Pharmacokinetics of Isosorbide Dinitrate in Rhesus Monkey, Cynomolgus Monkey, and Baboon

E. DOYLE ˣ and L. F. CHASSEAUD

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Abstract □ At 2 min after intravenous injection of isosorbide dinitrate (1 mg/kg), mean plasma drug concentrations were 565 ± 66 (*SD*), 586 ± 43, and 1572 ± 253 ng/ml in the rhesus monkey, cynomolgus monkey, and baboon, respectively. Following a relatively short distribution phase, mean plasma concentrations declined with half-lives of 62, 23, and 24 min in these three species, respectively. The time course of plasma concentrations could be described by a two-compartment open model, although a one-compartment open model was adequate for obtaining some pharmacokinetic parameters. Statistically significant differences among the species were observed in areas under the plasma concentration–time curves, plasma half-lives, and volumes of distribution. The pharmacokinetics of isosorbide dinitrate in baboons most closely resembled those in humans.

Keyphrases □ Isosorbide dinitrate—pharmacokinetics, intravenous administration compared in nonhuman primates □ Pharmacokinetics—isosorbide dinitrate, intravenous administration compared in nonhuman primates □ Vasodilators—isosorbide dinitrate, pharmacokinetics compared in nonhuman primates

Isosorbide dinitrate, an organic nitrate vasodilator, has been in clinical use for many years (1–3). This lipophilic drug is almost completely eliminated by biotransformation, being rapidly denitrated (4, 5) by the glutathione *S*-transferases (EC 2.5.1.18) (6). The urinary metabolites of isosorbide dinitrate in animals (7–10) and humans (11, 12) are the corresponding isomeric mononitrates and isosorbide, which are excreted free and probably partly conjugated with glucuronic acid. Sorbitol has also been shown to be a urinary metabolite of isosorbide dinitrate in humans (11).

Little has been reported of the pharmacokinetics of the drug in animals, probably because of the difficulty in measuring it in plasma; most early data were obtained using [^{14}C]isosorbide dinitrate. The recent development of adequately sensitive analytical methods now permits studies of the drug's pharmacokinetics in humans (13–15).

The present study evaluated the pharmacokinetics of isosorbide dinitrate in nonhuman primates.

EXPERIMENTAL

Materials—Isosorbide dinitrate[1] solutions suitable for intravenous injection were prepared by dissolving the drug in aqueous 10% (v/v) ethanol. Solutions of nitroglycerin, 1% (w/v) in ethanol, were obtained commercially[2] and used as the internal standard.

Animals—Five female animals of each nonhuman primate species, ~1.5–3 years of age, were obtained commercially and maintained under conditions described previously (16). Animal body weights ranged as follows: rhesus monkey, 4–6 kg; cynomolgus monkey, 3–4 kg; and baboon, 4.5–7 kg.

Dosage—The animals were fasted for ~16 hr before and 7 hr after dosing, but water was provided at all times. Isosorbide dinitrate solution (1 mg/kg) was injected within 5 sec into a cephalic vein. This dose level was selected as one that might be used in chronic toxicity studies of the drug by this route. In humans, doses of 12.5 mg of isosorbide dinitrate have been infused during 2.5 hr (15).

Blood samples (2.5 ml) were withdrawn from a femoral vein before dosing and at 0.03, 0.08, 0.17, 0.33, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, and 4.0 hr (4.5 hr in the rhesus monkey) after dosing. The blood was taken into heparinized tubes, cooled in ice-cold water after mixing, and rapidly centrifuged to remove cells, which then were discarded. The plasma was stored at −20° until assayed. Isosorbide dinitrate is stable in plasma under these conditions.

Drug Analysis—Isosorbide dinitrate concentrations were measured in plasma by the electron-capture gas chromatographic procedure of Doyle *et al.* (17). Standard curves were prepared for three concentration ranges. Some analytical parameters are shown in Table I.

Data Processing—A two-compartment open model was fitted[3] to the plasma isosorbide dinitrate concentration–time data using a nonlinear least-squares curve fitting program (16). This program fits a curve to the observed data and selects a model by comparing the relative sizes of the initial and terminal phases, α and β, but does not allow user-defined selection of a model. A one-compartment model was fitted to the data as a possible alternative to a two-compartment model by normal regression techniques.

