Pharmacokinetics of Intranasally-Administered Dihydroergotamine in the Rat

David T.-W. Lau,[1,2,3] Zhiling Yu,[1] Renee L. Aun,[1] Alan E. Hassell,[1] and Francis L. S. Tse[1]

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Intranasal dosing of dihydroergotamine (DHE) allows convenient self-administration and provides an alternate route of administration for the treatment of migraine in addition to the existing parenteral dosage forms. In this study, the pharmacokinetics of ^{3}H-DHE were investigated following intravenous and intranasal dosing (0.343 mg DHE/animal) in the rat. Intranasal administration of DHE resulted in rapid absorption. The extent of absorption of the radiolabeled dose was approximately 45%–60%. Absolute bioavailability of the parent drug was 35%–40%, as determined by deconvolution and by the ratios of $AUC_{0-\infty}$ following intranasal and intravenous dosing. Due to the limited capacity of the nostrils, approximately half of the intranasal dose was swallowed into the gastrointestinal tract. Biliary excretion was found to be the predominant pathway of radioactivity excretion following both routes of administration. The results from this study suggest that intranasal administration provides a viable means of delivering DHE into the systemic circulation.

KEY WORDS: dihydroergotamine; ergot alkaloids; intranasal delivery; pharmacokinetics; rat.

INTRODUCTION

Dihydroergotamine (DHE, 9,10-dihydro-12′-hydroxy-2′-methyl-5′α-(phenyl methyl)ergotaman-3′,6′,18-trione), a semisynthetic alkaloid, has been used for the treatment of migraine headaches for almost half a century [1]. Currently in the United States, DHE is commercially available in parenteral dosage forms for intravenous or intramuscular injection. Alternate routes of administration which allow more convenient self-administration of the drug are being investigated. The well-perfused nasal mucosal region provides an excellent site for rapid absorption of drugs following intranasal dosing. Although drug metabolizing-enzymes are present in the nasal mucosa [2], presystemic metabolism after intranasal dosing is generally expected to be less extensive than following oral dosing. In Europe, intranasally-administered DHE has been tested in clinical studies [3,4] and is currently available for the treatment of migraine attacks. The present study was conducted to examine the pharmacokinetics of intranasally-administered ^{3}H-DHE in the rat. The information is needed to confirm drug exposure in rats during chronic toxicity studies, which are ongoing in the United States.

MATERIALS AND METHODS

Chemicals

DHE and ^{3}H-DHE were prepared as the methanesulfonate (mesylate) salt [5]. The radiochemical purity of the product was >95% as determined by TLC and HPLC. Solutions of ^{3}H-DHE and DHE (3.43 mg/ml) were prepared by dissolving the drug in an aqueous solvent which consists of 50 mg of dextrose and 10 mg of caffeine per milliliter of water. The specific activity of the radiolabeled DHE solution was 100 μCi/ml.

Animal Experiments

Twenty four male Sprague-Dawley rats (Charles River, NY), weighing 280 ± 16.6 g, were divided into two groups of equal size for intravenous and intranasal dosing. Within each group, 4 animals were administered ^{3}H-DHE and the radioactivity content in blood, urine, and feces was examined. The bile ducts of another 4 animals were cannulated and the radioactivity profile in urine, feces, and bile were examined following administration of the ^{3}H-DHE dose. The remaining four animals in each group were dosed with the non-radiolabeled drug to provide plasma samples for unchanged drug measurement (via a radioimmunoassay which utilizes ^{3}H-DHE as the tracer). The animals were kept individually in metabolism cages and were allowed free access to standard laboratory chow and water.

