Enhancement of Bioavailability of Dopamine *via* Nasal Route in Beagle Dogs

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Dopamine (DA), which is ineffective by oral administration due to first pass metabolism and is usually injected, was administered to dogs *via* rectal, dermal, buccal and nasal routes. The nasal route had the highest bioavailability and best avoided first pass metabolism. The effects of the addition of hydroxypropyl cellulose (HPC), sodium deoxycholate, POE (6) hydrogenated caster oil (HCO-60) and Azone on the nasal absorption increased bioavailability from 11.7% (control) to about 20%, 35%, 25% and 68%, respectively.

Further, with a combination of 2%HPC and 5%Azone, bioavailability was increased to almost the same level as with i.v. administration. At the same time, plasma concentrations were maintained at a high level for more than 7 h. The increase in bioavailability is presumed to be caused by an enhancement in absorption and prolongation of the time DA is retained in the nasal cavity due to Azone and HPC, respectively.

Keywords dopamine; nasal absorption; Azone; bioavailability; hydroxypropyl cellulose; sodium deoxycholate; HCO-60

Broad studies have been made in recent years on drug absorption *via* various routes of administration, such as dermal, nasal, buccal, rectal and vaginal routes. Using these routes, first pass metabolism is avoided[1,2] so that drugs ineffective when administered orally are effective.[3,4]

Dopamine (DA) is known to be subject to first pass metabolism. When DA was administered by i.v. injection, the recovery percent of DA of labeled compounds in beagle dogs in urine, *i.e.* the total absorption ratio, during 24 h was about 85%,[5] whereas the bioavailability of DA was only about 3%[6] when orally administered. Therefore, its low bioavailability is not caused by its poor absorption from intestine, but by its high first pass metabolism. It was reported that a main metabolite of DA in plasma is dopamine sulfate (DA-SO_4), so the absorbed percent of DA can be evaluated by determining plasma overall DA and DA-SO_4 concentrations.[6]

DA has been used clinically to treat cardiovascular diseases or renal malfunctions in injectable dosage forms. It has a very short activity and is ineffective by *p.o.* administration; it is thus usually administered by intravenous infusions to maintain its effect. Only specialists can perform such treatment and frequent infusions undergone over a long period causes a patient much suffering. A convenient method for administering DA is therefore highly desirable.

Towards this end, DA was administered in this study by several routes, and was shown to be maximally absorbed by the nasal route. The effects of several additives on nasal absorption were then investigated in beagle dogs.

Experimental

Materials DA hydrochloride and sodium deoxycholate (NaDC) of special grade were used and purchased from Nakarai Chemicals, Ltd. (Kyoto, Japan). Hydroxypropyl cellulose (HPC-H) was purchased from Nihon-Soda Ltd. (Tokyo, Japan). POE (60) hydrogenated caster oil (HCO-60) was purchased from Nikko Chemicals, Ltd. (Tokyo, Japan). Azone was generously supplied by Nelson-Sumisyo, Ltd. (Tokyo, Japan).

Analysis of DA and DA-SO_4 DA and DA-SO_4 concentrations were determined by high performance liquid chromatography-electron capture detector method according to Murata *et al.*[6] The area under the curve (*AUC*) was determined using the trapezoidal rule from the plasma DA concentration *versus* time curve up to 7 h. Bioavailability for each administration route was calculated using the *AUCs* of i.v. administration determined for the same dogs.

Animals Male beagle dogs weighing about 11 kg were purchased from Yoshiki Yakko Company (Gifu, Japan).

Animal Experiment The dogs were fasted for 18 h prior to the administration, then anesthetized by i.v. administration of pentobarbital (20 mg/kg, i.v.) in the experiments of nasal, buccal and dermal absorptions. Blood samples were withdrawn periodically from a foreleg vein at a definite time after administration. Plasma was separated immediately by centrifugation and samples were stored at −20 °C until analyzed. All experiments were conducted at least two weeks apart on individual animals.

DA was dissolved in 1/15 M phosphate buffer (pH 7.4), and when used this way, pH of the prepared DA solutions was neutral (pH 5—7). When DA was dissolved in water at the concentration of the experiment, it showed an acidity around pH 3.

1) Rectal Route: Five ml of 2% aqueous DA solution (100 mg of DA) was administered to the rectum using a zonde.

2) Dermal Route: A dog was anesthetized and restrained in the supine position. After removal of the chest hair with electric clippers, 1 ml of 10% aqueous DA solution (100 mg of DA) was put on the skin surrounded by a vaseline wall. The 30 cm^2 area in contact with the drug solution was covered with polyvinylidene chloride film (Kurewrap™, Kureha Kagaku, Ltd., Tokyo). The experiment was performed with great care so that the skin on which the solution had been placed was not spoiled by the vaseline.

