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Nasal Absorption of Procyclidine in Rats and Dogs

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Nasal absorption of procyclidine, a synthetic anticholinergic compound, was investigated in Wistar rats and Beagle dogs. The dosing solution was prepared by dissolving ^{14}C-procyclidine in 50% ethanolic saline. The dosing solution was administered intravenously and intranasally to rats at a dose of 0.6 mg/kg (i.e., 60 μl/kg in the form of a 1% w/v solution), and intravenously, orally and intranasally to dogs at a dose of 0.3 mg/kg (i.e., 6 μl/kg in the form of a 5% w/v solution). Blood samples were taken from an artery of the animals through the catheter for periods of 1200 (for rats) and 1440 min (for dogs), and the radioactivity in the samples was determined by liquid scintillation counting. The nasal bioavailability of procyclidine in rats and dogs, based on the radioactivity, was calculated to be 81.1 and 98.6%, respectively. In both rats and dogs, the plasma profiles of procyclidine following nasal administration were very close to those following intravenous administration, leading to nearly superimposable profiles between the two protocols. In dogs, nasal administration resulted in significantly higher plasma concentrations during the first 30 min period compared to oral administration, suggesting the superiority of the nasal route over the oral route in terms of a prompt expression of the pharmacological effect of the drug. The results obtained in this study indicate that procyclidine is rapidly and nearly completely absorbed via the nasal route. In conclusion, nasal administration represents a viable alternative to intravenous administration in the case of procyclidine.

Key words: Procyclidine, Nasal Administration, Bioavailability

INTRODUCTION

Procyclidine (1-cyclohexyl-1-phenyl-3-(pyrrolidin-1-yl)-propan-1-ol hydrochloride; Kemadrin®; Fig. 1) is a synthetic anticholinergic compound which is effective in the treatment of idiopathic, postencephalitic and arteriosclerotic Parkinson's disease (Strang, 1965). It also controls extrapyramidal symptoms, which are induced by neuroleptic drugs (Mindham *et al.*, 1977; Wawrinowski, 1981). An intravenous formulation is used to relieve severe dystonic reactions to neuroleptic drugs. When administered orally to humans at a dose of 10 mg, procyclidine is well absorbed (bioavailability of 75%, Whiteman *et al.*, 1985), reducing heart rate and increasing pupil diameter and visual near point (Hamilton *et al.*, 1982). Thus, procyclidine, as well as atropine, can be used to protect against organophosphate poisoning. Procyclidine posseses a more potent protecting effect than atropine against brain damage and has less side effects, when administered before or after organophosphate poisonong (Coudray-Lucas *et al.*, 1984; Gordon *et al.*, 1978). Reduction in saliva secretion after procyclidine treatment is mild compared to the use of atropine (Hamilton *et al.*, 1982). Procyclidine is now on the market in the form of an oral tablet formulation called Kemadrin®, which contains 5 mg of the drug in each tablet.

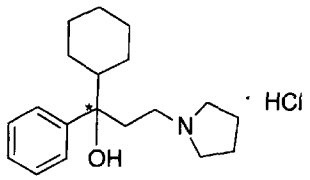

Fig. 1. Chemical structure of procyclidine

Pharmacokinetics and pharmacodynamics of procyclidine following intravenous and oral administration have been studied in man (Whiteman *et al.*, 1985). The maximal

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autonomic effects appeared within 0.5 h of intravenous administration, and within 1-2 h of oral administration. A significant autonomic effect was detectable up to 12 h after both administrations. In addition, effects on pupil diameter, visual near point, salivary secretion and heart rate after intravenous administration were more profound compared to oral administration.

The prompt elevation of the concentrations of anticholinergic drugs in the blood up to effective levels would be essential in the protection and treatment of organophosphorous poisoning. For this purpose, intramuscular injections and, recently, aerosol sprays for the intranasal or peroral inhalation of atropine have been developed by Aerochem Co. (BI, Germany). For the case of procyclidine, no preparations, other than Kemadrin® tablets for oral administration, have been developed to date. The pharmacological effects of procyclidine after oral administration, however, are substantially delayed compared in the case of intravenous administration (Whiteman *et al.*, 1985). In addition, oral tablets cannot be swallowed by patients, if they are unconscious due to severe poisoning. Thus, new dosage forms of procyclidine, other than Kemadrin®, would be desirable. Usually, a nasal spray formulation is first considered as the potential dosage form for achieving a rapid absorption of the drug. The application of nasal sprays is also quite simple and safe, even in the cases of unconscious patients. Thus, in the present study, the systemic absorption of procyclidine following a nasal administration in the form of an aqueous solution was investigated in rats and dogs.