A 100 μl aliquot (0.343 mg, equivalent to 0.4 mg of the methanesulfonate salt) of the dose solution was administered either intravenously or intranasally. For animals receiving the intranasal doses, a 250 μl Hamilton syringe with a small piece of PE 50 tubing was used for dosing. Prior to use, the free end of the tubing was heated to smooth the edges so as to minimize trauma to the nasal passage of the animals. The rats were placed under light anesthesia (1.5% isoflurane in oxygen) and approximately 50–65 mm of the tube was placed into the nostril. A 25 μl aliquot of the DHE solution was instilled into each nostril at the start of the experiment. A second dose of 25 μl/nostril was administered one hour after the initial dose. After each dosing session, the exterior nasal region of each animal was cleaned using a piece of gauze to collect any dose which was sneezed out or leaked from the nostrils. The nasal swabs were retained for radioactivity analysis for animals receiving the radiolabeled dose. The intravenous dose (100 μl) was administered as a single injection via the jugular vein, which was surgically exposed under isoflurane anesthesia (2% isoflurane in oxygen). The incision was closed with sutures immediately after dosing.

From intact animals receiving ^{3}H-DHE, serial blood samples (200 μl) were collected from the tail vein at designated times for 96 hours after dosing. At each time point, 50 μl aliquots of blood and plasma were used for radioactivity analysis. Urine and feces were collected at 24 h intervals for 4 days. From the bile-duct cannulated animals, bile, urine, and feces were collected for 72 hours after dosing. For animals receiving the non-radiolabeled drug, serial blood samples (300 μl) were collected for 72 hours. Due to limitations in assay sensitivity and the limited volume of blood that can

[1] Department of Drug Metabolism and Pharmacokinetics, Drug Safety, Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, East Hanover, NJ 07936.

[2] To whom correspondence should be addressed.

[3] Current address: Department of Pharmacology, Amgen Inc., 1840 De Havilland Drive, Thousand Oaks, CA 91320.

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be drawn from rats, plasma samples from the 4 animals in each dose group were pooled for analysis at each sampling time.

Sample Analysis

The radioactivity of all samples was determined by liquid scintillation counting in a Packard Spectrometer Model 460 (Packard Instrument Co., Downer's Grove, IL). Samples were counted directly or by combustion. Formula 989 (DuPont, Boston, MA) was used for direct counting. Monophase S (Packard) was used in a Tri-Carb Sample Oxidizer, Model 306 (Packard) as the scintillant for combusted samples. The detection limit for the radioactivity analysis was approximately 1.8 ng equivalent of DHE per milliliter of blood or plasma.

Plasma concentrations of DHE were determined using a radioimmunoassay which is specific for the determination of the parent drug [6]. Briefly, unknown DHE concentration in plasma was determined by the displacement of a tracer (^{3}H-DHE) in an anti-DHE antibody-antigen complex. The plasma sample, tracer, and rabbit antiserum were incubated overnight in citrate buffer at pH 6.0 and at 4°C. The antibody-bound drug was separated from free drug by the addition of plasma-coated charcoal. After centrifugation, the tritium content of the supernatant was counted. The minimum quantification limit was 0.018 ng/ml in this study. The interassay coefficient of variation was ~11% near the quantification limit, and was considerably lower as concentration increased.

Pharmacokinetic Analysis

The area under the curve (AUC) values were determined by linear trapezoidal and log-trapezoidal methods for the intranasal and the intravenous groups, respectively [7]. The percentage of intranasal absorption of the radiolabeled dose was estimated by comparing blood, plasma, and excreta radioactivity data following intranasal and intravenous administration using intact and bile-duct cannulated animals. The bioavailability of unchanged DHE was estimated by the following equation:

$$Bioavailability = \frac{AUC_{0-\infty,intranasal}}{AUC_{0-\infty,intravenous}} \times \frac{DOSE_{intravenous}}{DOSE_{intranasal}}$$

where $AUC_{0-\infty}$ is the area-under-the-curve of the plasma DHE concentration versus time plot. In addition, the cumulative percentage of dose available to the systemic circulation from the rats receiving intranasal doses was obtained by a modified version [8] of a finite difference numerical deconvolution method developed by Vaughan and Dennis [9], implemented using IMSL/IDL [10]. The pooled plasma DHE concentration data following intravenous dosing were defined as the impulse response, as fitted by the following triexponential equation:

$$C_{IV} = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} + A_3 e^{-\lambda_3 t}$$

A weighting factor of $1/y^2$ was used.