3) Buccal Route: A dog was anesthetized and restrained in the supine position. One ml of 5% aqueous DA solution (50 mg of DA) was administered to a polyethylene reservoir which was attached to the oral cavity using Carbopol 934. The area of contact with the drug solutions was 2 cm^2.

4) Nasal Route: A dog was anesthetized and restrained in the supine position. The tip of a piece of polyethylene tubing (SP-45, Natsume Seisakusyo, Ltd., Japan) equipped with a microsyringe was inserted into the nostril and each of the 125 μl of 40% aqueous DA solution was administered into the right and left nasal cavities (totally 100 mg of DA). During the experiment, the dog was kept in an supine position to prevent leakage of DA solution from the nasal cavity. The concentrations of DA solutions administered were very high due to the experimental requirement that the volume could not be greatly increased, but the dose should be 50—100 mg to be comparable to the results of the oral administration. Oral administration of 50—100 mg of DA was necessary to evaluate the drug's concentration in serum during 5 h precisely.[6] The effect of hypertonicity caused by the high DA concentration on the nasal absorption was presumably small since the volume applied was minimal (250 μl), and could be rapidly diluted with the nasal secretory fluids.

Bioavailability of nasal administration of the aqueous DA solution (control of nasal administration) was determined using eight dogs. Bioavailabilities of each of the other formulations were determined using three dogs randomly from these eight dogs.

Results and Discussion

Administration Route and Bioavailability We previously reported on the pharmacokinetics of DA in beagle dogs.[6] Following the intravenous administration of DA (dose, 10 mg), plasma DA concentration decreased rapidly (bio-

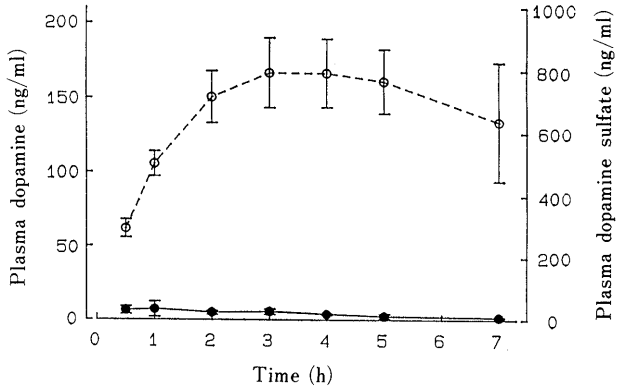

Fig. 1. Plasma Concentration of DA and DA-SO_4 Following Rectal Administration of DA

(●) DA; (○) DA-SO_4. Each bar represents mean ± S.E. of three experiments.

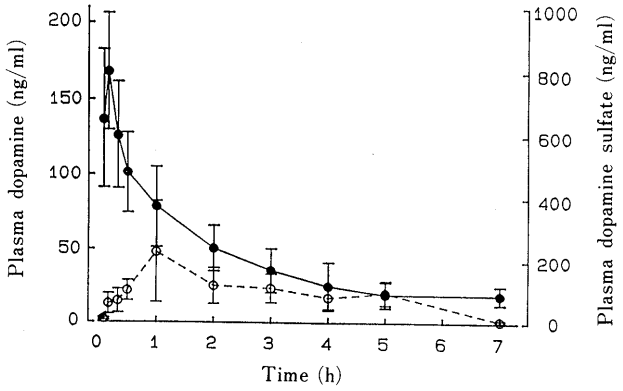

Fig. 2. Plasma Concentration of DA and DA-SO_4 Following Nasal Administration of DA

(●) DA; (○) DA-SO_4. Each bar represents mean ± S.E. of eight experiments.

logical half life, 10.8 min) and plasma concentration of DA-SO_4 was much lower than that of DA. Following oral administration (dose, 100 mg), plasma DA concentration was very low and its absolute bioavailability was less than 3%, while plasma DA-SO_4 concentration was much higher than DA.

After administration of aqueous DA solution *via* dermal, buccal, rectal and nasal routes, the plasma concentrations of DA and DA-SO_4 were determined. With the buccal and dermal administrations, concentrations were hardly detectable, indicating that DA was not efficiently absorbed through the oral mucosa or skin in beagle dogs.

Figure 1 shows the plasma DA and DA-SO_4 concentration–time profiles for rectal administration. Plasma DA concentration was very low, but DA-SO_4 was fairly high. This profile resembles that observed in oral administration, showing that rectal administration of DA with aqueous solution could not avoid the first pass metabolism.

