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Disposition of Moxalactam and *N*-Methyltetrazolethiol in Rats and Monkeys

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The disposition of moxalactam (MOX) and *N*-methyltetrazolethiol (NMTT) in rats and monkeys after intravenous injection was investigated, focusing on the in vivo liberation of NMTT, by using [NMTT-^{14}C]MOX and [^{14}C]NMTT. After [NMTT-^{14}C]MOX injection, MOX levels in plasma quickly became high in both rats and monkeys and then declined, with half-lives at the β phase of 18.8 and 67.1 min, respectively. The levels of NMTT liberated from MOX were much lower than those of MOX, but the apparent elimination was significantly slow. The levels of MOX and NMTT in rat liver were almost comparable but lower than those in plasma. With [^{14}C]NMTT administration, the level of NMTT in plasma declined, with half-lives at the β phase of 21.5 min in rats and 54.0 min in monkeys. After [NMTT-^{14}C]MOX injection, most of the radioactivity was excreted in urine as MOX, with 11% of the dose in rats and 8% of the dose in monkeys eliminated as NMTT until 24 h. Total biliary excretion was 26% of the injected radioactivity in rats, and most of it was due to MOX. In one monkey, the total biliary excretion was only 0.2% of the injected radioactivity. With [^{14}C]NMTT administration, most radioactivity was excreted in the urine as unchanged NMTT in both animals. Oral administration in rats showed that part of the biliary-excreted MOX was degraded to NMTT in the intestine and then absorbed. Repeated administration of [NMTT-^{14}C]MOX to rats did not change the levels of MOX and NMTT in plasma or liver nor did it change the excretion profiles. Thus, accumulation of MOX and NMTT did not occur.

Many of the *N*-methyltetrazolethiol (NMTT)-containing cephalosporins are used therapeutically, and their disposition in animals and humans has been studied extensively. There are only few reports, however, concerning the disposition of NMTT liberated from these antibiotics in vivo or of NMTT itself, except for reports of cefmetazole (6, 11), cefoperazone (6, 8), and moxalactam (MOX) (6; H. R. Black, M. K. Buening, and R. L. Wolen, Letter, Lancet **ii**:1090, 1983).

NMTT and NMTT-containing antibiotics produce hypoprothrombinemia in vitamin K-deficient patients (7, 21; A. Haubenstock, P. Schmidt, J. Zazgornik, P. Balcke, and H. Kopsa, Letter, Lancet **i**:1215, 1983; C. A. Hooper, B. B. Haney, and H. Stone, Letter, Lancet **i**:39, 1980) and experimental animals (2). It was initially proposed that the hypoprothrombinemic effect of NMTT is due to an inhibition of the vitamin K-dependent γ-carboxylation of glutamic acid (J. J. Lipsky, Preliminary communication, Lancet **ii**:192, 1983). Smith and Sundboom (13) and Uchida et al. (18) could not observe these inhibitory effects, however, on account of differences in the involved factors. Recently, Suttie et al. (15) strongly suggested that the hypoprothrombinemia caused by NMTT and NMTT-containing antibiotics is not related to the in vitro inhibition of the vitamin K-dependent carboxylation but is due to an inhibition of the vitamin K epoxide reductase. Therefore, it is important to estimate the NMTT concentration in the liver to understand the possible role of this compound. We examined the levels in plasma and liver and the urinary and biliary excretion of MOX, NMTT, or both after intravenous (i.v.) administration of [NMTT-^{14}C]MOX, labeled at the NMTT side chain, or [^{14}C]NMTT in rats and monkeys.

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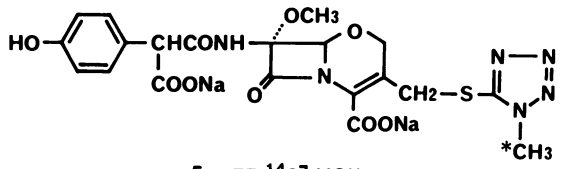
MATERIALS AND METHODS

Compounds. ^{14}C-labeled MOX disodium ([NMTT-^{14}C]MOX; specific activity, 5.90 μCi/mg) and ^{14}C-labeled NMTT sodium salt dihydrate ([^{14}C]NMTT; specific activity, 56.33 μCi/mg) were synthesized in our laboratory (5). Their radiochemical purities, determined by thin-layer chromatography (TLC), were 97.4 and 97.6%, respectively. The chemical structures of [NMTT-^{14}C]MOX and [^{14}C]NMTT are shown in Fig. 1.

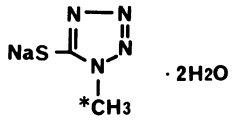
Animals. Male Sprague-Dawley rats (weight, 250 to 320 g; CLEA Japan Inc., Tokyo, Japan) and female cynomolgus monkeys (weight, 2.4 to 3.5 kg) were used.

