Pharmacokinetics of Stereomeric 1,4:3,6-Dianhydrohexitol Mononitrates in Rats

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Received February 24, 1992; accepted March 20, 1992

The pharmacokinetics and urinary recoveries of four isomeric mononitrates, L-isoidide mononitrate (L-IIMN), isosorbide-2-mononitrate (IS-2-MN), isomannide mononitrate (IMMN), and isosorbide-5-mononitrate (IS-5-MN), were investigated at an intravenous dose of 2 mg/kg in rats. All four compounds exhibited monoexponential kinetics at this dose. The volumes of distribution were similar for all four isomers and were estimated at about 1.0 liter/kg. The systemic clearances of L-IIMN, IMMN, IS-2-MN, and IS-5-MN were 65.1 ± 13.0, 32.7 ± 12.0, 11.0 ± 2.3, and 8.23 ± 1.82 ml/min/kg, respectively ($P < 0.05$, all pairwise comparisons). Free mononitrate in the urine accounted for 0.306 to 4.56% of the administered dose, while the recovery in conjugated forms (after glusulase hydrolysis) accounted for 42.8% of the IMMN dose and 7.70 to 14.5% of the dose of the remaining three isomers. The dose-dependent pharmacokinetics of three of the mononitrates were explored at selected higher doses which cause equivalent vasodilator responses, L-IIMN (22 mg/kg), IS-2-MN (100 mg/kg), and IS-5-MN (300 mg/kg). The clearances of L-IIMN, IS-2-MN, and IS-5-MN at these higher doses were 42.3 ± 5.7, 6.38 ± 0.59, and 3.33 ± 0.62 ml/min/kg, respectively, all significantly less than those found at the 2 mg/kg dose. Typical Michaelis–Menten-type curvatures were observed in the concentration–time curves after IS-2-MN and IS-5-MN dosing. The pharmacokinetics of L-IIMN were also dose dependent, but they could not be described by simple Michaelis–Menten kinetics.

KEY WORDS: organic mononitrates; pharmacokinetics; urinary recoveries; urinary conjugation.

INTRODUCTION

Increased attention has been paid to the therapeutic application of organic mononitrates in the treatment of angina pectoris and other cardiovascular diseases. Isosorbide-5-mononitrate (IS-5-MN), one of the major metabolites of isosorbide dinitrate (ISDN), has been developed and used clinically as a drug in its own right. Several pharmacokinetic advantages are apparent with this drug, including complete oral bioavailability, relatively long half-life, relatively low intersubject variability, and lack of complications arising from active metabolites (1). The predictability of plasma concentrations among patients has been claimed as a therapeutic advantage for this drug as well.

Examination of the chemical structures of *cis*-1,4:3,6-dianhydrohexitol mononitrates (Fig. 1) indicated that there are at least three additional isomers of IS-5-MN, L-isoidide mononitrate (L-IIMN), isomannide mononitrate (IMMN), and isosorbide-2-mononitrate (IS-2-MN) (2,3). L-IIMN has both nitrate and hydroxyl groups in the exo position, while both substituents on IMMN are in the endo position. IS-2-MN has an *exo*-nitrate and *endo*-hydroxyl group, while IS-5-MN has an *endo*-nitrate and *exo*-hydroxyl group. IS-2-MN is also one of the active metabolites of ISDN in human and animals (4), although its plasma concentrations are lower than those of IS-5-MN.

Interestingly, although the three isomers were found to have higher *in vitro* vasodilating potencies than IS-5-MN (3,6,7), they have not been developed as therapeutic candidates. No pharmacokinetic information is available for L-IIMN and IMMN. Thus, little is known about the relative disposition of these isomers *in vivo* and how their pharmacokinetic properties are affected by their stereochemical arrangements. The objectives of the present study, therefore, were to characterize the pharmacokinetics of these four mononitrates at the same dose (2 mg/kg) in rats and to explore whether dose-dependent pharmacokinetics can be observed for selected mononitrates.

