and terminal $t_{1/2}$ were evaluated by one-way ANOVA and Tukey's multiple comparison test. Values bearing the same superscript were judged to be different at $p < 0.05$. Abbreviations: AUC, area under the concentration–time curve from time 0 to infinity; *CL*, time-averaged plasma clearance. Values for half-lives are harmonic mean. Standard deviation was calculated using the jackknife method.[10]

the first and second classes of binding sites, respectively; and K_1 and K_2 are the association constants for the first and second classes of binding, respectively.[6] This analysis assumes that the classes of binding sites are mutually independent. The nonlinear regression analysis was performed with the RS/1 computer program (version 4.2, BBN Software) using a weighting factor of $1/y^2$.

Pharmacokinetic Analysis—Because of blood sample volume constraints, unbound concentrations were calculated from total concentrations in plasma (measured in vivo after administration of MK-826) using the binding capacities and association constants for the binding of MK-826 to plasma proteins. The relationship between bound and unbound concentrations for two classes of binding sites is described by

$$B = \frac{n_1P_1 \times K_1 \times U}{1 + K_1 \times U} + \frac{n_2P_2 \times K_2 \times U}{1 + K_2 \times U} \quad (2)$$

Substitution for bound concentration (Bound = Total − Unbound) permits calculation of unbound concentrations from total concentrations. In this study, $K_2 \times U \ll 1$, so eq 2 reduces to

$$(n_2P_2 \times K_2 \times K_1 + K_1) \times U^2 + (n_1P_1 \times K_1 + n_2P_2 \times K_2 - T \times K_1 + 1) \times U - T = 0 \quad (3)$$

which is a quadratic equation with one positive solution for unbound concentration. This equation for calculating the unbound concentrations for a drug that has two classes of binding sites is similar to that reported previously for a drug with single binding site.[2]

The terminal $t_{1/2}$ of MK-826 was determined by linear regression of the log-linear portion of the plasma concentration—time profile. Clearance (*CL*), based on either total or unbound plasma concentrations, was calculated as

$$CL = \text{Dose/AUC} \quad (4)$$

where AUC is the area under the curve from time zero to infinity. Clearance based on total concentration was shown to be dose-dependent, so these are time-averaged values. The standard method of calculating the volume of distribution at steady state (Vd_{ss}) based on total drug concentrations is invalid when nonlinear protein binding exists because it overestimates the steady-state distribution volume of total drug.[7,8] However, a corrected distribution volume $\overline{Vd^{t}_{ss}}$ may be calculated that theoretically better reflects the distribution volume of total drug at steady state:[8]

$$\overline{Vd^{t}_{ss}} = \overline{f_p} \times \overline{Vd^{n}_{ss}} \quad (5)$$

where $\overline{f_p}$ corresponds to the area-weighted average fraction unbound in the plasma[7] and $\overline{Vd^{u}_{ss}}$ is the steady-state distribution volume for unbound drug calculated by the standard method from the unbound concentrations.[7,8]

Liquid Chromatographic Method for MK-826—Plasma concentrations of MK-826 were measured by a direct-injection, column-switching method with UV absorption detection at 298 nm. Briefly, two columns connected by a switching valve provided automated sample cleanup and analysis; the columns were a Phenomenex (Torrance, CA) C18 Maxil-10 extraction column (50 × 4.6 mm) and a Whatman (Clifton, NJ) C18 Partisphere analytical column (125 × 4.6 mm). Plasma, buffered by the addition of one volume of 1 M 2-[*N*-morpholino]ethanesulfonate (pH 6.5) buffer, was injected directly onto the extraction column. Endogenous interfering components were diverted to waste for 5 min, and then from 5 to 10 min the mobile phase was switched to transfer MK-826 from the extraction column to the analytical column. Mobile phase for the extraction column consisted of 0.025 M sodium phosphate (pH 6.5) buffer at a flow rate of 2 mL/min. The analytical mobile phase consisted of the preceding buffer and acetonitrile (95/5, v/v) at a flow rate of 2 mL/min. Under these conditions, the retention time of MK-826 was 12 min. Replicate analysis of calibration standards and spiked quality control samples ($n = 5$ per concentration) indicated that the method provided >90% accuracy and variability of <10% over the 0.1- to 100-μg/mL concentration range.

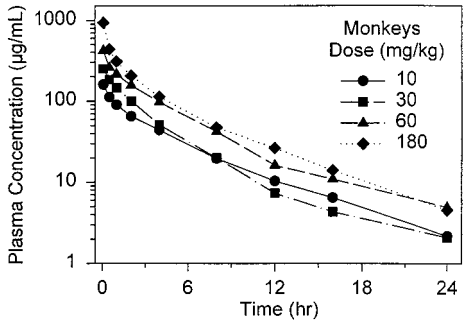

Figure 2—Mean plasma concentrations of MK-826 in rhesus monkeys after iv administration of 10 to 180 mg/kg.