Preparation of dose solution. [NMTT-^{14}C]MOX and [^{14}C]NMTT were diluted with 15- to 30-fold and 12- to 50-fold amounts of the unlabeled compounds, respectively, and dissolved in distilled water. Doses for i.v. administration to both animals were 300 mg (0.53 mmol) of [NMTT-^{14}C]MOX per kg and 90 mg (0.52 mmol) of [^{14}C]NMTT per kg, and for oral administration to rats the doses were 100 and 30 mg/kg, respectively.

Animal experiments. (i) Single i.v. administration to rats. The compound was injected into the tail vein. Rats (four rats per group) were housed in a cage. They were anesthetized with ether and killed by withdrawing blood from the abdominal aorta; and the livers were removed 5, 15, and 30 min and 1, 2, 4, 6, and 24 h after the injection. The second set of rats was kept in individual metabolism cages equipped with a urine-feces separator to collect urine and feces after i.v. administration of the drug. Urine eliminated at 0 to 1, 1 to 6, and 6 to 24 h after administration and feces eliminated 0 to 24 h after administration were collected. To collect bile from the third set of rats, the common bile duct was cannulated with polyethylene tubing (PE-10; Clay Adams, Parsipany, N.J.) under ether anesthesia, and rats were kept in restrain-



[NMTT-^{14}C] MOX



[^{14}C] NMTT

FIG. 1. Chemical structures of [NMTT-^{14}C]MOX and [^{14}C]NMTT. *, Position of the ^{14}C label.

ing cages. Compounds were administered after the rats had awakened from anesthesia, and bile and urine samples were collected at the times given above. Food and water were given freely during the experiments.

(ii) Repeated i.v. administration to rats. [NMTT-^{14}C]MOX was administered to the rats in the tail vein under light ether anesthesia once a day for 7 days. A group of four rats was housed in a cage during the experiment. Another four rats were placed individually into metabolism cages to collect urine and feces. After the final dose blood, liver, urine, and feces were collected at the same times as for the single-dose experiment described above.

(iii) Single i.v. administration to monkeys. Monkeys were catheterized in the bladder and cannulated in the femoral vein under ketamine hydrochloride (Sankyo Co., Ltd., Tokyo, Japan) anesthesia. An additional cannula was inserted into the common bile duct of another monkey. Animals were kept individually in monkey chairs, and the compound was injected into the cannula in the femoral vein after they had complete awakened from anesthesia. Blood and urine were taken from the same monkey, and bile and urine were taken from another monkey at the same times samples were taken from the rats. Water was given freely during the experiment, but the first meal on the day of drug administration was not given until 3 h after administration.

Determination of radioactivity. A total of 100 to 200 μl of plasma, urine, bile was added to 12 ml of Scinti-sol EX-H (Wako Pure Chemical Industries, Co., Ltd., Osaka, Japan) or Monophase-40 (Packard, Downers Grove, Ill.), and a homogenate of liver and feces was combusted with a sample oxidizer (model 306; Packard). The radioactivity was counted with a liquid scintillation spectrometer (Rackbeta 1215; LKB Wallac, Turku, Finland; or Tri-Carb 460C; Packard). Quenching was corrected by using an external standard.

Determination of MOX and NMTT. A total of 1 ml of plasma was mixed with 1 ml of 0.3 N HCl and extracted twice with 6 ml of ethyl acetate. The organic layer was combined and evaporated to dryness at 40°C under reduced pressure. The residue was reconstituted with an appropriate volume of methanol and applied to a TLC plate precoated with silica gel 60 without F (E. Merck AG, Darmstadt, Federal Republic Germany) with unlabeled MOX and NMTT, developed with CH_3CN-H_2O-acetic acid (80:20:1, by volume) for [NMTT-^{14}C]MOX-dosed samples and with toluene-ethanol-acetic acid (80:20:1, by volume) for [^{14}C]NMTT-dosed samples. Spots of MOX, NMTT, and other materials on dried TLC plate were detected by using iodine vapor and autoradiography. Localized areas were scraped off from the plate and transferred to counting vials to which 12 ml of Scinti-sol EX-H was added for radioactivity measurement. The whole rat liver or nearly 10 g of monkey liver was homogenized with 10 ml of phosphate buffer (50 mM, pH 6.0), and 1 ml of this homogenate was used in the assay. Recovery of the MOX and NMTT that spiked the plasma or liver homogenate was almost complete by ethyl acetate extraction. Urine and bile were diluted with distilled water and subjected to TLC separation. Detection limits for MOX and NMTT in [NMTT-^{14}C]MOX dosing samples were 0.05 nmol/ml for the plasma and 0.1 nmol/g for the liver, and those for NMTT in [^{14}C]NMTT dosing samples were 0.02 nmol/ml for the plasma and 0.005 nmol/g for the liver. The coefficient of variation of this method was less than 4% at a concentration of more than 1 nmol/ml or nmol/g and less than 8% at a concentration of less than 1 nmol/ml or nmol/g for both MOX and NMTT.

