Intranasal Absorption of a Kappa Agonist Analgesic

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Received October 19, 1992; accepted January 27, 1993
KEY WORDS: kappa agonist analgesic; nasal absorption; bioavailability; pharmacokinetics; ED_{50}.

INTRODUCTION

U-50,488H (1) was the first of several benzeneacetamide kappa agonist analgesics which produce potent analgesic activity without narcotic-like side effects or narcotic dependence liability in mice and rats. Newer benzeneacetamide and arylacetamide kappa-selective analgesics include spiradoline (2), CI-977 (3), and DuP 747 (4). Kappa agonist analgesics produce moderate–strong analgesic activity in a variety of animal models. However, in contrast to mu opioid analgesics, they produce minimal effects on respiration and do not produce morphine-like tolerance or physical dependence following chronic administration (5–7).

Most animal studies of kappa agonist analgesics have focused on parenteral routes of administration where analgesic activity is obtained at doses in the microgram per kilogram to low milligram per kilogram range. Comparative studies in our laboratory have shown that nearly all kappa agonist analgesics have substantially reduced activity by the oral route, with the most potent analgesics generally having the largest differences between oral and s.c. doses (8). Presumably, this is due to either poor oral absorption or a high first-pass effect as the compounds are circulated through the hepatic–portal system before reaching the systemic circulation and their receptor sites of action in the brain and the spinal cord.

E3800 (Fig. 1) is the 5-H-6-hydroxy analogue of DuP 747 (racemic *trans*-3,4-dichloro-*N*-methyl-*N*-[1,2,3,4-tetrahydro-5-methoxy-2-(pyrrolidin-1-yl)napth-1-yl]benzeneacetamide). Initial studies showed that E3800 has a moderately selective kappa vs mu receptor profile (K_i's = 1 and 21 n*M*, respectively) and that it produces potent antinociceptive responses in the mouse phenylquinone writhing test (ED_{50} = 0.03 mg/kg, s.c., and 4.2 mg/kg, p.o.; compound 22 in Ref. 9). E3800 is 15 times more potent s.c. but is equipotent orally with DuP 747. The high oral/s.c. ratio (140×) in these initial tests suggests that E3800, like other benzeneacetamide kappa agonist analgesics, may be either poorly absorbed or subject to high first-pass metabolism following oral administration in mice.

Intranasal administration has been shown to improve the bioavailability of many rapidly metabolized drugs [for example, propranolol (10), physostigmine, and arecoline (11)]. Intranasal butorphanol tartrate, a potent agonist/antagonist analgesic with a high first-pass effect, was recently introduced for the treatment of pain in man (12). Clinical studies show that 2 mg intranasal butorphanol is equipotent with and has a longer duration of action than 2 mg i.v. butorphanol (13).

In this report, we examined the nasal route for the delivery of E3800 in rats. Analgesic potency was determined following intranasal administration of 10-μL doses in lightly anesthetized rats which recovered to full consciousness prior to analgesic testing. Bioavailability and drug disposition were evaluated in surgically prepared pentobarbital anesthetized rats which remained anesthetized throughout the study period.

MATERIALS AND METHODS

Animals

Experiments were performed using male Sprague–Dawley CD rats, 65–95 g, or male CF1 mice, 18–23 g, for pharmacological testing and male Lewis rats, 300-g average weight, for pharmacokinetic studies (Charles River Breeding Laboratories, Wilmington, MA). All mice and rats were acclimated a minimum of 5 days prior to testing at 22 ± 2°C with 50 ± 10% average relative humidity. All animals received 12 hr light per day (0600 to 1800). Drug-naive mice and rats were used for each study. All studies were performed according to the standards of the *Guide for the Care and Use of Laboratory Animals* (Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council) in an AAALAC-accredited facility.

