Pharmacokinetic Relationships Between Cinromide and Its Metabolites in the Rhesus Monkey I: 3-Bromocinnamamide, an Active Metabolite

ELIZABETH A. LANE * **and RENÉ H. LEVY** ˣ

Received February 8, 1982 from the *Department of Pharmaceutics, BG-20, University of Washington, Seattle, WA 98195.* Accepted for publication May 25, 1982. *Present address: Laboratory of Chemical Pharmacology, NHLBI, National Institutes of Health, Bethesda, MD 20205.

Abstract □ Fifty percent of a cinromide dose was metabolized to an active metabolite in the rhesus monkey. The steady-state concentration of this metabolite was 3–6 times that of the parent drug, depending on the route of administration. Cinromide is a medium-extraction ratio drug with a short half-life (0.92 ± 0.23 hr) when compared with the active metabolite, which has a low extraction ratio and a longer half-life (4.43 ± 0.76 hr). Incomplete oral bioavailability of cinromide is a result of first-pass metabolism rather than incomplete absorption.

Keyphrases □ Cinromide—pharmacokinetics, 3-bromocinnamamide metabolite, bioavailability, first-pass effect □ 3-Bromocinnamamide—active metabolite of cinromide, pharmacokinetics, bioavailability □ Pharmacokinetics—cinromide, 3-bromocinnamamide, primate model □ Bioavailability—cinromide, 3-bromocinnamamide, primate model

Whenever a drug yields a metabolite known to have similar pharmacological activity, speculation arises regarding the extent to which the metabolite is responsible for the apparent effect of the parent drug. Therefore, it becomes important to understand the relative disposition characteristics of the parent drug and active metabolite. Cinromide (3-bromo-*N*-ethylcinnamamide) is a new anticonvulsant drug which has reached Phase II of pharmacological evaluation (1–7). Cinromide is known to have an active metabolite resulting from *N*-deethylation (3-bromocinnamamide) which accumulates in humans (8). This investigation addressed two questions pertaining to the relative importance of the parent drug and metabolite: (*a*) the prediction of the steady-state metabolite–parent drug concentration ratio (9) and (*b*) the fraction of cinromide metabolized to the active metabolite. Prior to testing cinromide efficacy for epilepsy in the chronic primate model (6), it was necessary to define the pharmacokinetic characteristics (in particular, the dose dependency and bioavailability) of both of these compounds in this animal model.

EXPERIMENTAL

Animal Studies—Five male rhesus monkeys (*Macaca mulatta*), weighing 4–6 kg and adapted to primate restraining chairs, were each equipped with a jugular catheter for blood withdrawal and a femoral catheter for intravenous drug administration. These studies included the following treatments: (*a*) three intravenous infusions of cinromide[1], (*b*) two intravenous bolus doses of metabolite[1], (*c*) and one oral dose each of cinromide and metabolite.

The three cinromide infusions and the two bolus doses of metabolite were administered according to a randomized Latin-square design. The poor solubility of cinromide (0.09 mg/ml of water) even in 60% polyethylene glycol 400 (29 mg/ml), and its toxicity after large doses, precluded administration of intravenous bolus doses >25 mg/kg. Therefore, to test for dose dependency, cinromide was administered by intravenous infusion (25 mg/ml in 60% polyethylene glycol 400) at rates of 1, 2, and 4 ml/hr for 5 hr. A total of 30 plasma samples (0.5 ml) were collected during the infusion and 30 hr into the postinfusion phase. Urine was collected from the beginning of the infusion until 30 hr postinfusion. Doses of the amide metabolite (10 and 20 mg/kg) were injected over 1–2 min, and 20–25 plasma samples were collected over 30 hr. Urine samples were collected every 12 hr for a 24-hr period after each dose. Oral doses of 125 mg/kg of cinromide and 20 mg/kg of 3-bromocinnamamide were administered to each monkey by nasogastric intubation. Plasma samples were collected for 30 hr after each dose.

The ratios of blood–plasma concentration (λ) for cinromide and the metabolite were estimated from two samples taken from each of four monkeys after a single dose of cinromide. The fraction of cinromide and metabolite bound to plasma proteins was measured after equilibrium dialysis of spiked monkey plasma against isotonic phosphate buffer (pH 7.4). Ten to fifteen replicate determinations of the binding of cinromide at 25, 50, and 101 μmoles/liter and metabolite at 28, 60, and 133 μmoles/liter were made. The samples were dialyzed at 37° for 6 hr (the time required to reach equilibrium).