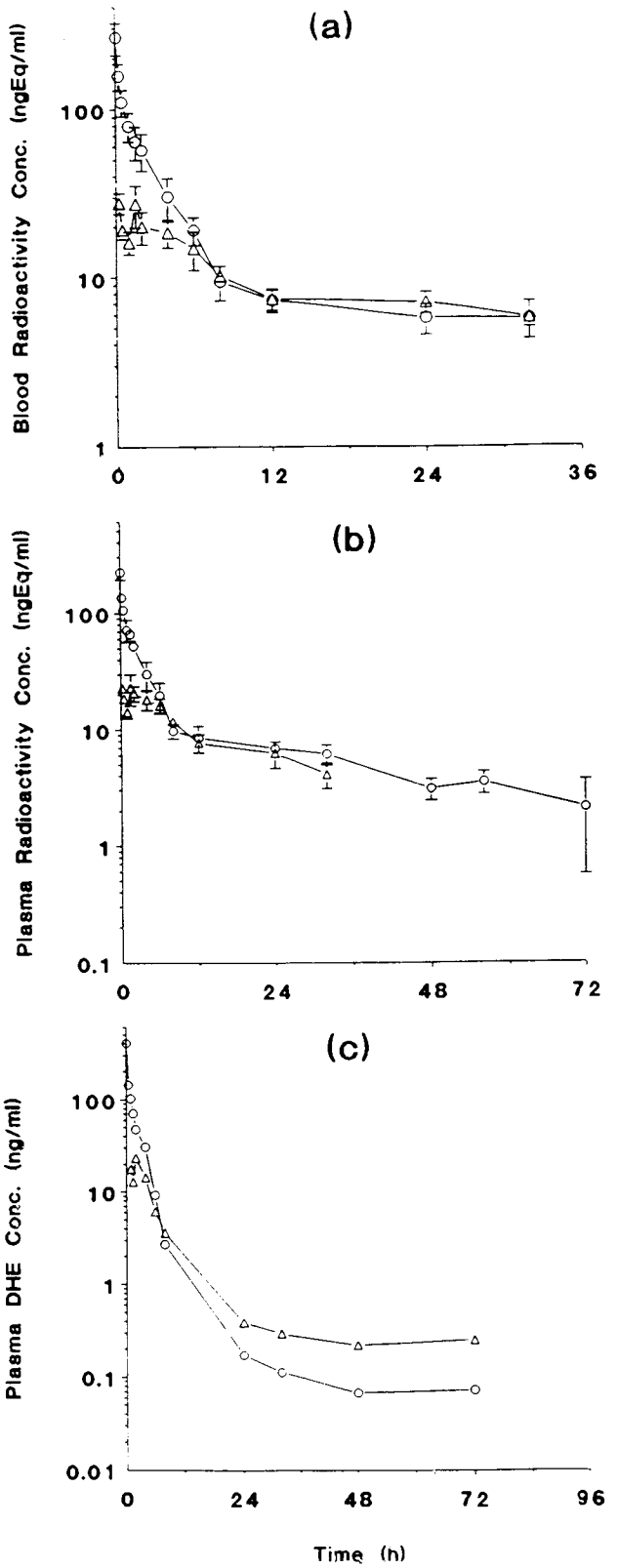

Figure 1 Concentrations of (a) blood radioactivity (mean ± SD), (b) plasma radioactivity (mean ± SD), and (c) plasma DHE (pooled sample) following intravenous (–○–) and intranasal (–△–) dosing of 0.343 mg ^{3}H-DHE. (n = 4)