The criteria used to assess the data fit (obs = observed, exp = expected) from each species were: coefficient of determination, $r^2 = (G - R)/R$, where $G = \Sigma (\text{obs} - \overline{\text{obs}})^2$ and $R = \Sigma (\text{obs} - \text{exp})^2$; and residual mean square, $R/(n - 1 - p)$, where n is the number of observations and p is the number of parameters fitted (four parameters for a two-compartment model and two parameters for a one-compartment model). The average coefficients of determination (r^2) obtained were 0.9852, 0.9801, and 0.9925 in the two-compartment model and 0.9331, 0.9772, and 0.9885 in the

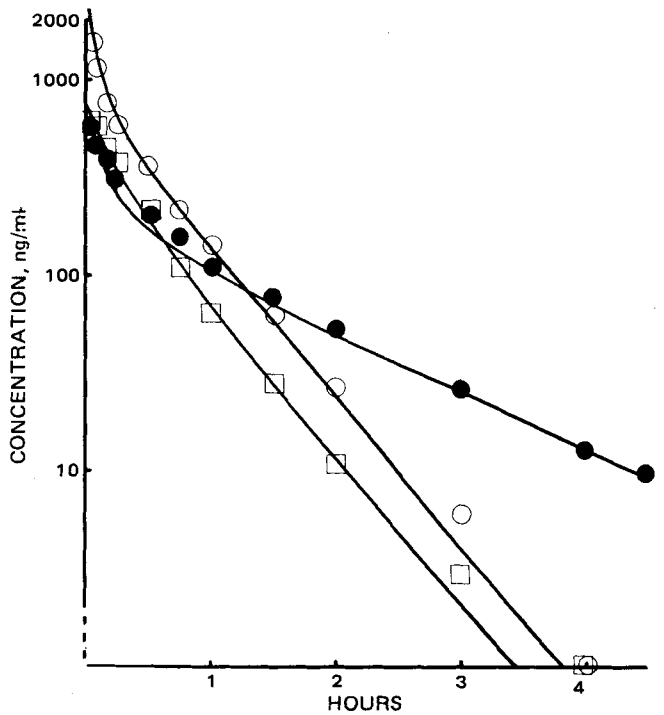

Figure 1—*Observed plasma concentrations of isosorbide dinitrate after a single 1.0-mg/kg iv dose to rhesus monkeys (●); cynomolgus monkeys (□); and baboons (○). Solid lines show computer-estimated concentrations.*

[1] Kindly provided by Pharma-Schwarz, Monheim, West Germany.
[2] Macarthys, Essex, England.
[3] ICL 2903 computer International Computers Ltd., Greenford, England.

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Table I—Analytical Parameters for the Measurement of Isosorbide Dinitrate in the Plasma of Nonhuman Primates

Parameter	Concentration Range 0–10 ng/ml	10–250 ng/ml	100–1000 ng/ml
Regression line[a] (± *SD*)	$Y = (0.079 \pm 0.001)X$	$Y = 0.003X - 0.028$	$Y = 0.001X - 0.065$
Accuracy[b]	±100% at 1 ng/ml ±10% at 10 ng/ml	±6% at 250 ng/ml	±18% at 1000 ng/ml
Precision	±8% at 10 ng/ml	±5% at 250 ng/ml	±2% at 1000 ng/ml
Limit of detection	0.5 ng/ml	—	—
Recovery of internal standard (± *SD*)	90 ± 4% at 5 ng/ml	91 ± 3% at 125 ng/ml	91 ± 3% at 500 ng/ml
Recovery of drug (± *SD*)	90 ± 2%	90 ± 4%	83 ± 4%

[a] Y = peak height ratio; X = drug concentration. [b] Defined as 95% confidence limits of the least-squares regression line.

one-compartment model for the rhesus monkey, cynomolgus monkey, and baboon, respectively. The residual mean squares obtained for the two-compartment model were 37, 87, and 73% of those obtained for a one-compartment model for the rhesus monkey, cynomolgus monkey, and baboon, respectively, indicating that the data were appreciably better fitted by a two-compartment model.

An analysis of variance was performed on each group of pharmacokinetic parameters. Group means were compared using the method of least significant differences in conjunction with the Newman–Keuls multiple comparison procedure (16). Significance testing was carried out at the 5 and 1% levels.

RESULTS

Plasma Concentrations—After intravenous injection of isosorbide dinitrate (1 mg/kg), the mean plasma concentration at 2 min (the first time of blood sampling) was similar in the rhesus monkey (565 ng/ml ± 66 *SD*) and cynomolgus monkey (586 ng/ml ± 43 *SD*), whereas it was about threefold higher in the baboon (1572 ng/ml ± 253 *SD*) (Fig. 1). After a short distribution phase in the cynomolgus monkey and baboon, mean plasma concentrations declined with a half-life of ~25 min to the detection limit at ~4 hr after dosing. In the rhesus monkey, the initial and terminal phase ($t_{1/2\beta}$ ~60 min) were relatively longer than in the other two species.