Table 2—Binding Capacities and Association Constants for the Protein Binding of MK-826 in Rat and Monkey Plasma[a]

animal	n_1P_1, M	K_1, M^{-1}	n_2P_2, M	K_2, M^{-1}
rat	2.89×10^{-4}	9.07×10^{4}	8.50×10^{-3}	2.17×10^{2}
monkey	6.52×10^{-4}	11.5×10^{4}	1.36×10^{-3}	1.37×10^{2}

[a] Symbols: n_1P_1 and n_2P_2 are the binding capacities or concentrations of the first and second class of binding sites, respectively; K_1 and K_2 are the association constants for the primary and secondary classes of binding sites, respectively.

Statistical Analysis—One-way analysis of variance (ANOVA) was used to assess the effect of dose on pharmacokinetic parameters. A log transformation of data was made when unequal variances across dose levels were detected. Differences between dose levels were evaluated using the Tukey multiple comparison test.[9]

Results and Discussion

Following the iv administration of MK-826 to rats, plasma concentrations increased less than dose-proportionally, as shown by the dose-normalized AUC that decreased with dose (Table 1). Over this 18-fold range of doses, the dose-normalized AUC decreased by 5- to 6-fold. This decrease was due to a 5-fold increase in the time-averaged plasma clearance. A similar dose-dependent trend was observed in monkeys. Because for drugs with a low extraction ratio the plasma clearance is proportional to the

Table 3—Pharmacokinetic Parameters of MK-826 Based on Unbound Concentrations in Rats and Monkeys[a]

	rat			monkey		
dose, mg/kg	dose-normalized unbound AUC, (μg x min/mL)/(mg/kg)	*CL*, mL/min/kg	terminal $t_{1/2}$, min	dose-normalized unbound AUC (μg x min/mL)/(mg/kg)	*CL*, mL/min/kg	terminal $t_{1/2}$, min
10	9.60 ± 2.38	110 ± 30	28 ± 9	55.6 ± 12.2	18.7 ± 4.3[a]	295 ± 21
30	7.44 ± 0.69	136 ± 12	31 ± 5	30.6 ± 7.0	33.9 ± 7.2[a]	270 ± 18
60	8.50 ± 0.85	119 ± 14	33 ± 4	40.5 ± 9.3	25.6 ± 5.1	282 ± 5
180	6.70 ± 1.92	159 ± 46	39 ± 4	42.2 ± 5.8	24.0 ± 2.9	267 ± 13

[a] Abbreviations and superscripts are as given in footnote to Table 1.

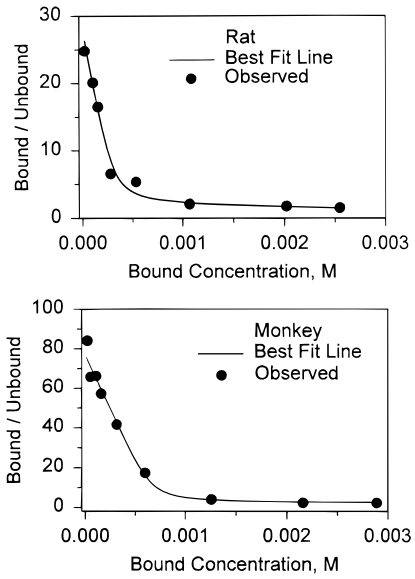

Figure 3—Rosenthal analysis of the protein binding of MK-826 in rat and monkey plasma.

unbound fraction in plasma, the concentration dependence of plasma protein binding was examined as a potential mechanism for the nonlinear clearance.

MK-826 was bound extensively to rat and monkey plasma proteins and the extent of protein binding was concentration dependent. In rat plasma, the unbound fraction was ca. 4% and remained constant up to 75 μg/mL; however, the unbound fraction increased to 40% at 2000 μg/mL. In monkey plasma, the unbound fraction in the linear range was 1.5% and then increased to 32% at 2000 μg/mL. The nonlinear binding range extended to concentrations achieved in the dose proportionality study (Figure 2). Rosenthal plots of the bound/unbound concentration ratio versus bound concentration were biphasic, consistent with two different classes of binding sites (Figure 3). When the data were fit to the equation describing two classes of binding sites, there was good agreement between the theoretical and observed values (the residual errors were ≤11% and there was no systematic deviation between the observed and calculated values). The binding capacities of the primary binding sites (n_1P_1) in rat and monkey plasma were 289 and 652 μM, respectively (Table 2), values which are comparable to physiological albumin concentrations.[11] Binding capacity is the product of the number of binding sites per protein molecule times the protein concentration, so this similarity implied that the higher affinity binding was to a single site on albumin. The binding of MK-826 (25 μg/mL) to rat albumin was assessed directly and found to be extensive (2.0 ± 0.1% unbound fraction, $n = 3$). Binding of MK-826 to monkey albumin also was extensive, however, the unbound fraction was higher than the free fraction in plasma (18 ± 0.5% versus 1.5 ± 0.2%). The reason for this result is unknown but could be due to the presence of endogenous competitive displacers, such as fatty acids, that form during the isolation of albumin.