RESULTS

The concentrations of blood radioactivity, plasma radioactivity, and plasma DHE concentrations following intravenous and intranasal dosing are shown in Figures 1a, b, and c, respectively. The time to the peak concentration (t_{max}), peak concentration (C_{max}), and $AUC_{0-\infty}$ of total radioactivity and unchanged DHE are summarized in Table I. Following intravenous administration, concentrations of radioactivity and unchanged DHE exhibited a rapid decline initially, followed by slower elimination phases at later times. Following intranasal administration, the radioactivity in blood and plasma peaked rapidly after each of the two doses, at approximately 0.25 h and 2 h, respectively. The two doses also resulted in similar peak concentrations of radioactivity. The rate of decline of radioactivity and unchanged DHE concentrations was similar to that during the slower phases of elimination following intravenous dosing. The concentrations of radioactivity and unchanged DHE were substantially lower than those obtained from the intravenous administration within the first 6 hours post-dose. Beyond 6 hours, the concentrations were similar between the two routes of administration. Plasma concentrations of unchanged DHE were similar to plasma radioactivity until 4 hours following both routes of administration. After four hours, plasma radioactivity concentrations were consistently higher than plasma DHE concentrations until the detection limit was reached.

The excretion of radioactivity in urine and feces following intravenous and intranasal administration of ^{3}H-DHE is shown in Table II. Following intravenous dosing, the majority of the radioactivity was found in the feces (79%), with only approximately 13% recovered in the urine. Similarly, fecal elimination was the major route of excretion in rats receiving the intranasal doses (73%), compared to only approximately 8% in the urine. Approximately 80% of the administered intranasal dose was recovered from the excreta within 48 hours post-dose. Less than 2% of the intravenous or intranasal dose was excreted between 48–96 h, indicating that the elimination of ^{3}H-DHE was rapid and essentially complete at 48 hours post-dose. Including the recovery from nasal swab (~12%) and cage wash (~1%) samples, excretion of the radiolabeled dose was complete (90%–105%) at 96 h after intranasal dosing.

In bile duct-cannulated rats receiving the intravenous dose, 81% of the radioactivity was found in the bile at 72 hours post-dose, with the majority of the radioactivity excretion occurring during the first 24 hours. Urinary excretion accounted for 19% of the dose. Following intranasal dosing, 24% and 16% of the dose was recovered in the bile and the urine, respectively. Approximately 47% of the radioactivity was recovered in the feces at 72 hours post-dose.

An impulse response model was generated by fitting the pooled plasma DHE concentration data for rats receiving the intravenous dose. As shown in Figure 2a, the following triexponential model yielded the best fit of the data:

$$C_{IV} = 401e^{-5.36t} + 150\,e^{-0.493t} + 0.195\,e^{-0.0169t}$$

The corresponding half-lives of the three disposition rate constants were 0.13 h, 1.4 h, and 41 h, respectively. The three disposition phases contributed to 19%, 78%, and 3% of the total AUC following intravenous administration. The cumulative amount of DHE available to the systemic circulation following intranasal administration was estimated by deconvolution, and the results are shown in Fig. 2b. Approximately 35% of the total intranasal dose of DHE was bioavailable as the parent drug.

DISCUSSION

Results from the present study show that the onset and rate of intranasal absorption of DHE were rapid in the rat. Blood and plasma radioactivity peaked rapidly following administration of the two intranasal doses. To estimate the extent of absorption of the radiolabeled dose following intranasal dosing, several common methods were used [11]. By comparing the total radioactivity data in blood or in plasma following intranasal and intravenous doses, the estimated absorption was 52%–59%. From the relative amount excreted in urine, the fraction of intranasal dose absorbed was estimated to be 60%. Using the sum of urinary and biliary excretion data from bile-duct cannulated rats, the fraction of dose absorbed was found to be 45%. It should be noted that using AUC values of blood or plasma radioactivity to assess absorption assumes the "metabolite mix" was similar after intravenous and intranasal administration [11]. Nevertheless, these results were consistent with the absorption estimates generated from the excretion data. Summarizing the results from the different methods, the fraction of intranasal dose absorbed was approximately 45%–60%.