Subcellular distribution. Four grams of the liver isolated from rats given [NMTT-^{14}C]MOX was homogenized repeatedly in Tris hydrochloride buffer (50 mM, pH 7.4, 20% [wt/vol]) containing 154 mM KCl at 0°C. The homogenate was centrifuged at 600 × g for 5 min to obtain subcellular fractions (nuclei and cell debris), mitochondria were centrifuged at 10,000 × g for 10 min, and microsomes were centrifuged at 105,000 × g for 1 h. The radioactivity in the respective subcellular fractions and 105,000 × g supernatant was determined by the combustion method as described above. The protein contents were measured by the method described by Lowry et al. (3).

Pharmacokinetic analysis. Concentrations of MOX and NMTT in plasma from 5 min to 2 h after administration of [NMTT-^{14}C]MOX and [^{14}C]NMTT, respectively, were analyzed by using a two-compartment open model for both rats and monkeys. An exponential equation, $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, is used to express the plasma concentration-time curve, where C_p represents the drug concentration in plasma at time t after administration; α and β are the hybrid rate constants for the distribution and elimination phases, respectively; and A and B are the time zero intercepts of the two components of the biexponential curves. Pharmacokinetic parameters were calculated by standard equations based on two-compartment open models (1). The area under the plasma concentration-time curve (AUC) was obtained by the integration of curves from zero to infinite time, giving the following equation: $AUC = A/\alpha + B/\beta$. The least-squares regression analysis program MULTI was used to fit the data to obtain the pharmacokinetic parameters described above (23).

RESULTS

Single i.v. administration of [NMTT-^{14}C]MOX in rats. (i) Levels in plasma and liver. Levels of radioactivity, MOX, and NMTT in plasma and liver are shown in Fig. 2. The concentration of radioactivity in the plasma was equivalent to 2,384 nmol/ml at 5 min after administration, fell quickly to 42.6 nmol/ml at 2 h, and then decreased slowly thereafter. The concentrations of radioactivity in the liver at 5 min and 2 h were equivalent to 535 and 27.9 nmol/g, respectively. The concentration of MOX in plasma at 5 min was 2,217 nmol/ml and then fell rapidly to 20.7 nmol/ml at 2 h and 0.1 nmol/ml at 24 h. The concentration of NMTT in plasma was 38.1 nmol/ml at 5 min, but the decline was considerably slower. Less MOX and NMTT were present in the liver than in

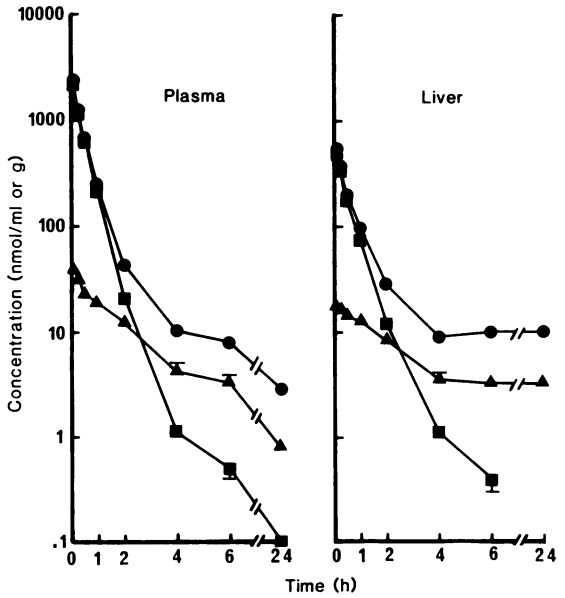


FIG. 2. Concentrations of radioactivity (●), MOX (■), and NMTT (▲) in plasma and liver in rats after i.v. administration of [NMTT-^{14}C]MOX at 300 mg (0.53 mmol)/kg. Each plot represents the mean ± standard error (SE) of four rats. Points without error bars indicate that the SE was less than the size of the symbol.

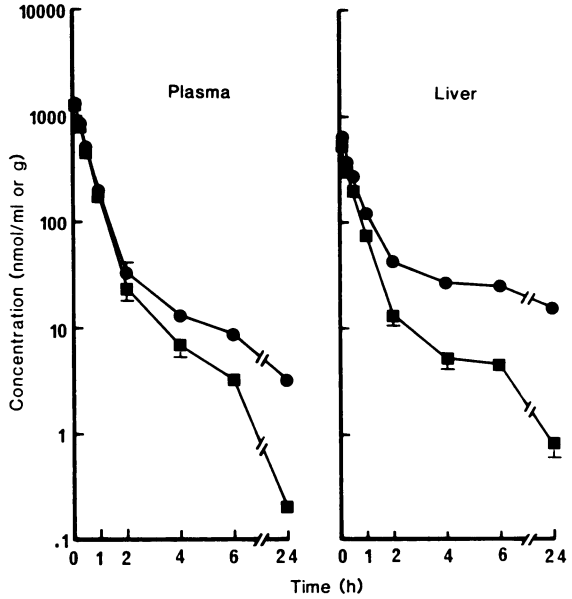


FIG. 3. Concentrations of radioactivity (●) and NMTT (■) in plasma and liver in rats after i.v. administration of [^{14}C]NMTT at 90 mg (0.52 mmol)/kg. Each point represents the mean ± SE of four rats. Points without error bars indicate that the SE was less than the size of the symbol.

plasma at almost all times, but NMTT remained, with a concentration of 3.3 nmol/g even at 24 h.