Analytical Procedure—Plasma samples were analyzed simultaneously for cinromide and the amide metabolite by high-performance liquid chromatography (HPLC). A 0.2-ml sample of plasma was extracted with 5 ml of benzene. The solvent was removed under a nitrogen stream at 50° and reconstituted with 100 μl of the mobile phase (methanol–water, 70:30). Two internal standards (3-bromo-*N*-butylcinnamamide[2] and 3-iodo-*N*-cyclopentylcinnamamide[2]), cinromide, and the metabolite were separated on a C_{18} μ Bondapak column[3] and measured by absorbance at 280 nm. Specificity of this assay was verified by comparison with another method (10).

Accuracy and precision of this assay were monitored by analysis of six control samples (duplicates of three different concentrations of cinromide and amide metabolite) with each set of unknowns. The difference between the known and assayed concentrations, divided by the known concentration, measured the accuracy of the assay. The difference between duplicates, divided by the average, measured the precision of the assay.

Urine (0.5 ml) was analyzed for cinromide in a similar manner. For the metabolite, a sample of urine was extracted and the benzene extract was washed with 2 ml of 0.2 *N* HCl before evaporation.

Pharmacokinetic Analysis—The area under the plasma concentration *versus* time curve (AUC) for each compound was measured by the trapezoidal rule with the terminal area estimated by C/β, where C is the concentration of the last sample and β is the rate constant for the slowest log-linear phase. Clearance (CL) was calculated from dose/AUC and steady-state volume of distribution by a previous method (11). Fitting of concentration time data was performed with the BMD-07R program (12).

[1] Supplied by Burroughs Wellcome Co., Research Triangle Park, N.C.

[2] Supplied by Dr. R. Welch, Wellcome Research Laboratories. Research Triangle Park, N.C.

[3] Waters Associates, Milford, Mass.

0022-3549/83/0500-0493$01.00/0

Table I—Pharmacokinetic Parameters of Cinromide in the Rhesus Monkey

Monkey	Clearance liter/hr Infusion 1[a]	Clearance liter/hr Infusion 2[b]	Clearance liter/hr Infusion 3[c]	Volume of Distribution, liter/kg Infusion 1	Volume of Distribution, liter/kg Infusion 2	Volume of Distribution, liter/kg Infusion 3	Elimination Half-Life, hr Infusion 1	Elimination Half-Life, hr Infusion 2	Elimination Half-Life, hr Infusion 3
336	9.6	4.6	6.6	7.2	3.3	5.3	0.87	0.77	1.28
326	10.1	8.9	—	8.3	6.5	—	0.69	0.90	—
316	4.6	8.3	3.4	3.1	6.8	7.1	0.40	1.08	1.08
184	6.3	6.7	7.8	4.9	5.1	6.9	0.80	0.72	1.08
266	3.9	3.4	3.7	3.9	3.1	3.3	1.05	0.96	1.17
Mean	6.9	6.4	5.4	5.5	5.0	5.6	0.76	0.89	1.15[d]
SD	2.8	2.4	2.2	2.2	1.7	1.7	0.24	0.15	0.10

[a] Infusion 1 rate is 25 mg/hr. [b] Infusion 2 rate is 50 mg/hr. [c] Infusion 3 rate is 100 mg/hr. [d] Statistically significant ($p < 0.05$) compared with infusions 1 and 2.

RESULTS AND DISCUSSION

Assay—The recovery of cinromide and metabolite averaged 98 ± 11% and 93 ± 9%, respectively ($n = 27$: nine replicates at three concentrations, 0.4–12.8 μg/ml for both compounds). Over 13 months, >300 control samples were analyzed yielding accuracy values of 8.2 ± 6.7% and 7.5 ± 6.0% for cinromide and the metabolite, respectively. The precision was 3.4 ± 3.5% and 4.0 ± 4.0% for cinromide and the metabolite, respectively. Within the ranges examined, no concentration dependence for accuracy or precision was apparent.

