Increased Bioavailability of Clomipramine after Sublingual Administration in Rats

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Abstract □ This study examined the absorption and disposition of clomipramine in rats after sublingual (5 and 50 mg/kg), oral (50 mg/kg), and iv (5 mg/kg) administration. The mean oral bioavailability of clomipramine was 24.8% and 29.7%, respectively, in conscious rats and in rats anesthetized with ketamine/xylazine (30/3 mg/kg). When given sublingually in isotonic saline at a dose of 50 mg/kg, clomipramine was rapidly absorbed, and the mean absolute bioavailability (36.2%) was increased over oral dosing. The mean AUC values of clomipramine were 2258 ± 1762 ng·h/mL and 1891 ± 867 ng·h/mL after oral administration to conscious and anesthetized rats, respectively, and 3303 ± 1576 ng·h/mL after sublingual administration to anesthetized rats. Sublingual administration (5 mg/kg doses) of clomipramine formulated with a permeation enhancer, 2-hydroxypropyl β-cyclodextrin, further increased the sublingual bioavailability to 57.1%. The sublingual route may be an alternative route of administration of clomipramine, providing enhanced bioavailability.

Introduction

Clomipramine is a tricyclic antidepressant widely used for the treatment of depression in a number of countries in Western Europe and Canada. In the United States, it has only recently been approved for the treatment of obsessions and compulsions in patients with obsessive-compulsive disorder.[1] Beneficial effects of clomipramine have also been reported in patients with phobia, panic disorder, chronic pain, premature ejaculation, enuresis, and anorexia nervosa.[2] Despite the widespread use of clomipramine for more than a decade, its pharmacokinetics are less well documented than those of other tricyclic antidepressants.[3,4] There are a few reports on the oral absorption of clomipramine in humans in which the oral bioavailability is approximately 50%.[5−7] To our knowledge, no information is available on the bioavailability of clomipramine after sublingual or buccal administration in humans or laboratory animals. Of the antidepressant drugs available at present, only imipramine has been indirectly shown to be efficiently absorbed across the oral mucosa in humans.[8]

The aim of this study was to determine the extent of absorption of clomipramine in rats after sublingual administration. Clomipramine given sublingually as a solution in isotonic saline was rapidly absorbed and its sublingual bioavailability was increased over oral dosing. The sublingual bioavailability of clomipramine was further enhanced when given with hydroxypropyl β-cyclodextrin as a permeation enhancer.

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Experimental Section

Chemicals—Clomipramine hydrochloride, imipramine hydrochloride, ketamine, xylazine, and triethylamine were purchased from Sigma Chemical Co. (St. Louis, MO). Methocel MC (methyl cellulose) and 2-hydroxypropyl β-cyclodextrin were obtained from Fluka Chemie AG (Buchs, Switzerland). Sodium hydroxide and phosphoric acid were purchased from Samchun Chemical Co. (Pyungtaek, Korea) and Yakuri Chemical Co. (Osaka, Japan), respectively. Acetonitrile and methanol (all HPLC grades) were obtained from Fisher Scientific (Fair Lawn, NJ) and hexane (HPLC grade) from Mallinckrodt (Paris, KY). Mannitol and heparin were obtained from Choongwae Pharma Co. (Seoul, Korea).

Animals and Surgical Preparation—Male Sprague Dawley rats (7−8 weeks, 240−300 g) were purchased from Jaeil Animals Co. (Ansung, Korea). The rats were placed in plastic rat cages and housed in a temperature-controlled (23 ± 2 °C) animal facility with light/dark cycle of 12/12 h and relative humidity of 50 ± 10%. The animals had free access to standard rat diet (DaeJong Co., Seoul, Korea) and water throughout the study. After at least one week of acclimatization, the rats were anesthetized with im injection of ketamine and xylazine (90/10 mg/kg) and cannulated with PE tubing (0.58 mm i.d. and 0.96 mm o.d., Natume Co., Tokyo, Japan) in the right jugular vein. In animals used in the iv injection study, the right femoral vein was also cannulated with PE tubing. After surgery, at least 2 days of recovery were allowed prior to drug administration.

Intravenous Injection Study—Clomipramine dissolved in isotonic saline (3 mg/mL) was administered iv in rats via the femoral vein at 5 mg/kg doses (n = 4). Serial blood samples of approximately 0.3 mL were collected from the jugular vein at 0, 5, 15, 30, and 45 min and 1, 1.5, 2, 4, 8, 12, and 24 h after drug injection. Equal volumes of drug-free heparinized saline were injected after each sampling. Serum samples were harvested by centrifugation at 2500 rpm for 10 min and were kept at −70 °C until drug analysis.

