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NASAL ABSORPTION OF SULFOBROMOPHTHALEIN AND AMARANTH

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ABSTRACT

Nasal absorption of sulfobromophthalein (BSP) and amaranth(AM) was investigated and compared with oral absorption in the rat. Bioavailability of BSP and AM after nasal administration was about 26% and 30% respectively. Oral absorption of them was not detected. Nasal route was considered more effective than oral route for these anionic model drugs, but their nasal bioavailability was not so good as expected from the reports for other drugs. High nasal

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mucus binding of BSP and AM were implied by their high binding to plasma protein (97% and 94%) or to intestinal mucus (78% and 81%). They seemed to have very low lipophilicity since their apparent partition coefficient(APC) between phosphate buffer of various pH and n-octanol were almost zero. They have too large molecular size to pass through the pore(<0.4nm) of nasal mucus membrane. Therefore it was concluded that the low nasal bioavailability of these anionic model drugs might be due to either nonspecific binding to nasal mucus, or low lipophilicity to pass the nasal mucus membrane, or their large molecular size to pass through the pore route of the nasal mucus. Possibility of nasal metabolism in the mucus membrane was excluded since the reported enzymes in the nasal mucus may not affect the metabolism of them.

INTRODUCTION

Nasal administration of drugs have been studied recently to obtain rapid absorption and improved bioavailability of drugs that are extensively metabolized in the gut wall, subject to an extensive first-pass elimination in the liver, destroyed by the gastrointestinal fluids or not capable of being adequately absorbed into the systemic circulation following oral administration. Intranasal route has

been experienced increasing interest for polypeptides[1-7], the steroid hormones[8,9,10], and clofilium tosylate[11], buprenorphine[12], ergotamine[13], nicardipine[14], propranolol[15], insulin[2,4,5,6], desmopressin[2] and nafarelin acetate[7]. Continuous nasal spray of steroid hormone induced the perforation on nasal septum[16] and the use of propranolol in high concentration extremely diminished the ciliary beat frequency of human adenoid cell [17]. Nevertheless, intranasal route has been regarded as convenient administration route comparable to intravenous injection for the drugs which are not proper to enteral administration. The efficacy and the mechanism of the nasal absorption of water-soluble organic anions, which undergo hepatic first-pass elimination and are not absorbed in gut wall, were studied in this study. Sulfobromophthalein (BSP) and amaranth (AM) were used as the models of organic anionic drugs.

MATERIALS AND METHODS

Materials and Apparatus

Sulfobromophthalein (Sigma Chemical Co., G.R.), amaranth O (Chroma-gesellschaft Schmid & Co., G.R.), sodium hydroxide (Tedia Company, INC.), heparin (Choong Woe Pharm. Ind. Co., K.P.), physiological saline (Daihan Pharm. Ind. Co., K.P.), Aron α (Toagosei Chemical Ind. Co., LTD.), potassium chloride(Shima-

kyu's Pure Chemicals), potassium phosphate monobasic (Shinyo Pure Chemicals Co., LTD., First Grade), hydrochloric acid (Junsei Chemical Co., LTD.,G.R.), n-octyl alcohol (Junsei Chemical Co., LTD., G.R.), sodium phenobarbital (Merck & Co.,Inc.) and sodium pentobarbital (Merck & Co., Inc.) were used as purchased. UV spectrophotometer (LKB1, LKB2), microcentrifuge (Beckman Microfuge F4), centrifuge (Kokusan Ensinki Co.,LTD.),autopipette(50～250μℓ and 0.5～5mℓ, Funakoshi Co., LTD.) and homogenizer (Omega Electric Co.SM-3type) were used as apparatus.

Experimental Animals

Male Wistar rats weighing 200～350 g from Laboratory Animal Center of Seoul National University were used in all experiments. Water and commercial chow (Sam Yang Animal Food Inc., Seoul) were given *as libitum* for more than one week before experiment.

Preparation of BSP Solution

2%(w/v) BSP normal saline solution was prepared for intravenous and oral administration. For nasal administration, 10% (w/v) of BSP solution was prepared. The solution was prepared by warming in water bath before use.