(ii) Excretion in urine and bile. The urinary and biliary excretions of radioactivity, MOX, and NMTT are shown in Table 1. Most of the radioactivity administered was excreted in the urine. Excretion ratios in urine and feces within 24 h were 88.5 and 8.7% of the dose, respectively. Within 1 h 71.7% of the dose already was excreted in the urine. In the urinary radioactivity, MOX accounted for 74.8% and NMTT accounted for 11.4% of the dose within 24 h. Biliary excretion of radioactivity within 24 h was 25.6% of the dose; most of it was MOX and only 0.5% NMTT was detected.

Single i.v. administration of [^{14}C]NMTT in rats. (i) Levels in plasma and liver. The levels of radioactivity and NMTT in plasma and liver are shown in Fig. 3. The concentration of radioactivity in plasma at 5 min after administration was equivalent to 1,344 nmol/ml, and then it fell to 32.6 and 3.2 nmol/ml at 2 and 24 h, respectively. The concentrations of radioactivity in the liver at 5 min and 2 and 24 h were equivalent to 619, 42.0, and 15.3 nmol/g, respectively; these concentrations were slightly higher than those after [NMTT-^{14}C]MOX injection. The concentration of NMTT in plasma at 5 min was 1,280 nmol/ml, after which it was eliminated rapidly until 2 h and was slowly eliminated thereafter.

(ii) Excretion in urine and bile. The urinary and biliary excretion of radioactivity and NMTT is shown in Table 2. More than 97% of the radioactivity was excreted in the urine, with most of it being excreted as NMTT. Biliary excretion of radioactivity was extremely low.

Repeated i.v. administration of [NMTT-^{14}C]MOX in rats. (i) Levels in plasma and liver. The levels of radioactivity, MOX, and NMTT in plasma and liver are shown in Fig. 4.

TABLE 1. Excretion of radioactivity, MOX, and NMTT in urine, feces, and bile after i.v. administration of [NMTT-^{14}C]MOX[a] to rats

Rat type	Excreta	Compound measured	Cumulative % of the dose at[b]:		
			0–1 h	0–6 h	0–24 h
Intact	Urine	^{14}C	71.7 ± 1.1	84.1 ± 1.4	88.5 ± 1.1
		MOX	67.7 ± 1.1	74.5 ± 1.5	74.8 ± 1.4
		NMTT	2.8 ± 0.1	8.1 ± 0.1	11.4 ± 0.4
	Feces	^{14}C			8.7 ± 0.6
Bile duct cannulated	Urine	^{14}C	62.3 ± 2.6	70.6 ± 2.0	70.9 ± 2.1
		MOX	59.0 ± 2.4	65.6 ± 2.0	65.8 ± 2.0
		NMTT	2.0 ± 0.1	3.5 ± 0.1	3.6 ± 0.2
	Bile	^{14}C	21.8 ± 1.2	25.6 ± 1.7	25.6 ± 1.7
		MOX	21.0 ± 1.2	24.5 ± 1.6	24.5 ± 1.6
		NMTT	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0

[a] A total of 300 mg (0.53 mmol)/kg was administered.
[b] Each value represents the mean ± SE of four rats.

TABLE 2. Excretion of radioactivity and NMTT in urine, feces, and bile after i.v. administration of [^{14}C]NMTT[a] to rats

Rat type	Excreta	Compound measured	Cumulative % of the dose at[b]: 0–1 h	0–6 h	0–24 h
Intact	Urine	^{14}C	80.8 ± 1.4	96.7 ± 0.4	97.5 ± 0.5
		NMTT	78.7 ± 1.4	93.1 ± 0.5	93.6 ± 0.5
	Feces	^{14}C			0.1 ± 0.0
Bile duct cannulated	Urine	^{14}C	75.8 ± 1.4	96.5 ± 0.5	97.2 ± 0.4
		NMTT	73.2 ± 1.4	92.5 ± 0.5	93.0 ± 0.4
	Bile	^{14}C	1.1 ± 0.0	1.9 ± 0.1	2.0 ± 0.1
		NMTT	0.7 ± 0.0	0.9 ± 0.0	0.9 ± 0.0

[a] A total of 90 mg (0.52 mmol)/kg was administered.
[b] Each value represents the mean ± SE of four rats.

The concentrations of radioactivity in plasma and liver were similar to those after single administration until 24 h, except that the concentrations in liver after 2 h persisted at about twofold higher levels. The concentration of MOX in plasma showed a similar concentration-time curve, but the NMTT concentration was approximately half that after a single dose. In the liver, concentrations of MOX and NMTT were similar to those in the plasma, i.e., almost the same for MOX but lower for NMTT.