Using the ratio of $AUC_{0-\infty}$ of plasma DHE following intranasal and intravenous dosing, the bioavailability of the parent drug was found to be approximately 40%, in good agreement with that estimated using deconvolution analysis

TABLE I. Pharmacokinetic Parameters of ^{3}H-DHE Following Intranasal and Intravenous Administration

Route of Administration	Pharmacokinetic Parameters	Blood[1] Radioactivity	Plasma[1] Radioactivity	Plasma[2] DHE
Intranasal	$t_{max,1}$ (h)	0.25 ± 0.00	0.25 ± 0.00	1.0
	$t_{max,2}$ (h)	2.1 ± 1.3	2.3 ± 1.2	2.0
	$C_{max,1}$ (ng Eq/mL or ng/mL)	28 ± 4.0	23 ± 2.0	18
	$C_{max,2}$ (ng Eq/mL or ng/mL)	28 ± 6.8	25 ± 5.5	23
	$AUC_{0-\infty}$ (ng Eq · h/mL or ng · h/mL)	370 ± 51	360 ± 21	170
Intravenous	$AUC_{0-\infty}$ (ng Eq · h/mL or ng · h/mL)	630 ± 140	690 ± 110	420

[1] Mean ± SD, n = 4.
[2] Pooled sample from 4 animals.

Table II. Percent of Administered Radioactivity Recovered from Rats Following an Intravenous or Intranasal DHE Dose (0.343 mg)

	Without cannulated bile duct[1]		With cannulated bile duct[1]	
Sample	Intravenous	Intranasal	Intravenous	Intranasal
Urine[2]	13 ± 6.7	8.0 ± 1.9	19 ± 7.0	16 ± 5.3
Feces[2]	79 ± 2.8	73 ± 3.3	9.2 ± 2.6	47 ± 6.9
Bile[3]	N/A	N/A	81 ± 9.6	24 ± 8.3
Nasal Swab[4]	N/A	12 ± 4.5	N/A	7.4 ± 2.1
Cage Wash[2]	1.4 ± 0.24	0.79 ± 0.22	1.5 ± 1.0	2.7 ± 0.95
Total[2]	94 ± 8.4	93 ± 2.4	110 ± 3.2	97 ± 5.9

[1] Mean ± SD, n = 4.
[2] 0–96 h and 0–72 h for rats without and with bile duct cannulation, respectively.
[3] 0–72 h for rats with bile duct cannulation.
[4] Collected immediately after intranasal dosing.

(35%, Fig. 2b). The results in the rat are similar to previous findings in humans that showed rapid absorption of DHE after intranasal administration, with a relative bioavailability (as compared to intramuscular injection) of approximately 38% [12]. The present bioavailability estimates are slightly lower than the estimated extent of absorption. This phenomenon can be explained by possible first-pass metabolism in the nasal mucosa, which is known to exhibit activities for oxidative drug metabolism [2]. However, since different animals were used for the administration of radiolabeled and non-radiolabeled compounds in the study, the small difference between bioavailability and absorption estimates could also be due to inter-animal variability.