Drugs and Treatments

E3800 [(±)*trans*-3,4-dichloro-*N*-methyl-*N*-[6-hydroxy-2-(pyrrolidin-1-yl)-1,2,3,4-tetrahydronapthalen-1-yl]benzeneacetamide), phosphate salt] was from The Du Pont Merck Pharmaceutical Company. Some studies performed with the HCl salt of E3800 are included without differentiation since separate studies confirmed that they are pharmacologically equivalent. E3800 was dissolved in distilled water for intranasal administration. Parenteral doses were prepared in saline, 5% dextrose/water, or 0.25% Methocel/2% Tween 80. Levallorphan tartarate, used as an internal standard for HPLC analysis, was a gift from Hoffman La Roche Inc.

Pharmacological Testing

Mouse and rat analgesic activity was determined using the phenylquinone writhing (PQW) test (14). Fasted male CF1 mice were injected with different doses of E3800 and then challenged with 1.25 mg/kg, i.p. phenylquinone 5 min prior to the designated time. Rats (65–95 g) were injected

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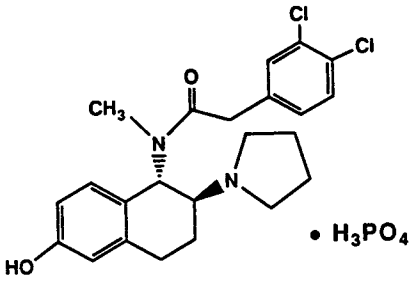

Fig. 1. Chemical structure of E3800.

i.p. with 1.0 mg/kg phenylquinone 5 min prior to the designated test time. For both mice and rats, analgesia was indicated by a complete blockage of the abdominal constriction (writhing) response during a 10-min observation period starting at the designated test time. For intranasal studies in rats, the drug was prepared at different concentrations and administered in a volume of 10 μL (5 μL in each nostril) using a Hamilton syringe, after the rats were lightly anesthetized with carbon dioxide. Animals awakened within 5 min of dosing.

Rat Bioavailability Studies

Male Lewis rats were anesthetized with pentobarbital (50 mg/kg, ip). For i.v. administration of E3800, a dose of 2 mg/kg in a volume of 1 mL/kg was administered by cardiac puncture using a 23-gauge needle and supplemental ether anesthesia. The oral dose was 20 mg/kg in a volume of 1 mL/kg administered by gavage.

For nasal administration, an incision was made in the neck of the animals and the trachea was cannulated with a polyethylene tube to allow free breathing. A closed tube was inserted through the esophagus to the posterior part of the nasal cavity. The nasopalatine passage was closed with a cyanoacrylate adhesive to prevent drainage of the drug from the nasal cavity to the mouth. E3800 at a dose of 4 mg/kg in a volume of 150 μL/kg was administered to the nasal cavity by means of a Hamilton syringe with PE tubing attached to the end. Rats remained anesthetized and lying on their backs throughout the study. Blood samples (~0.5 mL) were collected into heparinized test tubes by cutting the tip of the tail. Plasma was separated and assayed.

Analytical Methodology and Pharmacokinetics

Plasma samples (0.2 mL) were pipetted into extraction tubes; 50 μL of an aqueous solution of the internal standard (levallorphan tartarate; 1 μg/mL), 0.2 mL of carbonate buffer (pH 9.3) and 4 mL of anhydrous ethyl ether were added. The tubes were shaken mechanically for 1 min and centrifuged at ~2000 rpm for 5 min. The organic layer was then transferred to 12 × 75-mm tubes containing 0.2 mL of 0.017 *M* phosphoric acid. The tubes were shaken mechanically for 1 min and centrifuged at ~2000 rpm for 5 min. The ether was aspirated and the acid layer (100 μL) was subjected to analysis.

Analysis was performed using HPLC with electrochemical detection. Chromatographic separation was achieved on a 25 cm × 4.6-mm reverse-phase (Zorbax C_8, Du Pont) column attached to a guard column with C_{18} packing at ambient temperature. The mobile phase contained acetonitrile (500 mL), 3% acetic acid (500 mL), EDTA (0.17 g), triethylamine (1 mL), and the pH of the mobile phase was adjusted to 6 using concentrated sodium hydroxide. The mobile phase was delivered at a rate of 1.5 mL/min. Electrochemical detection (Bioanalytical System LCB_4) at an oxidation potential of 0.8 V using a working glassy carbon electrode and a Ag/AgCl reference electrode were used.