Preparation of AM Solution

3%(w/v) normal saline solution was prepared for intravenous and oral administration. For nasal

administration, 7.5%(w/v) normal saline solution was prepared.

Oral Administration and Blood Sampling of BSP and AM

For all the routes of administration, experimental animals were anesthetized with intraperitoneal injection of sodium pentobarbital (50mg/kg) and sodium phenobarbital(100mg/kg). Polyethylene catheter (Intramedic PE-50, Clay Adams) was inserted into left femoral artery. After 1 hour, 2% BSP solution or 3% AM solution was administered *per os* by sonde at a dose of 120 mg/kg or 90.7 mg/kg respectively. After the administration, 0.3 ml of blood samples were collected at 1, 2, 3, 4, 5, 7, 10 and 15 minutes for BSP and 5, 10, 15, 30, 45, 60, 75 and 90 minutes for AM respectively. The blood samples were centrifuged at 1,800xg for 1 minute and 100 μ l of plasma samples were obtained. During the experiment, the rat was kept under a heat lamp not to lose its temperature.

Nasal Administration and Blood Sampling of BSP and AM

After anesthetizing, the surgery was proceeded according to the previous method[15]. The neck of rat was incised and the trachea was cannulated with polyethylene catheter (Polyethylene Tubing 7, HIDIKI). The esophagus was cannulated with catheter (Polyethylene Tubing 7, HIDIKI) of which one end is filled with absorbent cotton and glue not to leak

the administered drug from nasal cavity. The incision was sutured (Natume, No.3). The nasopalatine was closed with adhesive agent (Aron α, Toagosei Chemical Ind.Co.,LTD., Japan) to prevent drainage of the drug from the nasal cavity to the mouth. After about 1 hour, drug was administered *via* nostril with autopipette (50～250μl) and the nostrils were glued up. 100μl/250g of 10%(w/v) BSP solution and 100μl/250g of 7.5%(w/V) AM solution were used.

Blood sampling was done by the same method with oral administration.

Intravenous Injection and Blood Sampling of BSP and AM

After anesthetizing, left femoral vein and artery were cannulated with polyethylene catheter (PE-50). The drugs were administered by femoral vein catheter. 2%(w/v) BSP solution and 3%(w/v) AM solution were injected at the dose of 40mg/kg (2ml/kg) and 30.2mg/kg (1ml/kg). Blood sampling was done by the same method with oral administration.

Determination of BSP in Plasma

3ml of 0.05N-NaOH solution was added to 100μl of plasma and the absorbance was determined at 578nm. Calibration curve was made at the range of 50～1,000μg/ml.

Determination of AM in Plasma

0.9ml of normal saline was added to 100μl of plasma and the absorbance was determined at

520nm. Calibration curve was made at the range of 5～100μg/ml and micro UV cell was used.

Determination of Binding to Plasma Protein and Intestinal Mucus

1) Plasma Protein Binding

Whole blood of rat was centrifuged at 3600xg for 10 minutes to obtain non-hemolyzed plasma. Cellulose membrane (Seamless Cellulose Tubing, 36/32, Union Carbide Corp.) was hydrated in distilled water for 24 hours and was inserted into the dialysis cell (Natume Co., Japan). 2ml of plasma was added into one side of the cell and 2ml of pH 7.4-Tris buffer, into the other side of the cell. 3μl of 4%(w/v) AM solution was added to plasma to be 0.1mM. The cell was dialyzed for 48 hours at 4℃ under shaking. The absorbances of AM in plasma and in the buffer (pH 6.4) were determined at 520nm.

2) Intestinal Mucus Binding

Rat small intestine was isolated and washed with ice cold phosphate buffer(pH 6.4) 5～6 times. Then it was split open on a chilled glass plate in ice bath. The mucus was scraped off with slide cover glass. Wet mucus was weighed and homogenized in ten fold its weight of pH 6.5 buffer solution(9,000rpm). The solution was added into one side of the dialysis cell and pH 6.4 phosphate buffer, into the other side of the cell. BSP and AM were added to the buffer

side to be 0.1mM. Equilibrium dialysis was done by the same method with plasma binding test. BSP and AM in pH 6.4 phophate buffer were determined at 578nm and 520nm respectively.