(ii) Subcellular distribution of radioactivity in the liver. The concentrations of radioactivity in the subcellular fractions of the liver obtained after repeated administration are shown in Table 3. The ratio of radioactivity in the cytosol fraction was 64% at 30 min, but it decreased with time to 14% at 24 h. In the nuclear fraction (600 × *g* pellet), however, it progressively increased with time and reached 79% at 24 h. Radioactivities in the mitochondrial (10,000 × *g* pellet) and microsomal (105,000 × *g* pellet) fractions were considerably low, and their ratios to total radioactivity in the homogenate were almost constant (3 to 5%) at all times.

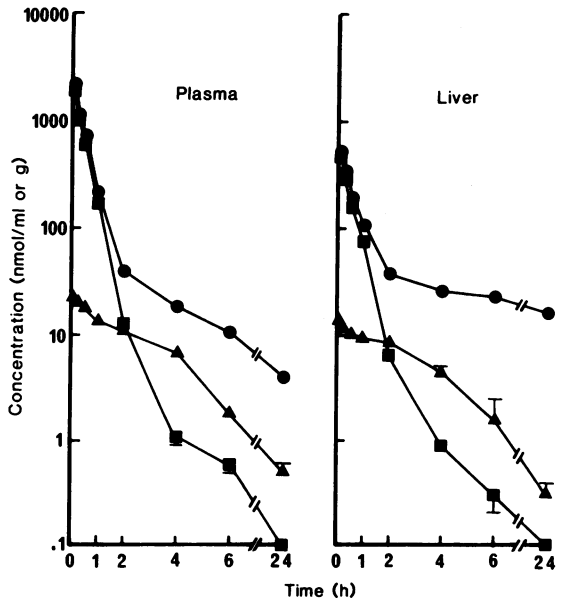


FIG. 4. Concentrations of radioactivity (●), MOX (■), and NMTT (▲) in plasma and liver in rats after repeated i.v. administration of [NMTT-^{14}C]MOX at 300 mg (0.53 mmol)/kg once a day for 7 days. Each point represents the mean ± SE of three rats. Point without error bars indicate that the SE was less than the size of the symbol.

(iii) Excretion in urine. The urinary excretions of radioactivity, MOX, and NMTT are shown in Table 4. Urinary and fecal excretions of radioactivity until 24 h after the last dose were 91.4 and 8.2% of the injected radioactivity, respectively, and recoveries from the urine as MOX and NMTT were 79.1 and 10.7% of the total dose, respectively. These values almost coincided with those obtained after single-dose administration.

Urinary excretion after oral administration of [NMTT-^{14}C]MOX and [^{14}C]NMTT in rats. The urinary excretions of radioactivity, MOX, and NMTT after oral administration of [NMTT-^{14}C]MOX or [^{14}C]NMTT are shown in Table 5. After [NMTT-^{14}C]MOX administration, 45.3 and 36.1% of the administered radioactivity was found in urine and feces, respectively, at 24 h. With urinary excretion, 34.5 and 8.0% of the dose were accounted for as NMTT and MOX, respectively. It appeared that about half the amount of the radioactivity was absorbed; most of this was NMTT liberated from MOX in the intestinal tract. In the case of the oral administration of [^{14}C]NMTT, 94.0% of the administered radioactivity was found in the urine and 0.1% was found in the feces until 24 h. The major component excreted in the urine was NMTT. Thus, the absorbability of NMTT from the intestine seems to be excellent.

Single i.v. administration of [NMTT-^{14}C]MOX in monkeys. (i) Levels in plasma and liver. The levels of radioactivity, MOX, and NMTT in plasma are shown in Fig. 5. The radioactivity in plasma 5 min after administration was equivalent to 3,526 nmol/ml, and then it declined at a slower rate than that in rats. The concentration of MOX in plasma corresponded to 95% of the radioactivity until 2 h and more than 70% until 6 h. The concentration of NMTT in plasma

TABLE 3. Subcellular distribution of radioactivity in liver after repeated i.v. administration of [NMTT-^{14}C]MOX[a] to rats

Fraction	Radioactivity concn (nmol equivalent to MOX/g of liver) at[b]: 30 min	2 h	24 h
Homogenate	205.9	38.4	16.3
600 × *g* pellet	45.5 (0.22)	18.5 (0.48)	12.8 (0.79)
10,000 × *g* pellet	9.2 (0.04)	1.9 (0.05)	0.5 (0.03)
105,000 × *g* pellet	9.9 (0.05)	1.8 (0.05)	0.7 (0.04)
Cytosol	131.4 (0.64)	15.6 (0.41)	2.3 (0.14)

[a] A total of 300 mg (0.53 mmol)/kg was administered once a day for 7 days.
[b] Values in parentheses are the fraction/homogenate concentration ratio.