MATERIALS AND METHODS

Materials. IS-2-MN and IS-5-MN were provided by Schwarz Pharma (Monheim, Germany) as solids (>99% purity) and were used without further purification. L-IIMN was supplied by Dr. D. Hayward (D. H. Stereochemical Consulting LTD. Vancouver, Canada) as a gift. IMMN was synthesized in this laboratory (8) according to the methods described by Jackson and Hayward (2). The purity of L-IIMN (about 98.0%) and IMMN (about 98.6%) was separately determined by capillary gas chromatography against authentic samples obtained from Schwarz Pharma.

Unless specified, all chemicals were analytical grade. Capillary GC/GC-MS-grade ethyl acetate was obtained from Burdick & Jackson Laboratories (Muskegon, MI). Glacial acetic acid was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). Sodium acetate, silver nitrate (1 *N*), and sodium hydroxide (1 *N*) were obtained from Fisher Scientific Company (Fair Lawn, NJ). Glusulase was obtained from E. I. duPont de Nemours & Co. (Boston, MA).

Animals and Pharmacokinetic Studies. Male Sprague–Dawley rats (300–325 g) were purchased from Blue Spruce Farms (Altamont, NY). Animals were anesthetized with ether for implantation of an indwelling cannula in the right jugular vein 1 day before the pharmacokinetic study. In the kinetic experiment, the animals were divided into four groups ($n = 6$ for each group, except for the IS-2-MN group, where $n = 4$) and housed individually in metabolic cages, with food and water allowed ad lib. Each rat received a 2 mg/kg dose of mononitrate in about 0.5 ml of normal saline through the cannula, which was immediately rinsed with about 0.5 ml of normal saline. Serial blood samples (ca. 0.25 ml) were collected before and after dosing from the same cannula at the following times: L-IIMN—5, 10, 15, 25, 35, 45, 60, and 90 min; IMMN—5, 10, 15, 20, 30, 40, 60, 80, 100, and 120 min; IS-2-MN—15, 30, 45, 60, 80, 120, 160, 200, 240, and 280 min; and IS-5-MN—30, 60, 90, 135, 180, 270, 360,

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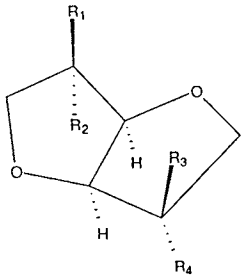

	L-IIMN	IS-2-MN	IMMN	IS-5-MN
R_1 (endo)	H	OH	OH	ONO_2
R_2 (exo)	ONO_2	H	H	H
R_3 (endo)	H	H	ONO_2	H
R_4 (exo)	OH	ONO_2	H	OH

Fig. 1. The chemical structures of 1,4;3,6-dianhydrohexitol (*cis*-isohexide) mononitrates.

450, and 540 min. Plasma (100 μl) was harvested and stored at −20°C until analysis.

Dose-Dependent Pharmacokinetic Studies. In order to examine the dose-dependent pharmacokinetics of L-IIMN, IS-2-MN, and IS-5-MN, another 16 rats were divided into three groups and were dosed as follows: L-IIMN, 22 mg/kg (n = 6); IS-2-MN, 100 mg/kg (n = 4); and IS-5-MN, 300 mg/kg (n = 6). These doses were chosen because a separate study (21) showed them to be equipotent pharmacologic doses in causing a peak reduction of about 40% in pulse pressure. The amount of IMMN available was not sufficient to examine its dose-dependent pharmacokinetic characteristics. Blood samples (ca. 0.25 ml) were collected before and after dosing from the cannula at the following times: L-IIMN—5, 10, 15, 25, 35, 45, 60, 70, and 90 min; IS-2-MN—30, 60, 90, 140, 190, 290, 390, 490, 590, 690, and 790 min; and IS-5-MN—90, 180, 270, 405, 540, 810, 1080, 1350, and 1620 min. Again, plasma (100 μl) was harvested and stored at −20°C until analysis.