Typical retention times for the internal standard (levallorphan) and E3800 were 5.9 and 8.3 min, respectively. The limit of E3800 detection was 7 ng/mL using 0.2 mL plasma.

The terminal decay rate constant, k, and the terminal half-life, $t_{1/2}$, were calculated by linear regression of the terminal portion of ln C_p of plasma E3800 concentration versus time. The area under each E3800 concentration versus time curve (AUC) was calculated using the trapezoidal method, with the residual area calculated by dividing C_p at the time of the last sample by k. Nasal bioavailability (F) was calculated from the i.v. dose-normalized AUC 0–∞ values. Average AUC for the group administered E3800 i.v. was used.

Statistics

Quantal ED50 values were calculated by the method of moving averages (15). Quantitative comparisons of blood level data were performed using Student's t test (two-tailed).

RESULTS AND DISCUSSION

E3800 produces potent parenteral analgesic activity in mice and rats. ED_{50}'s after subcutaneous administration in the phenylquinone writhing (PQW) test were 0.058 and 0.012 mg/kg in mice and rats, respectively (Table I). After oral administration, much higher doses of E3800 were needed to exhibit a similar effect in mice (ED_{50} = 9.5 mg/kg). Values in these mouse PQW tests are in good agreement with ED_{50} values obtained in our initial tests [mouse PQW ED_{50}'s = 0.03 and 4.2 mg/kg, s.c. and p.o., respectively (9)]. These data confirm that E3800 has a low oral bioactivity in mice (ratio for oral/s.c. ED_{50}'s is 164× in the present test vs 140× in our previous test).

Administered intranasally, E3800 produced potent analgesic effects from 0.020 to 1.3 mg/kg (Fig. 2). Intranasally administered E3800 appeared to be at least as potent as s.c.

Table I. Analgesic Activity (Mouse and Rat PQW Tests)[a]

	PQW analgesic ED_{50} (mg/kg)				Oral/s.c. ED_{50} ratio	
	Mice		Rats			
Compound	s.c.	p.o.	s.c.	p.o.	Mice	Rats
E3800	0.058	9.5	0.012[b]	NT	164×	—
DuP 747	0.46	6.2	0.30	1.1	13×	4×
Morphine	0.98	3.8	0.62	2.4	4×	4×

[a] N = 15 mice or rats/dose for E3800. Mouse s.c. ED_{50} is at 20 min peak-effect time; mouse oral ED_{50} and rat s.c. ED_{50} are at 30 min after dosing. DuP 747 and morphine values are historical peak-time data from our laboratory.

[b] E3800 HCl salt was used for rat (s.c.) study; other data were obtained using the more soluble phosphate salt. ED_{50} data have been adjusted to phosphate equivalent salt weight.