TABLE 4. Excretion of radioactivity, MOX, and NMTT in urine and feces after repeated i.v. administration of [NMTT-^{14}C]MOX[a] to rats

Excreta	Compound measured	Cumulative % of the last dose at[b]:		
		0–1 h	0–6 h	0–24 h
Urine	^{14}C	76.5 ± 1.1	88.2 ± 0.6	91.4 ± 0.4
	MOX	72.4 ± 1.0	78.7 ± 0.8	79.1 ± 0.8
	NMTT	2.8 ± 0.0	8.0 ± 0.6	10.7 ± 0.6
Feces	^{14}C			8.2 ± 0.6

[a] A total of 300 mg (0.53 mmol)/kg was administered once a day for 7 days.
[b] Each value represents the mean ± SE of four rats.

was less than 100 nmol/ml, corresponding to 10% of the radioactivity until 2 h and 10 to 20% at 4 to 6 h. At 24 h, MOX and NMTT concentrations in plasma were less than 2 nmol/ml. The concentration of radioactivity in liver 24 h after administration was equivalent to 24.5 nmol/g, and those of MOX and NMTT were both nearly 1 nmol/g (data not shown).

(ii) Excretion in urine and bile. The urinary excretion of radioactivity, MOX, and NMTT and the biliary excretion of radioactivity are shown in Table 6. Urinary excretion of radioactivity was 72 to 79% of the administered radioactivity within 6 h and 84.6% within 24 h. Of this radioactivity, the ratio of MOX was more than 90% and that of NMTT was 3 to 9%. Biliary excretion of radioactivity was only 0.2% of the administered radioactivity until 24 h.

Single i.v. administration of [^{14}C]NMTT in monkey. (i) Levels in plasma and liver. The levels of radioactivity and NMTT in plasma are shown in Fig. 6. Radioactivity at 5 min was equivalent to 1,661 nmol/ml, and it decreased to 327 nmol/ml at 2 h and 4.5 nmol/ml at 24 h. Most of the radioactivity in plasma was detected as unchanged NMTT at any time. The levels of radioactivity and NMTT in liver at 24 h were equivalent to 39.1 and 1.1 nmol/g, respectively (data not shown).

(ii) Excretion in urine. Most of the injected radioactivity was excreted in the urine as unchanged NMTT until 24 h (Table 7).

Pharmacokinetic parameters. Pharmacokinetic parameters following i.v. administration of [NMTT-^{14}C]MOX or [^{14}C]NMTT in rats and monkeys are summarized in Table 8. The half-life at the β phase ($t_{1/2\beta}$) following single administration of [NMTT-^{14}C]MOX was 18.8 min in rats and 67.1 min in monkeys, or approximately 3.5 times greater in monkeys than that in rats. The AUC for rats was about 3.5 times smaller than that for monkeys, and vice versa, the total

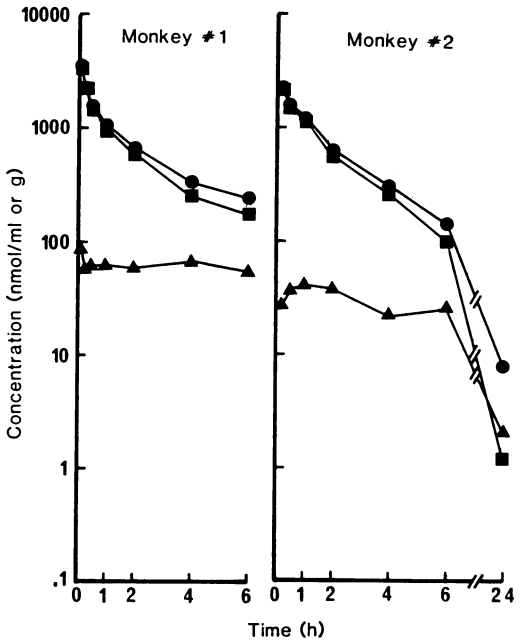


FIG. 5. Concentrations of radioactivity (●), MOX (■), and NMTT (▲) in plasma in monkeys after i.v. administration of [NMTT-^{14}C]MOX at 300 mg (0.53 mmol)/kg.

clearance (CL_{tot}) from rats was about 3.5 times larger than that from monkeys. The volumes of distribution at steady state (V_{ss}) in both animals were almost identical. These parameters are similar or comparable to those obtained from animals injected with 20 or 40 mg/kg (14). The $t_{1/2\beta}$ of NMTT was 21.5 min in rats and 54.0 min in a monkey; this difference was not as large as that with MOX. The V_{ss} of NMTT, however, was approximately 1.5 times larger than that of MOX in both the rat and the monkey. This might reflect the higher distribution ability of NMTT than of MOX.