Urinary Recovery Studies. To determine the urinary excretion of the free and conjugated forms of these four isomers, another 16 male Sprague–Dawley rats, 300–325 g, were divided into four groups. Each group received 2 mg/kg of each mononitrate in normal saline through the right jugular vein. Urine was collected at the following time intervals after dosing: for both L-IIMN and IMMN—4, 8, and 12 hr; and for both IS-2-MN and IS-5-MN—4, 8, 16, and 24 hr. The urine was collected under ice to minimize any potential degradation (9) during the study. The urine volume was recorded and the sample was frozen at −20°C until assay.

Determination of Mononitrate Concentrations. Plasma concentrations of mononitrates were analyzed using a capillary gas chromatography–electron capture detection (GC-ECD) method developed in our laboratory (8). The concentrations of free and total (conjugated and free) mononitrates in urine were also determined by the same GC method after the following manipulations. Free mononitrates in urine were determined after the addition of internal standard and extraction of 100 μl of urine with 1 ml of ethyl acetate. For the determination of total mononitrates (conjugated and free forms), 100 μl of urine was first incubated with 100 μl each of 2 *M* acetate buffer (pH 4.75) and Glusulase (E. I. duPont de Nemours & Co.), containing 135,000 U/ml of β-glucuronidase and 10,000 U/ml of sulfatase, for 4 hr at 37°C. An aliquot of 20 μl 1 *N* silver nitrate was added to quench the hydrolysis reaction. After the addition of internal standard, the mixture was shaken with 3 ml of ethyl acetate, and after centrifugation, 1 ml of ethyl acetate extract was transferred and neutralized with sodium hydroxide (50 μl, 1 *N*). This neutralization step was found to be necessary to preserve assay sensitivity and resolution, as well as the life span of the GC column. After centrifugation, the ethyl acetate layer was separated and evaporated to dryness under nitrogen in an ice bath. The residues were dissolved with 2 ml ethyl acetate, and a 1-μl aliquot was injected into the GC.

Calibration standards for free mononitrate were prepared by mixing equal volumes of mononitrate solutions and blank urine. Standards for total mononitrate were prepared in mixtures containing equal volumes of blank urine, acetate buffer (pH 4.75), and Glusulase solution. Standard samples were assayed concurrently with the unknowns.

Pharmacokinetic Data Analysis. The plasma concentration–time profile after the i.v. bolus of each mononitrate at 2 mg/kg was analyzed by assuming monoexponential decline. The apparent elimination constant (k) and volume of distribution (V_d) were determined via NONLIN. Systemic clearance (CL) was obtained as the dose divided by the area under the plasma concentration vs time curve (AUC). AUC was calculated by the Lagrange polynomial method (10). The amount of conjugated form was determined by subtracting the amount of free form from the total amount (conjugated and free forms) of mononitrate obtained after hydrolysis. The renal clearance (CL_r) and urinary conjugation clearance (CL_c) were determined as products of CL and the fractions of dose excreted in free form and conjugated form, respectively, in the urine.

The concentration (C) vs time (t) data obtained at 100 mg/kg of IS-2-MN and 300 mg/kg of IS-5-MN were fitted to the differential equation describing Michaelis–Menten kinetics, using NONLIN, to obtain V_{max} (theoretical maximum rate of elimination) and K_m (the dissociation constant).

$$\frac{dC}{dt} = -\frac{V_{max} \cdot C}{V_d \cdot (K_m + C)} \quad (1)$$

Statistical analyses were performed by Student's t test or one-way ANOVA when appropriate. All data are presented as mean ± standard deviation.

RESULTS

The retention times of IS-2-MN, IMMN, L-IIMN, and IS-5-MN in our GC assay were 3.2, 4.1, 4.3, and 4.9 min, respectively. As described earlier (8), the calibration curves of the four mononitrates in plasma were empirically bilinear, and unknown concentrations were estimated from the appropriate portion of the calibration curve. The calibration curves of urinary concentrations, which were present at

much higher ranges, were linear (correlation coefficient >0.999) for all four mononitrates over the concentration range 0.25–6 μg/ml for free mononitrate and 1–150 μg/ml for total mononitrate.