Cinromide Pharmacokinetics—The postinfusion decay of cinromide was fitted to a monoexponential equation. The average half-life (0.92 ± 0.23 hr) is consistent with achievement of steady state during the 5-hr infusion in most monkeys (Fig. 1). The half-life, clearance, and volume of distribution of cinromide measured from the infusion data are reported in Table I. The volume of distribution was constant in the dose range examined [one-way analysis of variance (ANOVA) on repeated measures, BMDP2V (13)]. Although there was a trend for the lowest clearance at the highest dose, there was no significant difference. However, the postinfusion half-life after 100 mg/hr was significantly longer than the postinfusion half-lives after 25 and 50 mg/hr ($p < 0.05$).

It was found that cinromide had a blood clearance (CL_{plasma}/λ) of 7.5 ± 2.9 liter/hr ($\lambda = 0.82$), about one-half of liver blood flow in the monkey (14). Less than 1% of a dose of cinromide was excreted unchanged in the urine and, assuming all metabolism occurs in the liver, this blood clearance may be regarded as the systemic hepatic clearance (CL_{sys}) of cinromide. CL_{sys} is a nonlinear function of liver blood flow and intrinsic hepatic clearance (CL_{int}) (15, 16), and therefore, it is not a sensitive index of dose dependency of intrinsic clearance. In addition, measurement of clearance as dose/AUC is a time-averaged value and less sensitive to dose-dependent nonlinearities than measurements of clearance at steady state. At the lower two infusion rates, cinromide reached steady state in all monkeys; at the highest infusion rate, steady state did not appear to have been reached in three of the five monkeys. This observation indicates that cinromide may be cleared in a dose-dependent fashion not detected through measurements of average intravenous clearances (dose/AUC).

Metabolite Pharmacokinetics—The amide metabolite could be adequately described by a one-compartment model in three of the five monkeys and a two-compartment model in the other two. The metabolite half-life (4.43 ± 0.76 hr; Table II) was 3–8 times longer than the parent drug half-life (0.92 ± 0.23 hr). Because of the long half-life, the amide metabolite did not reach steady state during the 5-hr cinromide infusion (Fig. 1). The difference in the time course of the two compounds was highlighted in the postinfusion phase. Cinromide could not be measured in the plasma 4 hr after the infusion was stopped, whereas the metabolite was present for >30 hr.

Table II—Pharmacokinetic Parameters for 3-Bromocinnamamide in the Rhesus Monkey Following Intravenous Bolus Doses

Monkey	Plasma Clearance, liters/hr 10-mg/kg Dose	Plasma Clearance, liters/hr 20-mg/kg Dose	Volume of Distribution, liter/kg 10-mg/kg Dose	Volume of Distribution, liter/kg 20-mg/kg Dose	Half-Life, hr 10-mg/kg Dose	Half-Life, hr 20-mg/kg Dose
336	0.78	0.49	0.92	0.65	4.2	5.0
326	1.02	0.78	1.03	0.78	3.7	3.2
316	0.88	0.70	1.06	0.69	4.8	3.5
184	0.84	0.57	1.09	0.85	4.5	5.0
266	0.52	0.63	0.98	0.91	5.6	4.8
Mean	0.81	0.63	1.02	0.78[a]	4.6	4.3
SD	0.18	0.11	0.07	0.11	0.71	0.88

[a] Statistically significant, $p < 0.05$ compared with the 10-mg/kg dose.