DISCUSSION

Because the pharmacokinetic parameters ($t_{1/2\beta}$, AUC, and V_{ss}) of MOX following injection of [NMTT-^{14}C]MOX at 300 mg/kg were almost comparable to those obtained at lower doses ranging between 20 and 40 mg/kg (14) in both rats and monkeys, the pharmacokinetic characteristics of this drug in these animals appeared to be linear within the 20- to 300-mg/kg dose range.

The concentration of NMTT in plasma following [^{14}C]NMTT injection at 90 mg/kg, equimolar to 300 mg of

TABLE 5. Excretion of radioactivity, MOX, and NMTT in urine and feces after oral administration of [NMTT-^{14}C]MOX or [^{14}C]NMTT to rats

Compound administered	Compound measured	Cumulative % of the dose at the indicated times in[a]:		
		Urine		Feces
		0–4 h	0–24 h	0–24 h
[NMTT-^{14}C]MOX (100 mg/kg)	^{14}C	16.7 ± 4.4	45.3 ± 4.8	36.1 ± 4.6
	MOX	5.4 ± 4.3	8.0 ± 5.7	ND[b]
	NMTT	10.4 ± 0.4	34.5 ± 1.2	ND
[^{14}C]NMTT (30 mg/kg)	^{14}C	90.1 ± 1.3	94.0 ± 0.4	0.1 ± 0.0
	NMTT	86.0 ± 1.3	89.3 ± 0.5	ND

[a] Each value represents the mean ± SE of four rats.
[b] ND, Not determined.

TABLE 6. Excretion of radioactivity, MOX, and NMTT in urine and bile after i.v. administration of [NMTT-^{14}C]MOX[a] to monkeys

Monkey type	Excreta	Compound measured	Cumulative % of the dose at:		
			0–1 h	0–6 h	0–24 h
Intact (no. 1)	Urine	^{14}C	37.1	71.8	—[b]
		MOX	35.7	67.9	—
		NMTT	0.4	2.0	—
Intact (no. 2)	Urine	^{14}C	35.4	79.2	84.6
		MOX	34.3	72.4	74.9
		NMTT	0.4	5.3	7.7
Bile duct cannulated (no. 3)	Urine	^{14}C	34.9	83.0	92.3
		MOX	33.8	78.8	85.2
		NMTT	0.4	2.7	5.1
	Bile	^{14}C			0.2

[a] A total of 300 mg (0.53 mmol)/kg was administered.
[b] —, Not collected.

[NMTT-^{14}C]MOX per kg, was almost half the level of the MOX concentration following [NMTT-^{14}C]MOX injection in rats and monkeys. Because the V_{ss} for NMTT also was approximately 1.5-fold larger than that for MOX, the distribution ability of NMTT was expected to be higher than that of MOX in both animals. However, the $t_{1/2\beta}$ for NMTT following [^{14}C]NMTT injection was 21.5 min in rats and 54.0 min in monkeys, which was similar to the values for MOX. The total excretion of radioactivity after [NMTT-^{14}C]MOX or [^{14}C]NMTT injection was more than 85% of the dose up to 24 h in both animals. These data indicate that both MOX and NMTT were quickly distributed throughout the body and then cleared rapidly.

We determined the NMTT concentration in plasma or liver after [NMTT-^{14}C]MOX injection and obtained the following results: 38.1 nmol/ml in rat plasma, 17.4 nmol/g in rat liver, and 85.8 nmol/ml in monkey plasma. The apparent elimination of liberated NMTT from the plasma or liver was rather slow in both animals, although the inherent elimination of MOX was fast. The gradual liberation of NMTT from MOX may cause this phenomenon. Total excretion of NMTT into rat urine following [NMTT-^{14}C]MOX injection accounted for 11.4% of the administered radioactivity. Part of NMTT is likely to be produced in the intestinal tract from biliary-excreted MOX, and the rest is likely to be produced in the circulatory system or other part of the body. When [NMTT-^{14}C]MOX was given orally, 34.5% of the administered radioactivity was excreted in the urine as NMTT. This percentage may reflect the generation ratio of NMTT by the biliary-excreted route, because orally administered [^{14}C]NMTT was completely absorbed and excreted in the urine. The biliary excretion of MOX after i.v. injection of [NMTT-^{14}C]MOX was 24.5% of the dose; therefore, about 8% of the dose may have been reabsorbed as NMTT and then excreted in the urine. Thus, the remaining 3%, i.e., the difference between the biliary-generated 8% and the urinary-excreted 11%, is due to the NMTT generated by other routes.

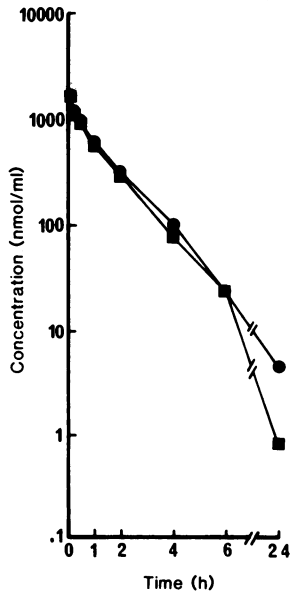


FIG. 6. Concentrations of radioactivity (●) and NMTT (■) in plasma in a monkey after i.v. administration of [^{14}C]NMTT at 90 mg (0.52 mmol)/kg.