Figure 2 shows the mean plasma concentration-time profiles of all four mononitrates after intravenous bolus injection of 2 mg/kg. All the curves showed an apparent monoexponential decline. Table I summarizes the values of the pharmacokinetic parameters. The systemic clearances of L-IIMN, IMMN, IS-2-MN, and IS-5-MN were 65.1, 32.7, 11.0, and 8.23 ml/min/kg, respectively, and are significantly different from one another ($P < 0.05$). The values of the apparent volume of distribution (from 0.835 to 1.32 liters/kg) of these four isomers, except for a marginal statistical difference between IS-2-MN and IS-5-MN ($P = 0.04$), were not different ($P > 0.05$ for all other pairs). Since the apparent volumes of distribution were similar, the differences in systemic clearance resulted in a proportional difference in their elimination rate constants, which ranged from 0.00636 to 0.0525 min^{-1}. Characteristic of mono- or disubstituted organic nitrates, the interanimal variabilities of pharmacokinetic data (Table I) were relatively low. For instance, in the clearance of all four isomers, the highest coefficient of variation was 36% (for IMMN), while the remaining three isomers showed corresponding values of less than 25%.

Figure 3 shows the plasma concentration vs time curves of L-IIMN at intravenous doses of 2 and 22 mg/kg. Although a large fraction of the curve of 22 mg/kg (up to 60 min) appeared to be describable by monoexponential kinetics, there was a systematic bias (exhibited by all animals) to slower decline rates at longer collection times. This nonlinearity was most evident in rat 95 (Fig. 3, inset). If linear kinetics were assumed at this dose (Table I), the apparent volume of distribution could be estimated as 1.10 ± 0.12 liters/kg, which was similar to that found for the 2 mg/kg dose. However, by this assumption of linearity, both the total-body clearance (42.3 ± 5.7 ml/min/kg) and the apparent elimination rate (0.03832 ± 0.00277 min^{-1}) were significantly reduced compared to those found for the 2 mg/kg dose ($P < 0.05$; see Table I).

In contrast, the plasma concentration vs time profiles (Fig. 4) of IS-2-MN (100 mg/kg) and IS-5-MN (300 mg/kg) exhibited a typical Michaelis–Menten-type curvature over the time periods studied. The average clearances (6.38 ± 0.59 ml/min/kg for IS-2-MN and 3.33 ± 0.62 ml/min/kg for IS-5-MN) were significantly less than those obtained at the 2 mg/kg dose ($P < 0.05$; see Table I). The respective terminal half-lives (56.5 ± 11.6 min for IS-2-MN and 96.2 ± 8.5 min for IS-5-MN), however, were similar to those observed at the 2 mg/kg dose. If the concentration–time data were fitted to a one-compartment model with a single Michaelis–Menten elimination process, the K_m value obtained for IS-5-MN (166 ± 57 mg/l) was shown to about twice that for IS-2-MN (88.4 ± 50 mg/liters; $P < 0.05$). The V_{max} values, 0.875 ± 0.345 mg/min/kg for IS-2-MN and 1.05 ± 0.32 mg/min/kg for IS-5-MN, were essentially the same ($P > 0.05$). The apparent volumes of distribution for both mononitrates at the higher doses were similar to those found at the 2 mg/kg dose, about 1.0 liter/kg, although a statistical difference ($P = 0.012$) was detected between the values of this parameter for IS-2-MN at the two doses.

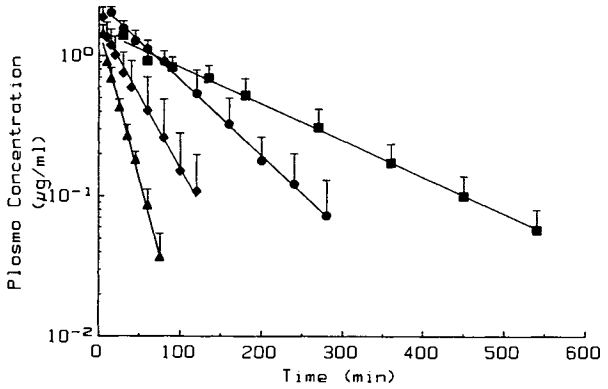

Fig. 2. Mean plasma concentration–time profiles of L-IIMN (▲), IMMN (◆), IS-2-MN (●), and IS-5-MN (■) in rats after i.v. administration of a 2 mg/kg dose.