Oral Dosing—Clomipramine dissolved in isotonic saline was administered po at 50 mg/kg doses in rats (n = 4). A second group of rats (n = 4) was also given clomipramine orally (50 mg/kg) followed by induction of light anesthesia with ketamine/xylazine (30/3 mg/kg). The animals gained consciousness within 10−30 min. Serial blood samples of approximately 0.3 mL were collected from the jugular vein as described above. Equal volumes of drug-free saline were injected after each sampling. Serum samples were harvested by centrifugation at 2500 rpm for 10 min and were kept at −70 °C until drug analysis.

Sublingual Administration—Clomipramine dissolved in isotonic saline (50 mg/kg doses) was applied to the sublingual mucosa of rats (n = 4) under light ketamine/xylazine (30/3 mg/kg) anesthesia. The volume of the drug solution administered was approximately 30 μL applied with an autopipet. Prior to application, the buccal and subligual areas were blotted dry with cotton swabs, and the animals were kept face downward until recovery from anesthesia. The animals gained consciousness within 10−30 min after induction of anesthesia. Separately, clomipramine (5 mg/kg doses) formulated with 2-hydroxypropyl β-cyclodextrin (100 mg/mL), mannitol (50 mg/mL), and Methocel M. C. (20 mg/mL) in isotonic saline was applied to the sublingual mucosa of rats (n = 4) as described above. Serial blood samples of approximately 0.3 mL were collected from the jugular vein at 0, 5, 15, 30, and 45 min and 1, 1.5, 2, 4, 8, 12, and 24 h after application.

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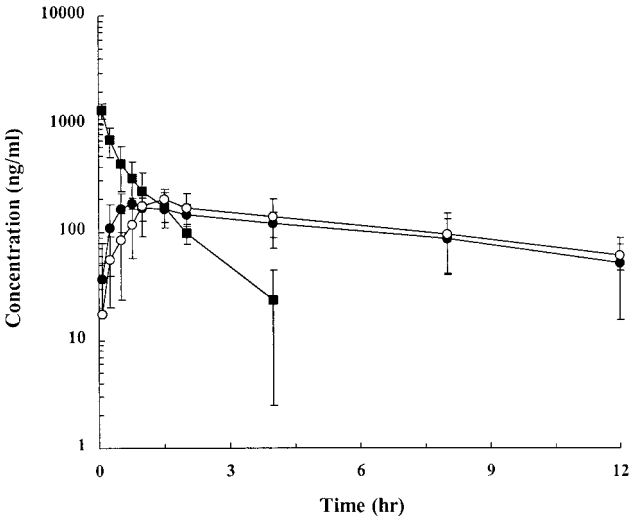

Figure 1—Mean serum concentration vs time curves of clomipramine after iv administration (5 mg/kg doses) to conscious rats (■) and after oral administration (50 mg/kg doses) to conscious (●) and anesthetized (○) rats ($n = 4$ each).

Equal volumes of drug-free saline were injected after each sampling. Serum samples were harvested by centrifugation at 2500 rpm for 10 min and were kept at −70 °C until drug analysis.

Drug Analysis—Drug analysis was performed on a HPLC system consisting of a Gynkoteck Model M480G pump, a Model UVD 340 photodiode array detector and a Model Gina 50 autosampler (Germering, Germany), a degasser (Lab-Quatec Co., Tokyo, Japan), and a Chromeleon Data System integration software (Softran GmbH, Germering, Germany). Chromatographic separations were achieved using a HAISIL HL RP C_{18} analytical column (4.6 mm i.d. × 250 mm, 5 μm) and a guard column (3.2 mm i.d. × 20 mm, 5 μm) (Higgins Analytical Inc., Mountain View, CA). The mobile phase consisted of acetonitrile:0.1% triethylamine in deionized water (50/50, v/v). The mobile phase was adjusted to pH 3.5 by phosphoric acid, passed through a 0.2 μm membrane filter, and degassed prior to use. Clomipramine was extracted by a single liquid−liquid extraction with hexane. Briefly, to an aliquot (100 μL) of the rat serum were added 100 μL of the internal standard solution (imipramine 200 ng/mL) and 100 μL of 5.0 M NaOH, and the mixture was vortexed for 10 s. The mixture was then extracted with 2 mL of hexane on a vortex mixer for 60 s and centrifuged at 2500 rpm for 3 min. The resulting supernatant was transferred into a fresh tube and dried at 35 °C under nitrogen gas. The residue was then reconstituted with 100 μL of the mobile phase on a vortex mixer for 30 s. The reconstituted solution was centrifuged at 2500 rpm for 60 s, and a portion (40 μL) was injected onto the chromatograph. The flow rate of the mobile phase was 1.0 mL/min at ambient temperature, and the effluent was monitored at 253 nm. Clomipramine and imipramine were well-resolved, with retention times of 3.8 and 5.0 min, respectively. Standard curves were linear over the concentration range of 10−1000 ng/mL, with a typical correlation coefficient of $r = 0.9998$. The intra- and interday assay coefficients of variation were <3.2% and <4.7%, respectively, for clomipramine and imipramine over the concentration range studied ($n = 5$ each).