Plasma DA concentrations following intranasal administration were very high as shown in Fig. 2. The maximum plasma concentration was achieved at 10 min following the administration and then gradually decreased at a relatively slow rate. Plasma DA-SO_4 concentration also increased, but it was relatively low compared with oral or rectal administration.

Bioavailability of DA for each of the five administration route is shown in Table I. That of the nasal route was the highest, and about 3 times as high as oral administration. Hussain *et al.* reported that buccal absorption was an effective method to avoid first pass metabolism for some drugs.[7] But in this study, the buccal absorption of DA was lower than dermal absorption. Under the condition used, the effective buccal absorption area was 2 cm^2 due to experimental limitations, one-fifteenth of the dermal route. Further, DA has a contracting action on the peripheral vein which may reduce peripheral blood flow. These may be the reasons that the bioavailability *via* the buccal route was low.

TABLE I. Bioavailability and *AUC* Ratio of DA and DA-SO_4 Following the Administration of DA by Various Routes

Route of administration	Bioavailability of DA (%)[a]	$\frac{AUC \text{ of DA-}SO_4}{AUC \text{ of DA}}$
i.v.	100	0.3
p.o.	3.0 ± 0.5	206.1
Rectal	1.4 ± 0.4	161.8
Dermal	1.1 ± 0.5	5.9
Buccal	0 ± 0	—
Nasal	11.7 ± 3.7	2.3

a) Values represents mean ± S.E. of three to eight experiments.

The ratio of *AUC* of DA-SO_4 to *AUC* of DA can be used as an index indicating the extent of first pass metabolism. Values obtained for the various administration routes are shown in Table I. In oral and rectal administration, the ratio was 200 and 160, respectively, the high values suggesting that DA underwent first pass metabolism by these routes. In rectal administration, DA may have moved to the upper site of the rectum since it was administered by aqueous solution, and underwent first pass metabolism. The values were very low in i.v., dermal and nasal administration. Nasal administration thus seems to be the most promising route to avoid first pass metabolism and obtain high bioavailability.

Effect of Enhancers on the Nasal Administration of DA Drugs of low molecular weight such as propranolol[1] and steroids[2] are absorbed at high levels from the nasal membrane.[8,9] In some drugs, the resultant *AUCs* are almost equal to that of i.v. administration. In the present study, bioavailability of DA *via* the nasal route was only 11.7% and thus not particularly good. The effects of several adjuvants were investigated to improve this bioavailability.

Two types of mechanism were evaluated to enhance the nasal absorption using adjuvants: One was to increase the viscosity of the DA solution so that it stays on the nasal membrane for a longer time; and the other was to add absorption promoters such as surfactants, organic solvent, organic acids, *etc.* Thus, the effect of adjuvants was examined using four materials. The time courses of plasma DA concentration are shown in Fig. 3.

Figure 3a shows the effect of HPC, used as viscous agent, on the nasal absorption of DA. Plasma DA concentration increased immediately after administration, reached the maximum concentration (C_{max}) at 5 min and then gradually decreased. As C_{max} was higher than control, HPC might partly act as an absorption promoter. At the same time,

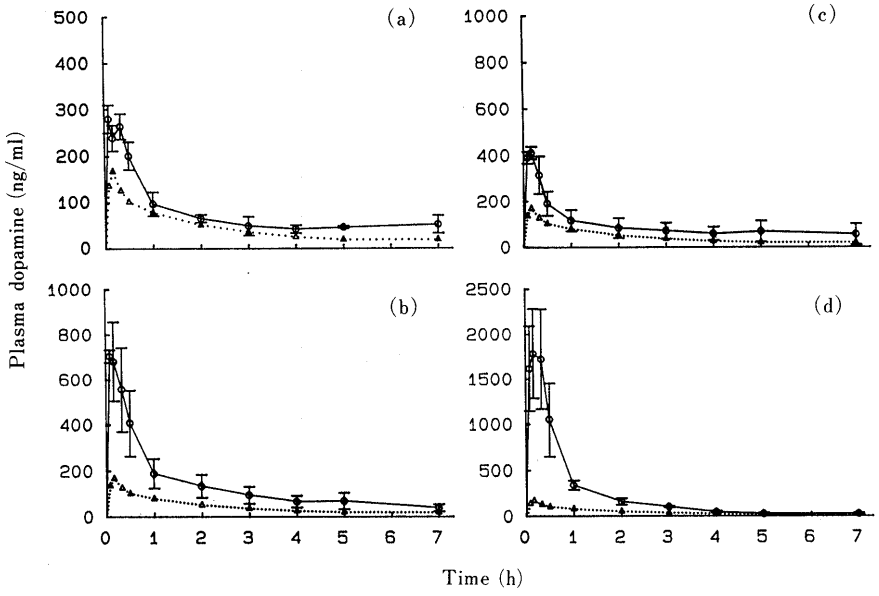

Fig. 3. Effect of Various Adjuvants on the Nasal Absorption of DA

(a) 4% HPC; (b) 1% NaDC; (c) 1% HCO-60; (d) 5% Azone. (○) adjuvant; (△) control. Each bar represents mean±S.E. of three experiments.