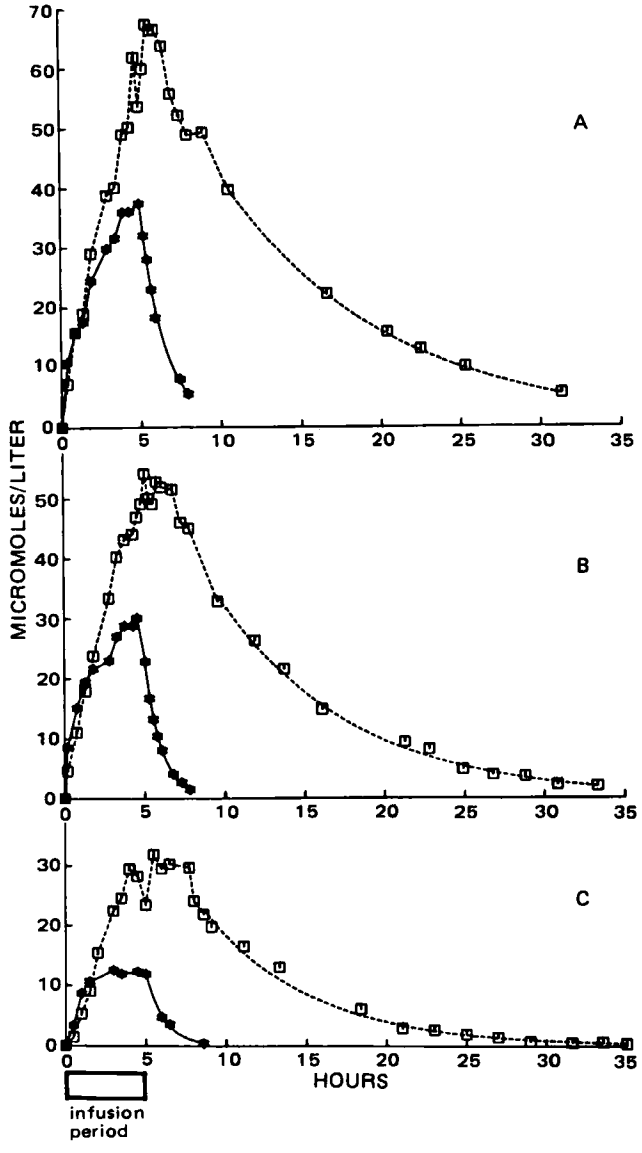

Figure 1—*Cinromide and 3-bromocinnamamide plasma levels during and following three separate cinromide infusions to monkey 184. Infusion concentrations were: (A) 291, (B) 189, and (C) 83 μmoles/hr. Key: (★) cinromide data, (□) 3-bromocinnamamide data, (—) least-squares fit to a monoexponential equation for cinromide (end of infusion to not detectable), and (---) least-squares fit to a monoexponential equation for 3-bromocinnamamide (from the time cinromide is no longer detectable).*

The average plasma clearance of 3-bromocinnamamide decreased from 0.80 ± 0.18 liter/hr to 0.63 ± 0.11 liter/hr when the dose was increased from 10 to 20 mg/kg (Table II). This apparent dose dependency was not significant at the 5% level ($p = 0.077$, paired t test). The volume of distribution of 3-bromocinnamamide decreased significantly ($p < 0.05$) when the dose was increased through this range. Administration of the amide metabolite suggested nonlinear behavior, both in clearance and distribution. However, these two nonlinearities affect half-life in opposite directions. This situation may explain the lack of dose effect on half-life.

The overall average amide metabolite half-life was significantly longer after administration of the parent drug than after administration of the

Table III—Variation in the Half-Life of the Amide Metabolite at Different Doses of Cinromide

Monkey	Cinromide Dose, mg/hr 25	50	100
336	4.6	4.9	8.3
326	6.6	6.3	6.8
316	3.9	4.1	6.6
184	4.6	5.8	7.3
266	6.2	5.4	5.7
Mean	5.2	5.3	6.9[a]
SD	1.2	0.9	0.9

[a] Statistically significant ($p < 0.05$) compared with infusion of 50 or 25 mg/hr.

metabolite. Also, the half-life of this amide metabolite (Table III) increased with increasing doses of cinromide [$p < 0.05$, one-way ANOVA on repeated measures, BMDP2V (13)].

The larger metabolite half-life observed after administration of the parent drug could be due to a type of nonlinearity different from saturation, *e.g.*, inhibition of metabolism by the parent drug or any of its metabolites. Such a phenomenon could also explain the dose-dependent half-life of the metabolite generated *in vivo*.

Fraction Metabolized and Steady-State Metabolite–Parent Drug Concentration Ratio—The fraction of a dose of cinromide metabolized to the amide metabolite (fm) was calculated in a model-independent manner (17):

$$fm = \frac{(AUC_m)_p}{D_p} \times \frac{D_m}{(AUC_m)_m} \qquad \text{(Eq. 1)}$$

where $(AUC_m)_p$ and $(AUC_m)_m$ are the AUC values of the metabolite following a dose of the parent drug and metabolite, respectively, D_p is the dose of the parent drug, and D_m is the dose of the metabolite. The areas under the curve and doses are expressed in molar units.