Data Analysis—Serum clomipramine concentration vs time data were analyzed by a compartmental method for the iv injection study and by a noncompartmental method for the oral and sublingual studies. The nonlinear least squares regression program WinNonlin (Scientific Consulting Inc., Cary, NC) was used in the analysis. Statistical differences in pharmacokinetic parameters between conscious and anesthetized rats and between different doses were tested by the unpaired Student t-test. The significance level was set at $p < 0.05$.

Results and Discussion

Figure 1 shows the serum concentration vs time curves of clomipramine in rats obtained after iv injection (5 mg/kg doses) and oral administration (50 mg/kg doses). Following iv injection, the disposition of clomipramine was described by a biexponential curve, with a mean distribution and terminal elimination half-life of 7.8 ± 4.2 min and 63.0 ± 15.6 min, respectively. The elimination half-life of clomipramine in rats was much shorter than in humans (mean range, 24−39 h), primarily due to a higher systemic clearance and lower V_{ss} (96.6 ± 27.3 mL/min/kg and 6.1 ± 1.3 L/kg, respectively) (Table 1) compared to those in humans (10.8 mL/min/kg and 7−20 L/kg, respectively).[5,7,9,10] Clomipramine was administered orally at 50 mg/kg doses so that the maximum serum drug concentrations were within an acceptable therapeutic range of 150−450 ng/mL.[3] Upon oral administration in conscious rats, clomipramine was rapidly absorbed (Figure 1), with an average t_{max} of 0.8 ± 0.2 h and C_{max} of 209.4 ± 44.1 ng/mL (Table 2). The mean C_{max} found in this study (209.4 ng/mL) was higher than those reported after administration of 90 mg/kg po doses (162 ng/mL) or 15 mg/kg i.p. doses (144 ng/mL).[11,12] The oral bioavailability of clomipramine was 24.8% in conscious rats and 29.7% in rats anesthetized with ketamine/xylazine (30/3 mg/kg). There was no significant difference in AUC between the conscious and anesthetized rats (2258 ± 1762 ng·h/mL vs. 1891 ± 867 ng·h/mL). The C_{max} values between the two groups of animals were comparable, although the rate of absorption was lower in the anesthetized rats (Table 2). Therefore, it is unlikely that the light anesthesia induced by ketamine/xylazine (30/3 mg/kg) caused any significant alterations in the extent of drug absorption and elimination. The terminal elimination half-life was prolonged after oral administration (9.2 ± 7.8 h) as compared with iv injection (1.1 ± 0.3 h), suggesting that the drug elimination was rate-limited by the absorption process. These increased elimination half-lives are similar to the half-life of 6 h reported previously in rats after i.p injection.[12] The oral bioavailability of clomipramine in the rat was lower than that found in humans (approximately 50%).[5,6]

When the drug was given sublingually at 50 mg/kg doses, higher C_{max} (414.5 ± 47.0 ng/mL) and AUC (3303 ± 1576 ng·h/mL) were observed compared to those obtained after oral administration (Figure 2). Also, the absolute bioavailability of clomipramine after sublingual administration (36.2%) was higher than after oral administration. Clomipramine is known to be extensively metabolized by the liver in rats and humans via demethylation and hydroxylation.[13,14] Since the rats used in the sublingual study were lightly anesthetized to facilitate drug administration, it may be argued that the increased bioavailability was due to reduced hepatic metabolism caused by anesthesia. However, given no differences in AUC, C_{max} and $t_{1/2,\lambda z}$ between conscious and anesthetized rats after oral administration, it is unlikely that the increased sublingual bioavailability was caused by induction of anesthesia.

Table 1. Pharmacokinetic Parameters of Clomipramine in Rats ($n = 4$) after iv Injection of 5 mg/kg doses[a]

body weight (g)	$t_{1/2,\lambda1}$ (min)	$t_{1/2,\lambda2}$ (min)	AUC (ng·h/mL)	AUMC (ng·h²/mL)	k_{10} (h^{-1})	k_{12} (h^{-1})	k_{21} (h^{-1})	Cl (mL/min/kg)	V_{ss} (L/kg)
295.0 (5.8)	7.8 (4.2)	63.0 (15.6)	912 (234)	1007 (372)	2.35 (1.15)	2.53 (1.73)	2.28 (1.76)	96.6 (27.3)	6.1 (1.3)

[a] Values are expressed as the mean (±1 SD).