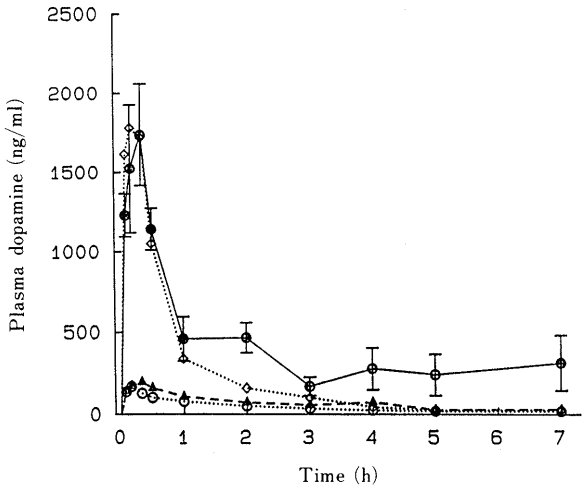

Fig. 4. Effect of 5% Azone and 2% HPC on the Nasal Absorption of DA

(○) control; (△) HPC alone; (◇) Azone alone; (●) HPC+Azone. Each bar represents mean±S.E. of three experiments.

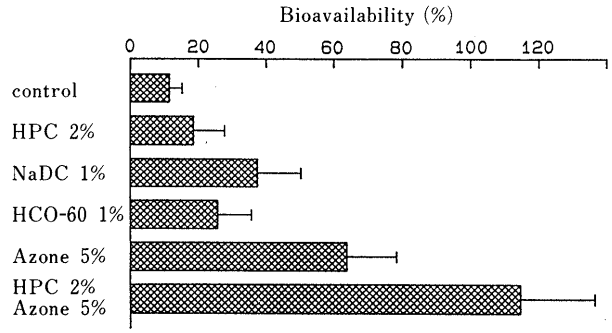

Fig. 5. Effect of Adjuvants on the Bioavailability of DA in Nasal Administration

Each bar represents mean±S.E. of three to eight experiments.

HPC was expected to prolong the plasma DA level by its viscous property. Such a trend could be noticed, although it was not too obvious. Morimoto *et al.*[10] reported that nasal administration of nifedipine with carbopol–polyethyleneglycol gel caused high plasma concentration and a prolonged action. The difference in their study and this may be by the difference in physical properties of DA and nifedipine. Nifedipine is relatively lipophilic and might have remained in the gel for a long period, while DA is very hydrophilic and might have been rapidly released from the HPC gel.

In general, surfactants alter the characters of biological membrane and often enhance the permeability of a drug.[11] Figures 3b and 3c show the effects of HCO-60 and NaDC, respectively. With the addition of 1% HCO-60 or NaDC, plasma DA concentration was increased immediately after administration and the bioavailability was promoted to 25.7% and 37.5%, respectively. Azone is well known to be an effective promoter of transdermal absorption for many drugs.[12] Thus, its effect was tested at 5% of its concentration (Fig. 3d). The plasma DA concentration was markedly increased, and bioavailability increased to 63.8%. This suggests that Azone can be used as a good absorption enhancer on nasal administration as well as on transdermal administration.

Azone's beneficial enhancement effect could be maintained for a longer period when it was used together with HPC. Figure 4 shows plasma DA concentration following administration of DA with 5% Azone and 2% HPC. Immediately following administration, plasma DA concentration was very high, similar to the case of 5% Azone, it then decreased at a relatively fast rate for 1 h, and maintained a high concentration for the next 6 h.

Figure 5 summarizes the bioavailability of DA in nasal administration using the above adjuvants. Without any adjuvants, bioavailability was only 11.7%. All additives used in this experiment increased DA absorption. Azone and HPC used together resulted in bioavailability of more than 100%. These values in excess of 100% might be the result of the large intraindividual variance due to the lower experiment number ($n=3$). However, taking this experimental variance into consideration, it can be concluded from

Fig. 5 that DA was absorbed *via* the nasal cavity at almost the same levels as when administered by i.v.

In conclusion, DA, of which bioavailability is low by oral administration, was successfully absorbed by the nasal route avoiding the first pass effect. Further, by adding appropriate adjuvants, DA bioavailability was increased and a high plasma level was maintained for a long period. Thus, the nasal route is believed to be a promising method of administrating this drug.

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