Since 3-bromocinnamamide is a low-extraction ratio drug, intravenous administration provided an appropriate estimate of intrinsic metabolite clearance for calculation of the fraction metabolized (18). This equation assumes a dose-independent metabolite clearance which measures the clearance of metabolite formed *in situ*. The preceding analysis implies that this is not the case. Therefore, the clearance of a 3-bromocinnamamide dose that was associated with approximately the same plasma concentrations of 3-bromocinnamamide as observed after cinromide, was used in calculation of the fraction metabolized. This matching of 3-bromocinnamamide concentrations would be appropriate if the apparent nonlinearity in metabolite clearance is due to a saturation phenomenon of the Michaelis–Menten type.

The fraction of cinromide metabolized to 3-bromocinnamamide in five monkeys after 14 doses of cinromide averaged 0.47 ± 0.12 (Table IV). This fraction of cinromide metabolized by *N*-deethylation showed no apparent dose dependence. Although theoretically the fraction metabolized is independent of metabolite clearance, it is not when calculated by this method. Therefore, the intra- or interanimal variability in the fraction metabolized may reflect a true variability in the fraction metabolized, as well as variability in the metabolite clearance.

From measurements of the total AUC of the metabolite and the parent drug after a single dose of cinromide, the steady-state metabolite–parent drug concentration ratio during intravenous infusion was predicted at 3.2 ± 0.9 (9). The fraction of free cinromide in monkey plasma (0.17 ± 0.04) was constant over the range examined (25–101 μmoles/liter). The binding of the amide metabolite was similar to the binding of the parent drug, *i.e.*, the free fraction was 0.18 ± 0.04 in the 28–133-μmoles/liter range. Therefore, the ratio of unbound metabolite to parent drug in plasma was predicted to be ~3.

Oral Bioavailability of the Parent Drug and the Metabolite—

Table IV—Fraction of a Cinromide Dose Metabolized by *N*-Deethylation in the Rhesus Monkey

Monkey	Cinromide Dose, mg/hr 25	50	100
336	0.32	0.44	0.39
326	0.43	0.47	0.40
316	0.61	0.28	0.44
184	0.71	0.60	0.35
266	0.48	0.36	0.50
Mean	0.52	0.43	0.42
SD	0.16	0.12	0.06

Table V—Bioavailability of Cinromide and 3-Bromocinnamamide

Monkey	Parent Drug Bioavailability	Metabolite Bioavailability After Administration of Parent Drug	After Administration of Metabolite
336	0.72	0.90	0.99
326	0.35	1.17	0.95
184	0.39	0.82	1.97
266	0.40	0.98	1.03
183	0.79	1.20	1.07
Mean	0.53	1.01	1.20
SD	0.21	0.17	0.43
CV, %	39	16	36

Since the systemic clearance of cinromide was a significant fraction of the hepatic blood flow, an appreciable first-pass effect was expected. The maximum bioavailability of an oral dose (F) can be predicted from the systemic clearance and hepatic blood flow (19, 20):

$$F = 1 - \frac{\text{Hepatic blood clearance}}{\text{Hepatic blood flow}} \qquad \text{(Eq. 2)}$$

This calculation assumes: (*a*) complete absorption from the GI tract; (*b*) metabolism only in the liver; and (*c*) an average hepatic blood flow of 3.18 liters/hr/kg (14). The maximum predicted bioavailability of cinromide was 0.57 ± 0.12, while the observed bioavailability was 0.52 ± 0.12 (Table V). There was no significant difference between the two values. Thus, the incomplete oral bioavailability of cinromide is probably not a result of a dosage form effect or incomplete absorption from the GI tract. Confirmation of the fact that the incomplete availability of oral cinromide is a result of a presystemic first-pass effect, rather than incomplete absorption, was obtained from the metabolite data. The AUC ratio of oral–intravenous metabolite (corrected for dose) averaged 1.01 ± 0.17 (Table V). The equal availability of the active metabolite by both routes of parent drug administration decreases the therapeutic consequences of the first-pass effect of the parent drug.

In view of the low extraction ratio of 3-bromocinnamamide, no reduction in oral bioavailability by a first-pass effect was predicted. The observed bioavailability (median = 1.05) confirmed this prediction (Table V).