MATERIALS AND METHODS

Materials

Procyclidine hydrochloride and atropine were purchased from Sigma Chem. Co. (MS). ^{14}C-Procyclidine hydrochloride (7.1 mCi/mmol) was synthesized by labelling carbon number 7 of the molecule by Sunkyung Ind. Co. (Seoul). The purity of the labelled compound was in excess of 98%, when tested by TLC using chloroform: methanol: acetic acid (45:5:1), ethyl acetate: ethanol (5:1) and dichloromethane: methanol:acetic acid (5:1:1) as irrigants. All other chemicals were of reagent grade and were obtained from Junsei Chem. Co., Showa Chem. Co. or Shinyo Pure Chem. Co. of Japan.

Preformulation studies for nasal dosage forms

1) Solubility - In order to administer the usual dose of procyclidine (15 mg) intranasally in a solution form in a minimum volume (100 µl, for example), the solubility of the drug in the solution should exceed 150 mg/ml, at least. However, the solubility of procyclidine in water does not exceed 30 mg/ml at room temperature, as evidenced by a preliminary experiment. Thus, a co-solvent, which can enhance the solubility of the drug in water seemed necessary. Among the possible solvents, ethanol was first the examined since it is widely used in the formulation of nasal fluids. Thus, the effect of ethanol content in water on the solubility of procyclidine was examined by measuring the solubility of the drug in solvent mixtures. The solubility of procyclidine in the mixture was measured at room temperature after vortexing for 30 min according to a standard method. HPLC was performed to determine procyclidine concentration in the solution.

2) pH-stability - In order to elucidate the effect of pH of the formulation on the chemical stability of procyclidine, concentrations of the drug in aqueous buffers of various pH (3.48-8.68) were monitored at 37°C as a function of time. Citric acid-sodium phosphate dibasic solutions were used as buffers of pH 3.48, 5.13, 6.18 and 7.16, and the glycine-sodium hydroxide solutions for a buffer of pH 8.68. Procyclidine was dissolved in each buffer to yield a concentration of 1 mg/ml for all pHs except for pH 8.68, in which 200 µg/ml was adopted as the concentration because of the low solubility of the drug at that pH. The buffers containing the drug were placed in 4 ml glass vials, and the vials were sealed and stored at room temperature. The content of procyclidine in the vial was assayed on 1, 2, 3, 4, 5, 6 and 7 weeks by HPLC after appropriate (i.e., 5 fold in general) dilution of the sample with distilled water. The rate of degradation of procyclidine at each pH was then estimated from the slope of the plot between procyclidine concentration (µg/ml) and time (week) for each storage pH. The slope was estimated through a linear regression of the plot.

3) *Stability of procyclidien in plasma* A 200 µl aliquot of an aqueous solution of procyclidine (4 mg/ml) was mixed with 1800 µl of human fresh plasma to yield a 200 µg/ml concentration of the drug. While shaking at 50 opm, 200 µl aliquots were removed from the solution at 0.5, 1, 2, 4, 6, 8, 10 and 24 h, and mixed with 200 µl of glycine buffer (pH 11) and 20 µl of atropine solution (2 mg/ml, internal standard) by vortexing for 5 min. To the mixture, 900 µl of chloroform was added and the mixture shaken for 10 min, and a 800 µl aliquot of the chloroform layer was withdrawn and dried under a stream of nitrogen gas. To the residue, 200 µl of 50% (v/v) ethyl alcohol was added, and the reconstituted solution was introduced to HPLC for the procyclidine assay.

Intravenous and intranasal administration to rats

Wistar rats weighing 245-300 g (Animal Center for Pharmaceutical Research, Seoul National University) were fixed in a supine position on a surgery table during intravenous and nasal administration experiments.

1) intravenous administration Under light ether anesthesia, a polyethylene tube (PE-50, Intramedic) filled with diluted heparin (100 IU/ml) was introduced into the

femoral artery for blood sampling. A PE-50 tube filled with physiological saline solution was introduced for the drug administration. After complete recovery from the ether anesthesia, ^{14}C-procyclidine in distilled water for injection was administered through the venous catheter at a dose of 0.6 mg/kg. Blood samples (0.1 ml) were taken from the arterial catheter before the drug administration and at 1, 5, 10, 20, 30, 45, 60, 90, 180, 360, 600 and 1200 min after the administration. During the blood sampling, the rats were heated appropriately using a white glow lamp in order to maintain body temperature. Plasma samples were obtained by centrifugation (12000 rpm, 30 seconds) of the blood samples, and the radioactivity in 50 µl plasma samples were measured by LSC for the assay of total procyclidine.