Pharmacokinetic Parameters—The observed plasma concentrations of isosorbide dinitrate could be adequately described by a biexponential equation, $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where C_p is the plasma drug concentration at time t; A and B are the zero-time intercepts of the initial and terminal phases of the concentration–time curve, respectively (Fig. 1); and α and β are obtained from the slopes of these phases, respectively. Pharmacokinetic parameters were calculated using standard equations (18) for a two-compartment open model with elimination from the central compartment (Table II). Plasma concentrations predicted by this model were generally in good agreement with observed values (Fig. 1). However, since the distribution phase was short, pharmacokinetic parameters were also calculated using a one-compartment open model. There were no notable differences in parameters calculated with either of the two models, except perhaps in the elimination rate constant (K_{el}) (Table III). However, K_{el} obtained for a one-compartment open model was almost identical to β calculated for a two-compartment open model.

An analysis of variance of areas (AUC) under the plasma concentration–time curves showed that, in respect to this parameter, the cynomolgus monkey was significantly different from the other species and that the rhesus monkey was significantly different from the baboon (Table II).

Table II—Mean Pharmacokinetic Parameters (± *SD*) for a Two-Compartment Open Model after a Single Dose of Isosorbide Dinitrate (1.0 mg/kg iv)

Parameter[a]	Rhesus Monkey[b]	Cynomolgus Monkey[b]	Baboon[b]
Body weight, kg	4.8 ± 0.8	3.4 ± 0.4	5.8 ± 0.9
A, ng/ml	435 ± 113	384 ± 222	1139 ± 551
B, ng/ml	220 ± 74	427 ± 120	843 ± 183
α, min^{-1}	0.12 ± 0.13	0.17 ± 0.12	0.22 ± 0.08
β, min^{-1}	0.01 ± 0	0.03 ± 0	0.03 ± 0.01
k_{12}, min^{-1}	0.05 ± 0.07	0.04 ± 0.04	0.07 ± 0.03
k_{21}, min^{-1}	0.05 ± 0.06	0.11 ± 0.08	0.12 ± 0.06
k_{el}, min^{-1}	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
Cl, ml/min	188 ± 29	200 ± 37	169 ± 30
$t_{1/2\alpha}$, min	10 ± 5	6 ± 3	3 ± 1
$t_{1/2\beta}$, min	62± 13[c]	23 ± 2	24 ± 3
V_1, liters	7.1 ± 1.3	4.3 ± 0.8	3.0 ± 0.7
V_2, liters	6.0 ± 2.4	1.4 ± 0.7	1.9 ± 0.3
$V_{D(ss)}$, liters	13.2 ± 2.9[c]	5.7 ± 0.8	4.9 ± 0.8
$V_{D\beta}$, liters	16.0 ± 2.5[c]	6.7 ± 1.2	5.7 ± 1.0
AUC, ng hr/ml	409 ± 41[d]	292 ± 47[e]	575 ± 37
A/α, ng hr/ml	61 ± 51	56 ± 40	101 ± 71
B/β, ng hr/ml	318 ± 39	237 ± 61	474 ± 74

[a] See text for details of abbreviations. [b] Data are the means of the results from individual animals. [c] Significance level (analysis of variance) for rhesus monkey compared to the other two species ($p < 0.01$). [d] Significance level (analysis of variance) for rhesus monkey compared to the baboon ($p < 0.05$). [e] Significance level (analysis of variance) for cynomolgus monkey compared to the other two species ($p < 0.01$).

DISCUSSION

Relatively little data have been reported concerning the detailed pharmacokinetics of isosorbide dinitrate in animals. In dogs (9) and humans (13, 15), the plasma concentration–time course of isosorbide dinitrate appears to be adequately described by a one-compartment open model; in rats (5), a two-compartment open model appears to be more appropriate. Although a two-compartment open model seemed suitable in nonhuman primates (Table II), a one-compartment open model could be used without notable differences in calculated pharmacokinetic parameters (Table III). However, the distribution phase A/α contributed 14, 15, and 18% of the total area under the plasma concentration–time curve in the rhesus monkey, cynomolgus monkey, and baboon, respectively. This finding indicates that the distribution phase of isosorbide dinitrate, although short, should be considered in a pharmacokinetic analysis of the drug and that distribution of isosorbide dinitrate into a peripheral compartment is a feature of its pharmacokinetics in nonhuman primates.

The systemic clearance of isosorbide dinitrate can probably be regarded as reflecting hepatic clearance, although clearance in the intestinal mucosa also might take place after oral administration; in fact, only ~2% of an oral dose of isosorbide dinitrate appears to reach the peripheral circulation intact in the baboon[4]. Similar low absolute bioavailability of isosorbide dinitrate was reported to occur in humans (15).

In rhesus monkeys, the systemic clearance of isosorbide dinitrate was similar to the hepatic blood flow (50 ml/min/kg) reported for this species (19), as might be expected for a drug of high hepatic extraction ratio (5). By contrast, the systemic clearance of isosorbide dinitrate in humans appears to be ~10-fold lower than hepatic blood flow (15). Despite this species difference in clearance, the drug is subjected to an extensive first-pass effect in both these species, as in rats (5).