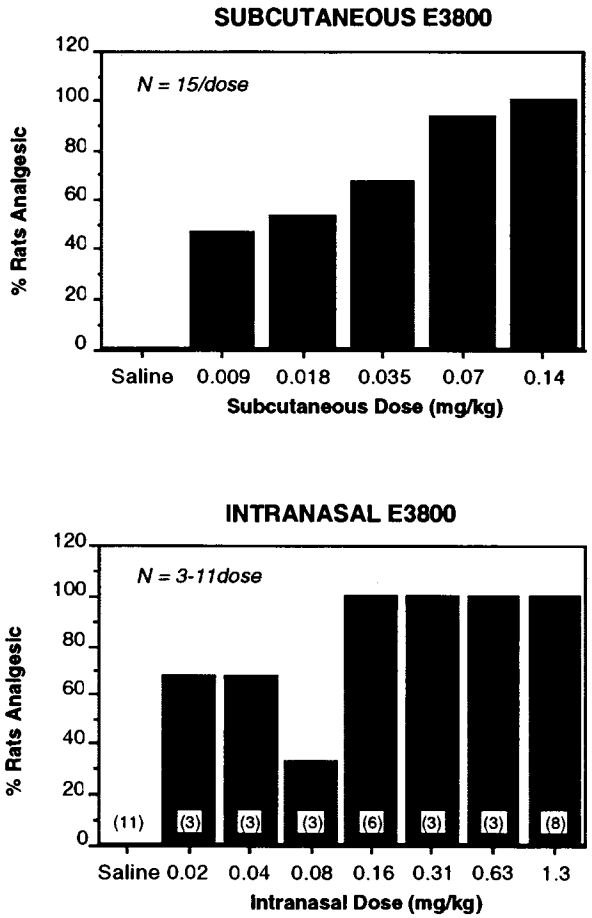

Fig. 2. Analgesic activity of subcutaneous and intranasal E3800 in the rat PQW test. Test times were at 30 min (s.c.) and 15 min (i.n.). N = 15 rats/dose (s.c.) and 3–11 rats/dose (i.n.; actual values shown in parentheses). Estimated ED_{50} values are 0.012 mg/kg s.c. and ≤0.020 mg/kg i.n.

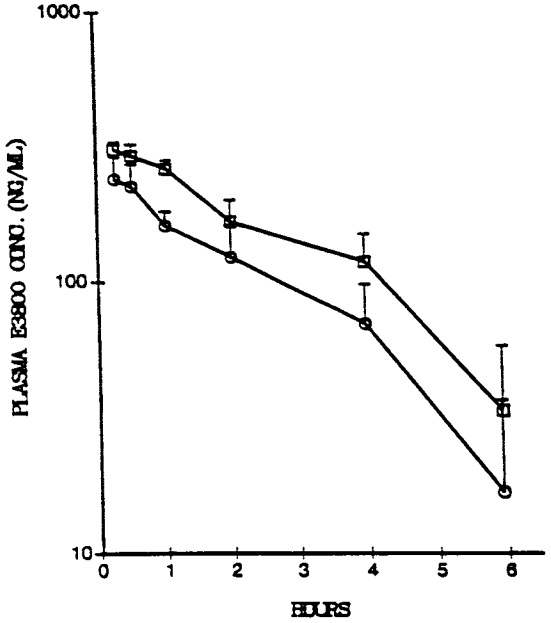

Fig. 3. Plasma E3800 concentrations (mean + SD) in rats administrated an i.v. (○; 2 mg/kg; n = 6) or an intranasal dose (□; 4 mg/kg; n = 3).

administered doses in the rat, indicating the effectiveness of the intranasal route as a delivery site. The i.n.-dosed rats were evaluated at 15 min after dosing, while the s.c.-dosed rats were evaluated at 30 min; the real s.c./i.n. potency comparison may be slightly different depending on actual peak-effect times for each route.

Pharmacokinetic studies were performed in a separate group of rats. Plasma E3800 concentrations after intranasal administration were compared to those after i.v. administration (Fig. 3). Absorption was very rapid as indicated by maximum plasma concentrations at the first sampling time (15 min).

This short time to reach maximum plasma concentration (t_{max}) is important for analgesic compounds where effect is needed immediately. The plasma terminal $t_{1/2}$ of intranasally administered E3800 was 2.6 ± 0.7 hr, similar to that observed after i.v. administration (2.2 ± 0.7 hr; no significant difference, t test).

The bioavailability of nasally administered E3800 was 78 ± 9%.

Blood levels of E3800 were not detectable following oral administration to rats at a dose of 20 mg/kg (five times higher than the nasal doses). These results confirm the low oral bioactivity observed in analgesic studies and indicate that intranasal administration represents a feasible alternative to parenteral injections for this kappa agonist analgesic.

ACKNOWLEDGMENTS

The technical assistance of Mr. Christopher Koval, Mrs. Robin Crow, Mr. William Keim, Mrs. Carla Maciag, Ms. Maria Marynowski, and Mrs. Ellen Godfrey is greatly appreciated. We also thank Dr. Richard Scribner, Mr. Peter Mattei, and Mr. Pennio Pennev for providing E3800.

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