In the rhesus monkey, cinromide is a medium-extraction ratio drug with a relatively short half-life. One-half of a cinromide dose is converted to 3-bromocinnamamide by *N*-deethylation. This metabolite has a low extraction ratio and a longer half-life than its precursor. The predicted high metabolite–parent drug steady-state concentration ratio suggests that this metabolite may contribute significantly to the anticonvulsant activity observed after administration of the parent drug. These findings led to the independent evaluation of the efficacy of the amide metabolite, 3-bromocinnamamide, for epilepsy in the primate model (7).

REFERENCES

(1) G. Cloutier, M. Gabriel, E. Geiger, L. Cook, J. Rogers, W. Cummings, and A. Cato, in "Advances in Epileptology: Xth Epilepsy International Symposium," J. A. Wada and J. K. Penry, Eds., Raven, New York, N.Y., 1980, p. 351.

(2) R. M. Welch, S. Hsu, E. Grivsky, and F. E. Soroko, Eleventh Collegium Internationale Neuro-Psychopharmacologicum, Vienna, Austria (1978).

(3) F. E. Soroko, E. M. Grivsky, B. T. Kenney, R. E. Bache, and R. A. Maxwell, *Fed. Proc.*, **38**, 753 (1979).

(4) L. A. Lockman, A. D. Rothner, G. Erenberg, F. W. Wright, G. Cloutier, and E. H. Geiger, American Epilepsy Society Meeting, San Diego, Calif. (1980).

(5) A. J. Wilensky, E. A. Lane, R. H. Levy, L. M. Ojemann, and P. N. Friel, *Eur. J. Clin. Pharmacol.*, **21**, 149 (1981).

(6) J. S. Lockard, R. H. Levy, L. L. DuCharme, and W. C. Congdon, *Epilepsia*, **20**, 339 (1979).

(7) *Idem.*, **21**, 177 (1980).

(8) J. A. Cramer and R. H. Mattson, American Epilepsy Society Meeting, San Diego, Calif., 1980.

(9) E. A. Lane and R. H. Levy, *J. Pharm. Sci.*, **69**, 610 (1980).

(10) R. L. D'Angelis, N. M. Robertson, A. R. Brown, T. E. Johnson, and R. M. Welch, *J. Chromatogr.*, **221**, 353 (1980).

(11) L. Z. Benet and R. L. Galeazzi, *J. Pharm. Sci.*, **68**, 1071 (1979).

(12) "Biomedical Computer Programs," 3rd ed., W. J. Dickson, Ed., University of California Press, Berkeley, Calif., 1973, p. 387.
(13) "Biomedical Computer Programs—P Series," W. J. Dickson and M. B. Brown, Eds., University of California Press, Berkeley, Calif., 1977, p. 540, 601.
(14) R. P. Forsyth, A. S. Nies, F. Wyler, J. Neutze, and K. L. Melmon, *J. Appl. Physiol.*, **25**, 736 (1968).
(15) M. Rowland, L. Z. Benet, and G. G. Graham, *J. Pharmacokinet. Biopharm.*, **1**, 123 (1973).
(16) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).
(17) S. A. Kaplan, M. L. Jack, S. Cotler, and K. Anderson, *J. Pharmacokinet. Biopharm.*, **1**, 201 (1973).
(18) K. S. Pang and J. R. Gillette, *ibid.*, **7**, 275 (1979).
(19) M. Rowland, *J. Pharm. Sci.*, **61**, 70 (1972).
(20) M. Gibaldi, R. N. Boyes, and S. Feldman, *ibid.*, **60**, 1338 (1971).

ACKNOWLEDGMENTS

This study was supported in part by NINCDS Research Contract NO1-NS-1-2282.

The authors acknowledge the analytical assistance of Ms. Jennifer Andrews and the editorial assistance of Ms. Colleen Montoya.

Use of Isosorbide Dinitrate Saliva Concentrations for Biopharmaceutical Investigations

H. LAUFEN ˣ, M. SCHMID, and M. LEITOLD

Received December 17, 1981, from the *Department of Pharmacology, Heinrich Mack Nachf., 7918 Illertissen/Bayern, West Germany.* Accepted for publication May 18, 1982.