2) Nasal administration to rats Under sodium pentobarbital anesthesia (intraperitoneal injection of 50 mg/2 ml/kg), a PE-50 catheter was introduced to femoral artery as described above for the intravenous administration. ^{14}C-Procyclidine in 50% ethanolic saline (10 mg/ml) was introduced to both nostrils of the rats at a procyclidine dose of 0.6 mg/kg (60 µl/kg). Blood sampling and LSC were performed in the same manner as described above.

Intravenous, oral and intranasal administration to dogs

^{14}C-Procyclidine was administered to four beagle dogs (5-months old, 6.5-10.0 kg in weight, Hazleton Research Products Inc., USA) through intravenous, oral or intranasal routes in a parallel design. The dose was fixed at 0.3 mg/kg (6.6 µCi/kg) for all the routes. A washout period of a week was employed between the doses in each dog, which was confirmed to be sufficient to wash most of the predosed radioactivity from the plasma. The dog was fixed using a jacket and holder, and an intravenous catheter (22 gage, 1.5 in), connected to a three-way cock valve, was inserted into the cephalic vein of a foreleg. For intravenous administration, ^{14}C-procyclidine in sterilized saline (0.15% w/v and 33 mCi/ml) was pushed into the body through one way of the catheter valve. For oral administration, a saline solution of ^{14}C-procyclidine (1.5 mg/ml) was pushed into the esophagus of the dog at a dose of 0.2 ml/kg (0.3 mg/kg as procyclidine) using a 3 ml syringe. The esophagus was then rinsed with 20 ml of distilled water using a 25 ml syringe in order to ensure the complete administration of the dose. For intranasal administration, ketamine (10 mg/kg) was injected through a valve of the catheter. Immediately before recovery from the ketamine anesthesia (approximately 10 min after the ketamine injection), a ^{14}C-procylidine solution (5 mg/ml) in 50% ethanol-sterilized saline was administered dropwise into the nostril at a dose of 6 µl /kg (0.3 mg/kg as procyclidine) with the aid of a 4 cm-long polyethylene tube (PE-50) connected to a 50-µl microsyringe. The nostril was sealed for 5 min by finger pressure to protect against drainage of the dosed solution.

Blood samples of 3.5 ml were withdrawn through the one of the remaining valves of the three-way cock valve of the catheter before and at 1, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 720 and 1440 min after the administration of the drug. The third valve of the catheter was used for flushing the catheter tube with 2 ml of heparinized saline (100 IU/ml) after blood sampling, and for draining the remaining saline in the tube before blood sampling. Plasma was separated by centrifuging the blood samples at 3000 rpm for 3 min, followed by storage at -20°C until reused for analysis.

Procyclidine assay

Procyclidine concentrations in the studies of solubility and pH-stability were assayed by HPLC. Relevant samples (200 µl) were injected into a µBondapak C_{18} column (3.9 × 300 mm, particle size 10 µm, Waters). The mobile phase in the column was a 70:30 (v/v) mixture of acetonitrile and 0.25 M acetic acid (pH 2.8). Flow rate of the mobile phase was set at 0.5 ml/min, with detection at 258 nm.

Procyclidine in the plasma sample was assayed in a similar manner, except for the sample treatment (see *Stability of procyclidien in the plasma).* Procycline and atropine (internal standard) peaks in the HPLC chromatograms were clearly isolated from the other plasma constituents, with respective retention times of 12 and 9 min.

A linear relationship (r=0.999) between procyclidine concentrations (in water and plasma) and the heights of the corresponding peaks for the concentration range of 25-400 µg/ml of the drug was found. Inter- and intra-day variations for the HPLC method were less than 10% regardless of the types of samples (i.e., aqueous solution and plasma solution).

Pharmacokinetic analysis

Systemic clearance (CL_s), distribution volume at steady-state (Vd_{ss}) and bioavailability of procyclidine were calculated by standard methods.