The kinetics of hydrolysis of conjugates were studied by incubating 2-ml urine samples (from experimental animals dosed with mononitrates) with equal volumes of Glusulase and acetate buffer (pH 4.7) at 37°C for 24 hr. Samples (100 μl) were taken periodically over 24 hr. Maximal concentrations of mononitrates were obtained after incubation for 2–4 hr, indicating that hydrolysis of conjugate was complete after this period of incubation.

The urinary recoveries of mononitrates, as percentage of dose after i.v. administration of a 2 mg/kg dose, are shown in Table II. With all isomeric mononitrates, only small amounts of the free form were recovered, ranging from 0.31% (L-IIMN) to 4.56% (IS-5-MN). Urinary excretion of IMMN conjugates was 42.8% of the dose, while that of the other three mononitrates ranged from 7.7 to 14.5%. Table II also shows the clearance values estimated from the combined plasma and urinary data. The values of the renal clearance of the four isomers were similar. On the other hand, the urinary conjugation clearances ranged from 0.633 ml/min/kg (for IS-5-MN) to 14.0 ml/min/kg (for IMMN), a 22-fold difference.

DISCUSSION

The GC-ECD method offered a sensitive assay with a detection limit of about 10 ng/ml when using 100-μl urine samples. In most cases, little mononitrate was found in the last collection period of urine samples, indicating complete collection of drug excreted in the urine. The metabolic fate of L-IIMN and IMMN in rats has not been reported to date. Studies in humans (11,12), dogs (13,14), and rats (15) have suggested that the major metabolic pathways of IS-2-MN and IS-5-MN were denitration and glucuronic acid conjugation.

The pharmacokinetic characteristics of IS-2-MN and IS-5-MN in rats were generally consistent with those reported by Morrison and Fung (16) and Taylor *et al.* (4), except that the clearance of IS-2-MN was about half (and the half-life double) the corresponding values reported previously (16). The similarity in the volume of distribution of these four isomers is probably a result of their similar lipophilicity (7). Because of their polar nature, these volumes of distribution were similar in magnitude to the volume of total-body water in the rat (17).

Table I. Pharmacokinetic Parameters of Isomeric Organic Mononitrates at Various Doses (Values Are Mean ± SD)

	L-IIMN	IMMN	IS-2-MN	IS-5-MN
Dose (mg/kg)	2	2	2	2
No. of animals	6	6	4	6
CL (ml/min/kg)	65.1 ± 13.0	32.7 ± 12.0	11.0 ± 2.3	8.23 ± 1.82
V_d (L/kg)	1.22 ± 0.16	1.09 ± 0.24	0.835 ± 0.102	1.32 ± 0.23
k ($min^{-1} \times 10^{-2}$)	5.25 ± 0.39	3.03 ± 1.06	1.34 ± 0.36	0.636 ± 0.135
$t_{1/2}$ (min)	13.2 ± 0.9	25.2 ± 8.2	54.6 ± 13.7	112 ± 20
Dose (mg/kg)	22	—	100	300
No. of animals	6	—	4	6
CL (ml/min/kg)	42.3 ± 5.7*	—	6.38 ± 0.59*	3.33 ± 0.62*
V_d (L/kg)	1.10 ± 0.12	—	1.04 ± 0.10*	1.11 ± 0.11
V_{max} (mg/min/kg)		—	0.875 ± 0.345	1.05 ± 0.32
K_m (mg/L)	—	—	88.4 ± 49.8	166 ± 57

* Statistically different ($P < 0.05$) from the lower dose studied.