Following both intranasal and intravenous doses, plasma concentrations of radioactivity were substantially higher than unchanged DHE concentrations after 4 hours post-dose, apparently due to circulating metabolite(s) which exhibit longer residence times than the parent drug. Nevertheless, the elimination of ^{3}H-DHE was rapid. Biliary excretion was the main route of elimination, in agreement with previous findings in rabbits and in man [13]. For intranasal administration, approximately 25% of the total administered dose was excreted via the bile. Since DHE is poorly absorbed via oral administration, the radioactivity found in the feces of the bile duct-cannulated rats receiving intranasal doses provides an estimate of the fraction of dose which was swallowed into the gastrointestinal tract. Approximately 50% of the dose was found in the feces following intranasal administration, suggesting that at the dose volume used in this study (25 µl/nostril), half of the administered dose was swallowed. Approximately 10% of the dose was recovered in the nasal swab. Therefore, the remaining 40% of radiolabeled dose available for intranasal absorption was completely absorbed since 25% and 15% of the dose was subsequently recovered in bile and urine, respectively. The dose volume used in this study was slightly higher than the optimal dosing volume (2–15 µl/nostril) suggested by Char et al. [14]. Conceivably, the percentage of dose swallowed into the gastrointestinal tract can be reduced with a lower dosing volume.

In summary, ^{3}H-DHE was moderately well-absorbed (45–60%) following intranasal administration. The bioavail-

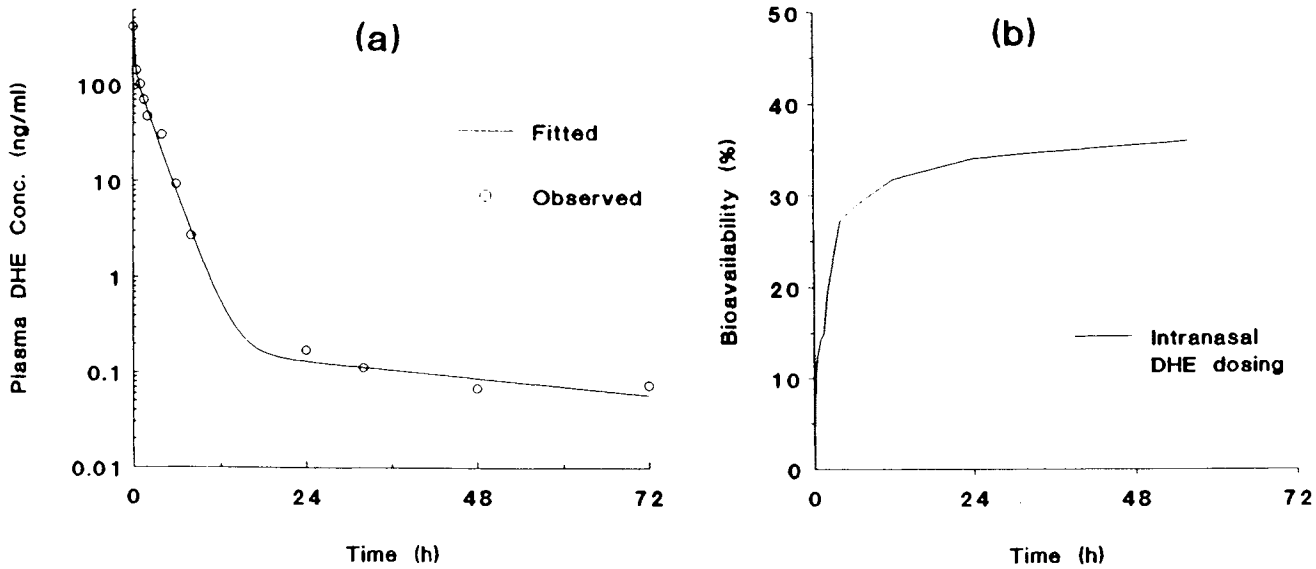

Figure 2 (a) Intravenous administration : plasma DHE concentration as fitted by a tri-exponential model, and (b) Intranasal administration: estimation of the cumulative bioavailability of DHE by deconvolution, using the model in (a) as the impulse response model.

ability of unchanged DHE was approximately 35%–40%. The onset and the rate of absorption were rapid. At the dose volume tested in this study, approximately 50% of the intranasal dose was swallowed into the gastrointestinal tract. Biliary excretion was the predominant pathway of excretion following intravenous and intranasal administration. Intranasal administration provides a viable means for the delivery of DHE into the systemic circulation.

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