RESULTS AND DISCUSSION

Preformulation study

The solubility of procyclidine increased with the ethanol content of the water (Fig. 2), reaching 180 mg/ml at 50% (v/v) ethanol concentration, which appears sufficiently high to reduce the volume of the dosing fluid to less than 100 µl. The pH-stability of procyclidine is shown in Fig. 3. The decomposition rate constant of procyclidine in the buffer of pH 5-7 at 37°C was between 0.015~0.025/week with a maximal stability at a pH of approximately 6.18. The stability decreased significantly as the buffer exceeded this pH range. The apparent pH

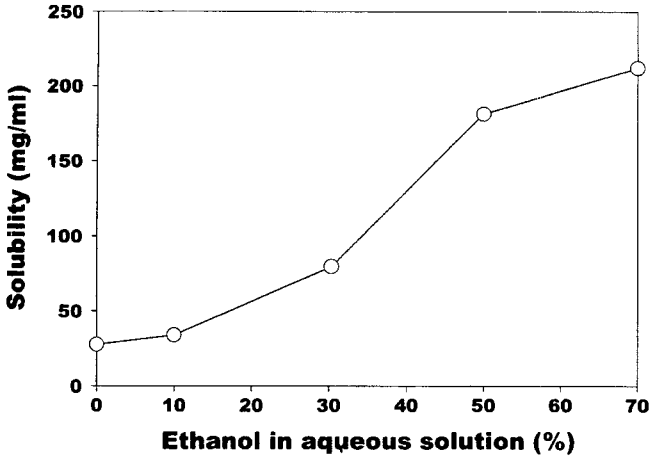

Fig. 2. Solubility of procyclidine in water/ethanol mixtures as a function of ethanol content at 25°C expressed as the mean ± SE of four measurements

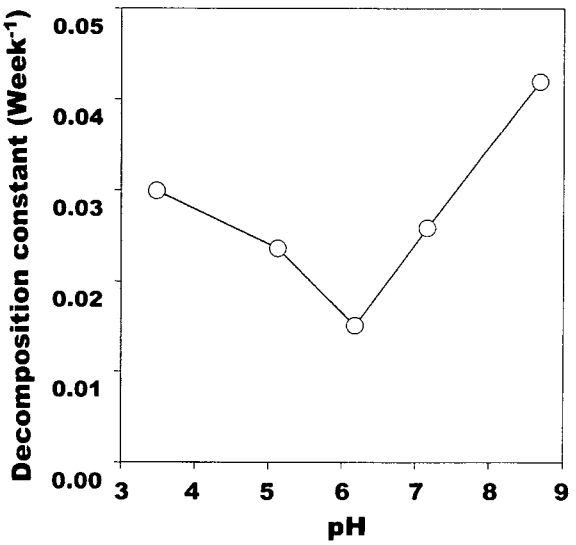

Fig. 3. Effect of pH on the decomposition rate of procyclidine in citric acid-sodium phosphate dibasic buffers

of the 50% (v/v) ethanolic solution was 5.8, falling within the most stable pH range for procyclidine. Ethanol is often used in formulating aerosols for intranasal or oral inhalation. For example, an atropine aerosol spray developed by Aerochem (Germany) was formulated using an ethanolic solution. In addition, ethanol might increase the permeability of drugs across the nasal mucosal membrane, and behave as a preservative in aqueous solutions of drugs. Thus, 50% (v/v) ethanol was utilized in the present study as a solvent in preparing the procyclidine solution for nasal administration.

Procyclidine was found to be fairly stable in plasma at 37°C, when estimated as described in Methods. Only slight and insignificant degradation could be found in the 24 h incubation study (data not shown).

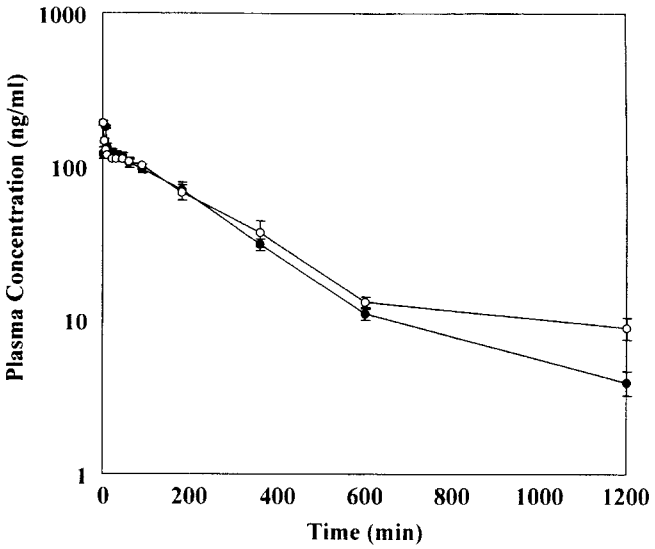

Fig. 4. Temporal profiles for ^{14}C radioactivity following intravenous (○) and intranasal (●) administration of procyclidine to rats at a dose of 0.6 mg/kg. Each data point represents the mean ± SE of six measurements