Abstract □ The concentration of isosorbide dinitrate in paired samples of plasma and mixed saliva was monitored for up to 24 hr after oral administration of 60 mg of sustained-release isosorbide dinitrate to eight healthy volunteers. Measured isosorbide dinitrate plasma concentrations were mainly in the range of 0.1–10 ng/ml. Isosorbide dinitrate was excreted into saliva resulting in a mean (±*SD*) saliva–plasma concentration ratio of 0.68 ± 0.37. A significant correlation between concentrations of isosorbide dinitrate in saliva and plasma was found ($p < 0.01$). The sustained-release properties of the administered formulation were confirmed from the concentrations of isosorbide dinitrate found in both saliva and plasma. Saliva–plasma ratios were independent of the absolute concentrations of isosorbide dinitrate but showed a slight tendency to decrease with time. The principal factor relating saliva and plasma isosorbide dinitrate concentrations appeared to be the degree of plasma protein binding of the drug.

Keyphrases □ Isosorbide dinitrate—saliva–plasma ratios following oral administration of a sustained-release preparation □ Sustained-release formulations—saliva–plasma ratios of isosorbide dinitrate following oral administration □ Excretion, salivary—use of isosorbide dinitrate saliva concentrations for biopharmaceutical investigations □ Pharmacokinetics—use of isosorbide dinitrate saliva concentrations for biopharmaceutical investigations

In recent years many investigations have been made on the salivary excretion of drugs in humans. For a number of drugs, it has been demonstrated that the measurement of their concentrations in saliva can be a convenient substitute for plasma analyses, both in monitoring therapeutic drug concentrations and in pharmacokinetic and biopharmaceutical studies. The advantage offered by the measurement of drug concentrations in saliva as well as the limitations of this procedure have been the subject of recent reviews (1–8).

Whether salivary concentration measurements are of value in monitoring pharmacokinetic properties of drugs or not depends on how closely saliva and plasma levels are related. Variations in the saliva–plasma ratio are produced by a number of factors, including variation of the pH of plasma and saliva, the extent of the drug's plasma protein binding, salivary flow rate, active secretion processes, buccal reabsorption, delayed appearance of the drug in saliva, and the technique of saliva sampling (9).

Isosorbide dinitrate is used in the treatment of coronary disease. The plasma pharmacokinetics of this drug are characterized by its rapid excretion (10). This is a disadvantage in long-term therapy with isosorbide dinitrate, and a sustained-release oral formulation of the drug has been used in an attempt to overcome this problem (11–17). During the development of a sustained-release preparation of isosorbide dinitrate, the usefulness of salivary concentration monitoring by multiple paired measurement of isosorbide dinitrate in saliva and plasma was tested.

The present report describes the saliva–plasma ratios of isosorbide dinitrate in eight healthy humans following oral administration of 60 mg of a sustained-release preparation.

EXPERIMENTAL

Eight healthy, fasted, volunteers (3 females and 5 males; 18–38 year; 42–75 kg) were each given one capsule of a slow-release formulation of isosorbide dinitrate[1] (60 mg) with 100 ml of water. Three hours after drug ingestion, a normal breakfast was taken. Blood samples were collected by venipuncture at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, and 24 hr after drug administration. At each time the volunteers expectorated 1 ml of mixed saliva into a glass vial. When necessary, to enhance the spontaneous saliva flow, a small crystal of citric acid was applied to the volunteer's tongue. Saliva and plasma (separated from whole blood by centrifugation) were stored at −30° until analysis. Concentrations of isosorbide dinitrate in plasma and saliva were determined by an identical GC procedure with an internal standard (18). The lower limit of isosorbide dinitrate detection was 0.05 ng/ml. All analyses were performed in duplicate.

Statistical calculations on the concentration data obtained were performed with a desk-top computer[2]. The correlation of saliva and plasma levels was determined by linear regression. The coefficient of regression was tested for significant difference from zero, the 95% confidence limits of the coefficient of regression were calculated, and an analysis of variance of the regression was performed (19). Using the same procedures, possible correlations of the saliva–plasma ratio with the absolute plasma concentrations of isosorbide dinitrate as well as with sampling time were tested. The areas under the saliva and plasma level curves were calculated using the trapezoidal rule. An approximate estimation of the apparent

[1] Iso Mack Retard 60 mg, batch No. Ph 2009.
[2] Hewlett-Packard 9815.

0022-3549/83/0500-0496$01.00/0