Determination of Apparent Partition Coefficient(APC)

pH 1.2 HCl buffer (USP), pH 5.8 phosphate buffer (USP) and pH 7.4 phosphate buffer (USP) were prepared. Each of them was saturated with equal amount of n-octyl alcohol and let stand over night to separate n-octyl alcohol saturated buffer and n-octyl alcohol saturated with corresponding buffer. Then 5㎖ of 0.1mM BSP or 0.1mM AM solution, which was prepared with n-octyl alcohol saturated buffer, was mixed with 5㎖ of n-octyl alcohol saturated with corresponding buffer in the screw-capped tube. The tubes were shaken vigorously three times for 30 sec. with vortex mixer. They were centrifuged at 3,300xg for 10min and 4㎖ of n-octyl alcohol phase was taken. For determining BSP in organic phase, 4㎖ of n-octyl alcohol phase was reextracted with 4㎖ of buffers of each pH respectively. The buffer solutions were made alkaline by adding appropriate volume of N-NaOH or 5N-NaOH. Absorbances of the alkaline solutions were determined at 578nm.

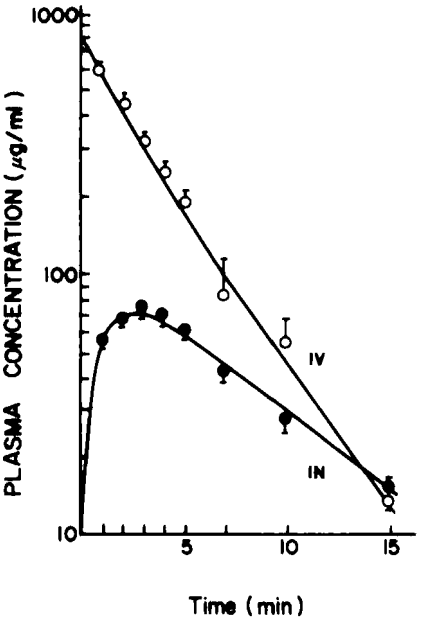

Fig. 1

Plasma concentration of BSP in rats following IV injection of 40mg/kg and IN administration of 40mg/kg. Absorption after PO administration of 120mg/kg was not detectable.

RESULTS

Nasal Absorption of BSP

Profiles of BSP plasma concentration after administering *via* various routes were given in Fig. 1. IV and IN represent intravenous and intranasal, respectively. IV profile was interpreted according to 2-compartment model and IN profile to 1-compartment with first order absorption process by

Table I - Bioavailability parameters of BSP and AM after IN administration to rats.

Drug	AUC(μg·min/kg) IV	AUC(μg·min/kg) IN	BA(%)	C_{max} (μg/mℓ)	T_{max} (min)
BSP	2867.78	745.82	26	72.0± 3.2	2.4± 0.6
AM	3003.13	926.70	31	17.0± 6.3	15

AUC was calculated by trapezoidal rule. In the case of AM, the value of $AUC^{0\to 90min.}$ was used. The values of C_{max} and T_{max} represent mean± S.E. (n=4).

non-linear regression program, M-MULTI[18]. Although threefold in amount of BSP was orally administered, the absorption was not detectable. Absolute bioavailability of BSP calculated by the following equations[19] after intranasal administration was about 31% (Table I).

$$AUC^{0\to\infty} = AUC^{0\to t} + C_t/\beta \quad \text{(Eq. 1)}$$

$$BA(\%) = (AUC_{IN}^{0\to\infty} / AUC_{IV}^{0\to\infty}) \times 100 \quad \text{(Eq. 2)}$$

Nasal Absorption of AM

Profiles of AM plasma concentration after administering *via* various routes were given in Fig.2. Although threefold in amount of AM was orally