Volumes of distribution [$V_{D(ss)}$ and $V_{D(\beta)}$] and apparent half-lives of the terminal phase, $t_{1/2\beta}$, were significantly greater in the rhesus monkey than in the other species (Table II). However, clearance (Cl) and the apparent half-life of the initial phase, $t_{1/2\alpha}$, did not differ significantly among the species.

The mean volumes of distribution (V_{Dss}) represented 273, 168, and 84% of the body weight in the rhesus monkey, cynomolgus monkey, and baboon, respectively. These values presumably represent the relative extent of tissue uptake of isosorbide dinitrate in these species. In the baboon, the volume of distribution was not too dissimilar from the probable total body water volume.

Adopting the two-compartment model, the mean volume of the central compartment (V_1) was larger than that of the peripheral compartment (V_2) in all three species, the ratios being 1.2, 3.1, and 1.6 for the rhesus monkey, cynomolgus monkey, and baboon, respectively. These ratios indicate that distribution of isosorbide dinitrate into the peripheral compartment is of some importance in its disposition in the body. Indeed, simulation of the plasma concentration–time profile of isosorbide dinitrate in the central and peripheral compartments showed that equilibration between these two compartments was rapidly achieved. Peak mean isosorbide dinitrate concentrations in the peripheral compartment

[4] Huntingdon Research Centre, unpublished data.

Table III—Mean Pharmacokinetic Parameters (± *SD*) for a One-Compartment Open Model Compared to a Two-Compartment Open Model following a Single Dose of Isosorbide Dinitrate (1 mg/kg)

Parameter[a]	One-Compartment Model[b]			Two-Compartment Model[b]		
	Rhesus Monkey	Cynomolgus Monkey	Baboon	Rhesus Monkey	Cynomolgus Monkey	Baboon
AUC, ng hr/ml	404[c] ± 51	281[d] ± 47	538 ± 21	409[c] ± 41	292[d] ± 47	575 ± 37
Cl, ml/min	189 ± 18	203 ± 22	173 ± 33	188 ± 29	200 ± 37	169 ± 30
$t_{1/2}$, min[e]	47[f] ± 4	29 ± 4	29 ± 2	62[f] ± 13	23 ± 2	24 ± 3
V_D, liters[g]	12.2[f] ± 2.0	8.5 ± 2.0	7.2 ± 1.5	13.2[f] ± 2.9	5.7 ± 0.8	4.9 ± 0.8
K_{el}, min^{-1}	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.01

[a] For details of abbreviations, see text. [b] Data are the means of results from individual animals. [c] Significance level (analysis of variance) for rhesus monkey compared to the baboon ($p < 0.05$). [d] Significance level (analysis of variance) for cynomolgus monkey compared to the other two species ($p < 0.01$). [e] $t_{1/2} = t_{1/2\beta}$ for the two-compartment open model. [f] Significance level (analysis of variance) for rhesus monkey compared to the other two species ($p < 0.01$). [g] V_D = total volume of distribution = $V_{D(ss)}$ for the two-compartment open model.

were 258, 394, and 727 ng/ml in the rhesus monkey, cynomolgus monkey, and baboon, respectively. Thereafter, concentrations in both compartments were similar.

Adopting a two-compartment open model, the mean ratios of β/K_{el} of 0.43, 0.63, and 0.51 in the rhesus monkey, cynomolgus monkey, and baboon, respectively, indicated that these fractions of isosorbide dinitrate in the body were in the central compartment and were available for elimination at any time after completion of the drug distribution phase.

The plasma half-lives of isosorbide dinitrate in nonhuman primates (rhesus monkey, 62 min; cynomolgus monkey, 23 min; and baboon, 24 min) (Table II) were longer than those observed in humans [~9 min, (15)], dogs [~7 min, (9)], and rats [~1 min, (5)]. The latter value for rats was disputed by Reed *et al.* (20) who claimed that it only represented a tissue distribution phase. The activity of the enzyme system (glutathione *S*-transferases) responsible for the denitration of isosorbide dinitrate was shown to be sex dependent in rodents, but this has not been observed in humans and would not be expected in nonhuman primates (21). In none of these species, however, would isosorbide dinitrate be expected to accumulate under the once daily dosage regimen used in chronic toxicity studies. Comparison of the relatively straightforward biotransformation of isosorbide dinitrate in different species would probably not provide the best criteria for the selection of a suitable species for chronic toxicity studies; instead, a comparison of the drug's pharmacokinetics would be more appropriate. Therefore, using pharmacokinetic criteria, the fate of isosorbide dinitrate in humans (15) appears to be more closely reflected by baboons (Tables II and III) than by either of the other two nonhuman primate species studied.

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