Schaumann (18) has proposed that the relative potency of different organic nitrates should be proportional to their systemic clearance. The rank order of systemic clearance observed in this study (Table II). L-IIMN > IMMN > IS-2-MN > IS-5-MN, however, did not agree with potency determinations from several sources (3,7), which indicated an order of L-IIMN > IS-2-MN > IMMN > IS-5-MN. The systemic clearances of L-IIMN and IS-2-MN (both with *exo*-nitrate groups) were higher than that of IS-5-MN, an *endo*-nitrate. This order of clearance is consistent with the general expectation (3,6,7) that an *exo*-nitrate would be sterically more accessible for metabolic elimination than an *endo*-nitrate. However, IMMN, an *endo*-nitrate, has a higher systemic clearance than IS-2-MN. The reason for this finding is not known at present.

The pharmacokinetics of oral IS-5-MN in humans had been reported to be linear over the clinical dose range. Taylor *et al.* (4) found that the linear range of this mononitrate as a human oral dose was 20 to 60 mg. There have been relatively few studies, however, of the effect of dose on mononitrate pharmacokinetics in animals. Data from this laboratory (19) indicated that the clearance of IS-2-MN in rats decreased from 19.3 ml/min/kg at 0.5 mg/kg to 11.7 ml/min/kg at 20 mg/kg ($P < 0.05$), without a significant change in the volume of distribution. A trend of an increase in the elimination half-life (from 86.6 to 102 min) as the oral IS-5-MN dose increased from 2 to 20 mg/kg was also observed, although statistical difference was not achieved. Taylor *et al.*

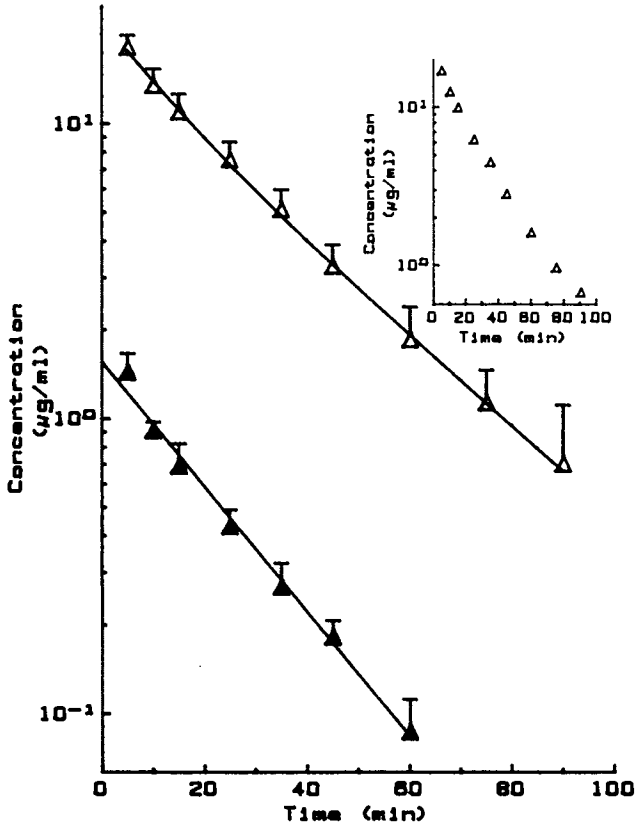

Fig. 3. Mean plasma concentration–time profiles of L-IIMN in rats after i.v. administration of 22 mg/kg (△) and 2 mg/kg (▲). The inset shows the kinetic profile for an individual rat after a 22 mg/kg dose.

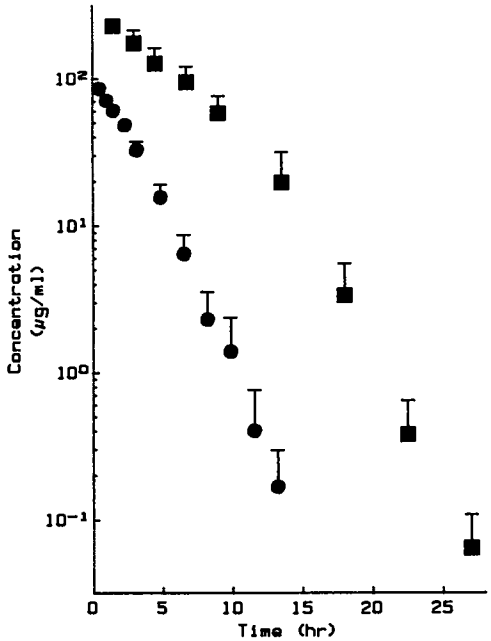

Fig. 4. Mean plasma concentration–time profiles of IS-2-MN at 100 mg/kg (●) and IS-5-MN at 300 mg/kg (■).