Nakamura et al. (6) have indicated that the amount of NMTT generated from NMTT-containing antibiotics depends on the amounts of biliary-excreted antibiotics. The biliary excretion values of NMTT-containing antibiotics in rats, expressed as a percentage of the dose, have been reported: 58.0% for cefmetazole (50 mg/kg administered subcutaneously) (12), 28.5% for cefmenoxime (20 mg/kg administered i.v.) (16), 85.6 and 83.9% for cefoperazone (20 mg/kg administered i.v. and 50 mg/kg administered intramuscularly, respectively) (10), 56.8% for cefotetan (20 mg/kg administered i.v.) (20), 59.6% for cefpiramide (20 mg/kg administered i.v.) (4), 80% for cefbuperazone (20 mg/kg administered i.v.) (9), and 32.3% for cefamandole (20 mg/kg administered intramuscularly) (17). That of MOX was 25.6% at 300 mg/kg administered i.v. (Table 1) and 21.5% at 20 mg/kg administered i.v. (14). This suggests that less NMTT might be liberated from MOX compared with these NMTT-containing antibiotics.

In monkeys, biliary excretion of MOX was very low, and

TABLE 7. Excretion of radioactivity and NMTT in urine after i.v. administration of [^{14}C]NMTT[a] to a monkey

Compound measured	Cumulative % of the dose at:		
	0–1 h	0–6 h	0–24 h
^{14}C	32.6	88.0	94.9
NMTT	31.1	83.7	89.7

[a] A total of 90 mg (0.52 mmol)/kg was administered.

TABLE 8. Pharmacokinetic parameters of MOX and NMTT in rats and monkeys after i.v. administration of [NMTT-^{14}C]MOX or [^{14}C]NMTT

Parameter	MOX			NMTT	
	Rat (4[a])		Monkey (2)[b]	Rat (4)[c]	Monkey (1)[c]
	Single dose[b]	Repeated dose[d]			
A (nmol/ml)	2,110	6,201	2,505	566	710
α (h^{-1})	13.5	36.8	6.0	8.3	7.1
B (nmol/ml)	1,839	2,014	1,798	1,168	1,288
β (h^{-1})	2.21	2.52	0.62	1.93	0.77
$t_{1/2\beta}$ (min)	18.8	16.5	67.1	21.5	54.0
V_{ss} (ml/kg)	210.5	182.1	228.6	369.0	361.8
AUC (nmol · h/ml)	988	968	3,318	673	1,773
CL_{tot} (ml/kg per min)	8.94	9.13	2.66	12.9	4.89

[a] Values in parentheses are the number of animals.
[b] Determined after a single administration of [NMTT-^{14}C]MOX at 300 mg (0.53 mmol)/kg.
[c] Determined after a single administration of [^{14}C]NMTT at 90 mg (0.52 mmol)/kg.
[d] Determined after the last dosing of repeated administration of [NMTT-^{14}C]MOX at 300 mg (0.53 mmol)/kg once a day for 7 days.

NMTT generation by this route was thought to be very little. However, urinary excretion of NMTT following [NMTT-^{14}C]MOX injection accounted for 8% of the dose. This is probably because MOX remained in the plasma and liver in monkeys longer than it did in rats and, therefore, the ratio of NMTT generation by routes other than the biliary excretion route increased. Because biliary excretion of MOX in humans has been estimated to be 1 to 2% of the dose (19) and the $t_{1/2\beta}$ of MOX in humans (1.3 h at 1 g administered i.v.) (22) does not differ much from that in monkeys, the concentration of NMTT in human plasma or liver can be expected to be low, as in rats or monkeys.

Repeated administration of [NMTT-^{14}C]MOX did not change the concentration of MOX and NMTT in the plasma or liver. As for excretion, 99.6% of the administered radioactivity was excreted in urine and feces up to 24 h; thus, MOX, NMTT, or both did not appear to accumulate in the body. Results of a subcellular distribution study after repeated administration revealed that most of the radioactivity in the liver tissues was localized in the cell debris and cytosol fraction, and the radioactivity concentration in the microsomes (less than 10 nmol equivalent to MOX per g) probably did not cause γ-glutamyl carboxylation inhibition with rat liver microsomes in vitro (13, 18; J. J. Lipsky, Preliminary Communication, Lancet ii:192, 1983).

In conclusion, MOX was quickly distributed throughout the body, followed by rapid and complete elimination in rats and monkeys. The concentrations of NMTT liberated from MOX in plasma and liver were very low (less than 100 nmol/ml or nmol/g). NMTT excretion in urine amounted to 11% of the dose in rats and 8% in monkeys. Even when [NMTT-^{14}C]MOX was repeatedly injected into rats, no accumulation of MOX, NMTT, or both occurred.

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