Plasma procyclidine following intravenous and nasal administration to rats

Fig. 4 shows temporal profiles for the mean plasma radioactivity following intravenous and nasal administrations of procyclidine to rats. The plasma radioactivity following nasal administration was comparable to that for intravenous administration. The peak level was attained 5 min after the nasal administration, suggesting that the nasal route can be utilized as a potential alternative to the intravenous route for procyclidine administration. The systemic clearance (CL_s) and distribution volume (Vd_{ss}) of procyclidine were 14.7 ± 1.7 ml/min/kg (mean ± SE, n= 6) and 4.7 ± 0.3 l/kg (mean ± SE, n=6), which are much larger compared to those in man (i.e., 67.4 ml/min/body and 1.2 l/kg, respectively, Whiteman *et al.*, 1985). This discrepancy can be attributed to species differences. The mean bioavailability of procyclidine for the nasal route in rats was calculated to be 81.1%, indicating a favorable absorption via the nasal route.

Plasma procyclidine following intravenous, oral and nasal administration to dogs

The appearance of procyclidine (radioactivity) in the plasma was very rapid in all administrations (Fig. 5). The overall profiles of plasma procyclidine were nearly superimposable for all administration routes. As a result, the bioavailability of the drug was calculated to be 98.6 for both the nasal and oral routes. However, during the initial phase of the absorption (i.e., ~30 min), much higher plasma drug concentrations were obtained for the nasal administration, comparable to that for intravenous admi-

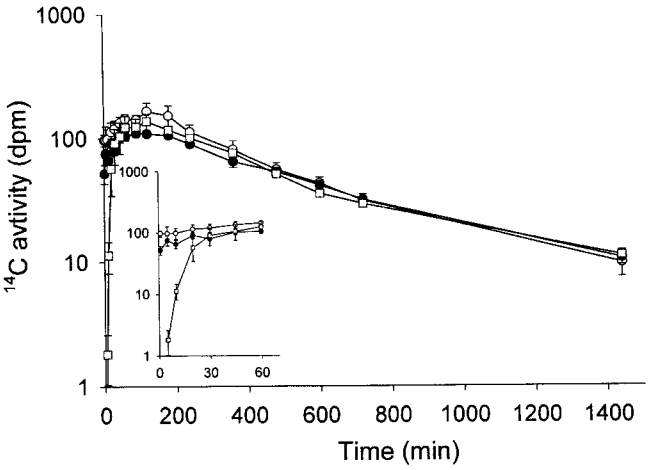

Fig. 5. Temporal profiles for plasma procyclidine following intravenous (○), oral (■) and intranasal (●) administrations to dogs (n=4) at a dose of 0.3 mg/kg. The inset shows detailed data for the time period of 180 min. Data are expressed as the mean ± SE

nistration, but significantly higher than for oral administration (see an inset in Fig. 5). The distribution volume (Vd_{ss}) and systemic clearance of the drug (CL_s) in dogs were calculated from the intravenous administration study to be 1.5 l/kg and 4 ml/min/kg, respectively, which are much closer to those in man compared to rats. This indicates the closeness of beagle dogs to man compared to rats in terms of species differences.

Nearly maximum concentrations of the drug were attained 30 min after administration regardless of the route, which is consistent with the result in rats for the nasal route (Fig. 4). Thus, we conclude that procyclidine is a drug that is absorbed very rapidly regardless of species or administration routes. The faster absorption from the nasal route during the first 30 min period, compared to oral administration, appears to represent an advantage of the nasal route over the oral route in terms of administering emergency drugs, including procyclidine (Gordon *et al.*, 1978).

In the present study, total radioactivity, instead of procyclidine itself, was measured. Thus, the results obtained from the present study should be carefully interpreted. In fact, procyclidine is known to be biotransformed into at least eight metabolites in isolated rat hepatocytes (Rogiers *et al.*, 1987). However, considering the fairly high oral bioavailability of the drug in dogs (i.e., 98.6%), the first-pass metabolism of the drug does not appear to be significant. Thus, it is possible to conclude that procyclidine is absorbed fairly well and rapidly through the nasal route, and that a 50% ethanolic solution represents a favorable formulation for nasal administration with regards to solubility and the stability of the drug. Nasal administration would be advantageous over the oral administration of tablets (e.g., Kemadrin®), whenever the application of drugs to unconscious patients is rewuired.

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