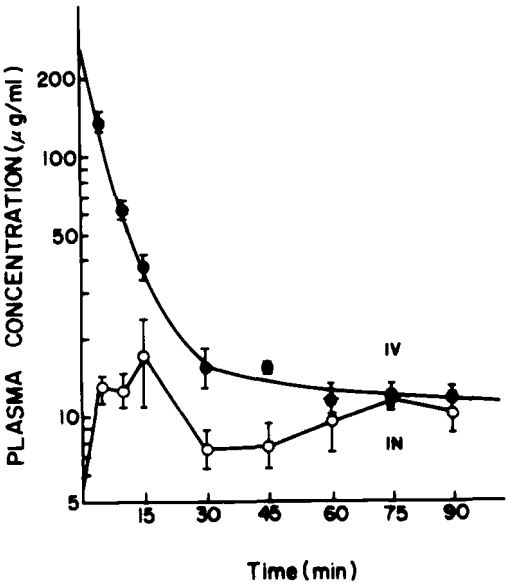

Fig. 2

Plasma concentration of AM in rats following IV injection of 50 μ mole/kg and IN administration of 50 μ mole/kg . Absorption after PO administration of 150 μ mole/kg was not detected.

administered, the absorption was not detectable (the detection limit ; 5 μ g/ml). Because AM plasma concentrations after intranasal administration were badly fluctuated, AUC from 0 to 90 minutes was obtained by trapezoidal rule. Absolute bioavailability of AM ($AUC_{IN}^{0\rightarrow 90}/AUC_{IV}^{0\rightarrow 90}$) after IN administration was 0.26 (Table I).

Protein Binding(%) of BSP and AM

Plasma protein binding(%) and small intestine mucous protein binding (%) were calculated by following equation[20];

$f_B = (C_B / (C_F + C_B)) X 100$ ——— (Eq.3)

Wherein, f_B , C_B and C_F represent protein binding(%), combined drug concentration (conc. in plasma or intestinal mucosa - conc. in buffer) and free drug concentration, that is , concentration in buffer (observed conc.) respectively. The results are shown in Table II. Plasma protein binding (%) for

Table II - Partition and binding of BSP and AM.

	BSP	AM
MW	838.0	604.5
Plasma binding (%)	96.6[21]	93.8± 3.4
Intestinal mucus binding(%)	77.9± 6.0	81.3± 0.2
APC (pH7.4→ n-octanol)	0.07± 0.00	0
APC (pH5.8→ n-octanol)	0.10± 0.02	0
APC (pH1.2→ n-octanol)	0.05± 0.00	0

Each value represents mean ± S.E. of three experiments.

BSP agreed with the cited value[21]. From the Table II, it was concluded that BSP and AM are highly bound with protein.

Partition of BSP and AM

Apparent partition coefficient (APC) was determined by following equation[20];

$APC = C_o / C_w$ ..... (Eq. 4)

Wherein, C_o and C_w represent the drug concentration in organic phase and the drug concentration in aqueous phase. As shown in Table II, AM was not partitioned at all to the organic phase. And BSP was partitioned only a little.

DISCUSSION

Nasal bioavailability of BSP and AM were 31% and 26% respectively, which were much higher than those by oral administration, but were lower than reported nasal bioavailabilities of drugs[7),11-15]. The improved nasal bioavailability are explained generally by the fact that ; the nasal mucus is very thin and includes a densely grown vessel network, and that the apparent water influx of nasal membrane is three times as much as that of intestinal membrane[23]. The high binding of BSP and AM with plasma protein or with intestinal mucus implies the possibility of nonspecific binding with nasal mucus which is composed mainly of glycoprotein[24]. Pene-

tration of BSP (MW:838) and AM (MW:604) through the lipoidal portion and pore of the nasal mucous membrane seemed to be poor due to their very low lipophilicity(APC) and their molecular size larger than the pore size (<0.4nm)[23] of the nasal mucus, respectively. The pore size of 0.4nm corresponds to the molecular weight 300[22]. Intranasal metabolism seemed not to be the reason for low nasal bioavailabilities of BSP and AM, since BSP glucuronidation and reduction of azo group in AM may not be affected by the nasal mucus enzyme like leucine aminopeptidase, steroid oxidase and steriod reductase *etc.* [7),25]. Therefore the unexpected low nasal bioavailabilities of BSP and AM may be attributed to 1) high mucous binding, or 2) poor lipophilicity to penetrate through the lipid portion of the nasal mucous membrane, or 3) large molecular size to penetrate through the hydrophilic pore of the nasal mucous membrane.

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