Table II. Urinary Recoveries, Renal Clearance (CL_r), and Urinary Conjugation Clearance (CL_c) of Mononitrates in Rats at a Dose of 2 mg/kg (Values Are Mean ± SD)

	L-IIMN	IMMN	IS-2-MN	IS-5-MN
Urinary recoveries (% of dose)				
Free mononitrate	0.31 ± 0.25	1.15 ± 0.60	1.13 ± 0.50	4.56 ± 0.69
Conjugates	14.5 ± 2.0	42.8 ± 5.3	8.57 ± 1.42	7.70 ± 1.77
Urinary excretion + conjugation	14.8 ± 2.2	43.9 ± 5.9	9.7 ± 0.95	12.3 ± 2.3
Clearance (ml/min/kg)				
CL_r	0.199	0.376	0.124	0.375
CL_c	9.44	14.0	0.943	0.633
Other[a]	55.5	18.3	9.93	7.22

[a] Estimated as $CL - CL_r - CL_c$.

(4) also claimed that, in rats, there was a dose-related increase in the plasma half-life (82 to 252 min) of oral IS-5-MN over the dose range of 2–40 mg/kg and suggested that this was due to prolonged absorption at the higher doses. In the present study, nonlinearity in pharmacokinetics of IS-2-MN (at 100 mg/kg) and IS-5-MN (at 300 mg/kg) appeared to be the result of enzyme saturation (Michaelis–Menten kinetics). The values of V_{max}/K_m of IS-2-MN and IS-5-MN were essentially identical to those of the systemic clearance found at the 2 mg/kg dose, suggesting that at 2 mg/kg, both mononitrates exhibited linear pharmacokinetics.

The nonlinearity in the pharmacokinetics of L-IIMN (at 22 mg/kg) was not describable by a simple Michaelis–Menten process. Detailed characterization of the nonlinear kinetic behavior of this mononitrate, however, was not appropriate at this time given the limited data accumulated.

Generally, only small amounts of the unchanged form of these mononitrates were found in the urine, ranging from 0.31% (L-IIMN) to 4.56% (IS-5-MN). Less than 5% of the dose of unchanged IS-5-MN was also found to be excreted in human urine (1). The urinary conjugation clearances of these four isomers were quite different. The rank order of renal conjugation clearance was IMMN > L-IIMN > IS-2-MN > IS-5-MN. In terms of stereochemistry, the order was *endo*-nitrate/*endo*-OH > *exo*-nitrate/*exo*-OH > *exo*-nitrate/*endo*-OH > *endo*-nitrate/*exo*-OH. The exceptionally extensive conjugation of IMMN (42.8%) excreted in the urine was supported by the observation of Rosseel and Bogaert (20), who showed that about 70% of an administered dose (4 mg/kg) of IMMN was conjugated with glucuronic acid in rat urine.

CONCLUSION

We have compared the pharmacokinetics of four isomeric mononitrates, in terms of both plasma decay and urinary excretion. Over the dose ranges used in our *in vivo* pharmacologic study (21), nonlinear pharmacokinetics were observed. The kinetics of IS-2-MN and IS-5-MN, but not those of L-IIMN, were describable by a simple Michaelis–Menten process. Urinary excretion of conjugates was found for all four mononitrates. The systemic clearance, at a 2 mg/kg dose, was in the order L-IIMN > IMMN > IS-2-MN > IS-5-MN, while the conjugation clearance was in the order IMMN > L-IIMN > IS-2-MN > IS-5-MN.

ACKNOWLEDGMENT

This work was supported in part by NIH Grants HL22273 and GM42850.

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