Table 2. Pharmacokinetic Parameters (mean ± SD) of Clomipramine after Oral and Sublingual Administration in Rats without and with a Permeation Enhancer ($n = 4$ for each group)

parameter	50 mg/kg oral dose in conscious rats without enhancer	50 mg/kg oral dose in anesthetized rats without enhancer	50 mg/kg sublingual dose in anesthetized rats without enhancer	5 mg/kg sublingual dose in anesthetized rats with enhancer
body weight (g)	270.0 ± 8.2	297.5 ± 5.0	280.0 ± 24.5	272.5 ± 23.6
$t_{1/2,\lambda z}$ (h)	9.2 ± 7.8	6.5 ± 1.6	5.4 ± 1.4	2.2 ± 1.3
t_{max} (h)	0.8 ± 0.2[a]	1.3 ± 0.3	1.5 ± 0.4	1.5 ± 0.0
C_{max} (ng/mL)	209.4 ± 44.1	210.0 ± 55.7	414.4 ± 47.0	135.5 ± 25.1
AUC (ng·h/mL)	2258 ± 1762	1891 ± 867	3303 ± 1576	521 ± 174
V_z/F (L/kg)	284.6 ± 22.5	270.3 ± 81.9	124.2 ± 28.5	29.0 ± 8.6
Cl/*F* (mL/min/kg)	522.1 ± 284.3	500.7 ± 178.4	289.3 ± 116.8	170.6 ± 44.7
F (%)[b]	24.8	29.7	36.2	57.1

[a] Significantly different from anesthetized rats ($p < 0.05$). [b] $F = (AUC_{sublingual} \cdot dose_{iv}) \cdot 100/(AUC_{iv} \cdot dose_{sublingual})$.

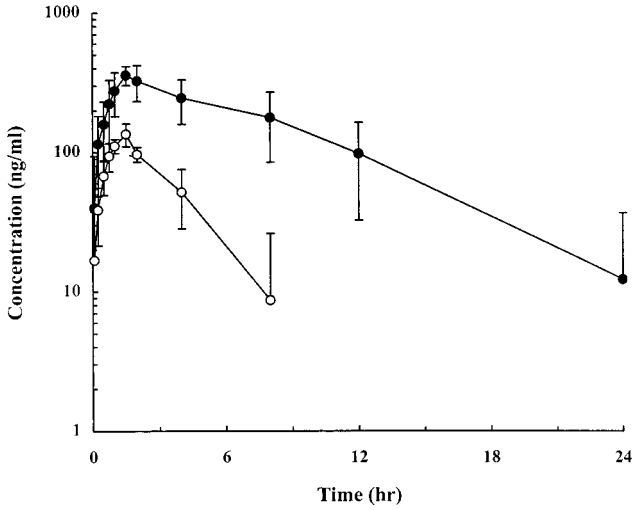

Figure 2—Mean serum concentration vs time curves of clomipramine after sublingual administration to rats with (5 mg/kg, ○) and without (50 mg/kg doses, ●) permeation enhancer ($n = 4$ each).

To further improve the sublingual bioavailability, clomipramine was formulated with a permeation enhancer, 2-hydroxypropyl β-cyclodextrin together with methyl cellulose and mannitol. After administration of this sublingual formulation in rats (5 mg/kg dose), C_{max} and AUC were higher by 3.3- and 1.6-fold, respectively, on a dose-normalized basis, than without permeation enhancer. Whether these increases were caused by the presence of the absorption enhancer, or were a result of a dose-dependent absorption, is unclear at present, although all the C_{max} values obtained in our study were within an acceptable therapeutic range. The terminal elimination half-life (2.2 ± 1.3 h) was shorter than found after oral and sublingual administration of 50 mg/kg doses but was still longer than found after iv injection. Therefore, at 5 mg/kg sublingual doses, the absorption process across the sublingual mucosa still seemed to have governed the elimination of clomipramine from the body. Nonetheless, the bioavailability of clomipramine was significantly increased (57.1%) for the sublingual formulation containing the permeation enhancer, and its relative bioavailability was increased to >192% over oral administration.

In conclusion, the pharmacokinetics of clomipramine showed multicompartment characteristics in rats. The oral bioavailability of clomipramine was low after administration of 50 mg/kg doses in rats. However, when given sublingually, clomipramine was well absorbed and its bioavailability was significantly increased over oral dosing. The sublingual route may be an alternative route of administration for clomipramine, providing enhanced bioavailability.

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