Pharmacokinetic parameters based on unbound concentrations would be independent of dose if saturation of plasma protein binding was the primary cause of the dose-dependent plasma clearance. Determination of the association constants and binding capacities for the plasma protein binding permitted the calculation of unbound pharmacokinetic parameters from total plasma concentrations. In rats, dose-normalized AUC and clearance based on calculated unbound concentrations in plasma appeared independent of dose, confirming that the concentration-dependent plasma protein binding was the primary cause of the nonlinear plasma clearance (Table 3). The unbound clearance in rats was very high (110−159 mL/min/kg) and exceeded hepatic blood flow. However, preliminary studies in rats indicate that dehydropeptidase-I, an enzyme that is present in highest concentration in rodent lungs and kidneys, is important to the clearance of MK-826 in this species (data not shown). Kinetic analysis of unbound concentrations showed similar results in monkeys over the dose range examined, although the unbound clearance at 30 mg/kg appeared slightly higher than in the other dose groups. During the initial period after administration of MK-826 (30 min in mice and 4 h in monkeys after 180 mg/kg), the unbound fraction in the nonlinear dose range was increased because the concentrations achieved saturated the primary albumin binding site. Clearance based on total concentrations increased because of the higher unbound fraction available for elimination.

Despite the dose-dependent plasma clearance, terminal half-life was dose independent because this parameter reflected decline during a period when concentrations were within the linear protein binding range. The calculated steady-state distribution volume (Vd_{ss}^{t}), corrected for the nonlinear protein binding, was relatively unchanged with dose (ranges of means across the dose levels were 145−151 and 84−122 mL/kg in rats monkeys, respectively). This result suggested that despite the saturation of plasma protein binding, the net shift of MK-826 out of the plasma into the tissues was minor.

In summary, the dose-dependent increases in plasma clearance of MK-826 in rats and monkeys were shown to be the result of concentration-dependent plasma protein binding. Initial clinical studies suggest that after high doses of MK-826, the AUC in plasma also increases less than dose proportionally.[12]

References and Notes

1. Pelak, B. A.; Gerckens, L. S.; Scott, P. M.; Gill, C.; Pacholok, C.; Lynch, L.; Dorso, K.; Kohler, J.; Shungu, D.; Kropp, H. Antibacterial profile of L-749, 345 (ZD-4433), a new potent 1-beta-methyl carbapenem. *Abstr. 36th Intersci. Conf. Antimicrob. Agents and Chemother.* **1996**, 120.
2. Stoeckel, K.; McNamara, P. J.; Brandt, R.; Plozza-Nottebrock, H.; Ziegler, W. H. Effects of concentration-dependent plasma

protein binding on ceftriaxone kinetics. *Clin. Pharmacol. Ther.* **1981**, *29*, 650−657.
3. Lin, J. H.; Hooke, K. F.; Yeh, K. C.; Duggan, D. E. Dose-dependent pharmacokinetics of diflunisal in rats. Dual effects of protein binding and metabolism. *J. Pharmacol. Exp. Ther.* **1985**, *235*, 402−406.
4. Weeks, J. R.; Davis, J. D. Chronic intravenous cannulas for rats. *J. Appl. Physiol.* **1964**, *10*, 540−542.
5. Judd, R.; Pesce, A. J. Free drug concentrations are constant in serial fractions of plasma ultrafiltrate. *Clin. Chem.* **1982**, *28*, 1726−1727.
6. Rosenthal, H. E. A graphic method for the determination and presentation of binding parameters in a complex system. *Anal. Biochem.* **1967**, *20*, 525−532.
7. McNamara, P. J.; Gibaldi, M.; Stoeckel, K. Volume of distribution terms for a drug (ceftriaxone) exhibiting concentration-dependent protein binding. I. Theoretical considerations. *Eur. J. Clin. Pharmacol.* **1983**, *25*, 399−405.
8. McNamara, P. J.; Gibaldi, M.; Stoeckel, K. Volume of distribution terms for a drug (ceftriaxone) exhibiting concentration-dependent protein binding II. Physiological significance. *Eur. J. Clin. Pharmacol.* **1983**, *25*, 407−412.
9. Zar, J. H. Biostatistical Analysis, 2nd ed.; Prentice-Hall: Englewood Cliffs, NJ 1984.
10. Lam, F. C.; Hung, C. T.; Perrier, D. G. Estimation of variance for harmonic mean half-lives. *J. Pharm. Sci.* **1985**, *74*, 229−231.
11. *Handbook of Laboratory Animal Science*, Vol. II, Melby, E. C., Jr.; Altman, N. H., Eds.; CRC: Cleveland, OH, 1974; pp 365−405.
12. Majumdar, A.; Birk, K.; Blum, R. A.; Cairns, A. M.; Conroy, J.; Mendel, C. M.; Musson, D.; Rogers, J. D. Pharmacokinetics of L-749, 345, a carbapenem antibiotic, in healthy male and female volunteers. *Abstr. 36th Intersci. Conf. Antimicrob. Agents and Chemother.* **1996**, 122.

Acknowledgments

The authors thank Ms. Janice Brunner and Mr. Sam White for assistance with the monkey studies.

JS980232K