Percutaneous Absorption of Bromhexine in Rats

Taro OGISO,* Masahiro IWAKI and Sachiko TSUJI

Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashi-Osaka 577, Japan. Received December 14, 1990

The percutaneous absorption of bromhexine (BH), an expectorant drug, through rat skin was examined *in vitro* and *in vivo*. BH in free base form penetrated better than the hydrochloride through the skin. When the *in vitro* penetration of BH was compared using Plastibase, macrogol and sucrose ester of fatty acid F-160 (DK-ester) formulations, the DK-ester formulation showed the best penetration of BH of the three. The addition of Azone (3%) or lauric acid (BH: lauric acid molar ratio, 1:1) considerably increased BH penetration to a relatively large penetration rate. The plasma levels of BH after *in vivo* application of the DK-ester formulation with Azone or lauric acid (0.6 g/3.8 cm²) were also higher than those after the formulation without an enhancer, and a constant plasma level (20–50 ng/ml) was obtained during the application for 48 h. However, the bioavailability was low, 2.5 and 2.7% respectively. When the amount of BH remaining in DK-ester ointment and the skin after an 18-h application was measured, the BH content in the ointment was 88.6 ± 8.0% for the formulation without Azone and 93.7 ± 6.9% for that with Azone. The low penetration and low bioavailability observed will thus be due to the high drug retention of the base.

Keywords bromhexine; percutaneous absorption; ointment; rat; absorption enhancer; high drug retention; low bioavailability

Bromhexine (BH) is an expectorant drug, promoting bronchial secretion and having mucolytic properties. Although BH is widely used in human and veterinary medicine, the bioavailability is found to be considerably low; 2.4% in rat,¹⁾ 6% in dog²⁾ and 26% in man.³⁾ However, successful therapy in man with BH can be achieved using very low doses (5–15 mg).⁴⁾ The low dosage, rapid biotransformation, and large distribution volume resulting from the lipophilic character of the drug^{4–6)} are the reasons for the very low plasma levels (nanogram range).

To avoid presystemic metabolism and to enhance the bioavailability of BH, we examined the potential of percutaneous absorption of the drug in rats. Additionally, the reason for the low plasma levels after percutaneous administration was analyzed by measuring BH levels in the skin and ointment after application.

Materials and Methods

Materials Reagent: BH hydrochloride and laurocapram (Azone) were generous gifts of Tokyo-Tanabe Pharmaceutical Co. and Nelson Research and Development Co., respectively. Santonin, an internal standard for gas chromatography (GLC), and imipramine hydrochloride, an internal standard for high performance liquid chromatography (HPLC), were obtained from Sigma Chemical Co. and Japan-Ciba Geigy Co., respectively. Plastibase (Taisho Pharmaceutical Co.), macrogol 400 and 4000 (Wako Pure Chemical Ind.) and sucrose ester of fatty acid (DK-ester) F-160 (Daiichi Industry Co.) were used as the ointment base. All other chemicals used were of reagent grade. A BH free base was prepared by the following procedure: To BH hydrochloride dissolved in water, 2N NaOH was added and BH was extracted twice with ether. The extract, after dehydration with Na₂SO₄ and filtration, was evaporated under a vacuum. The drug obtained was recrystallized from ethanol.

Animals Male Wistar rats, weighing 200–260 g, were used throughout this study. The animals had free access to an MF diet (Oriental Yeast Co.) and water during the experiment.

Preparation of Ointment Details of the ointment composition are listed in Table I. For Rp. 1, BH was directly dissolved in Plastibase with or without Azone. For Rp. 2, BH dissolved in carbitol was added to a mixture of macrogol 400 and 4000 with or without Azone. For Rp. 3, DK-ester was mixed to hydroxyethyl cellulose, swollen with water, and the mixture was heated in boiling water for 10 min. After cooling, BH dissolved in carbitol with or without Azone (or lauric acid) was mixed with the above base.

In Vitro Percutaneous Penetration Experiment The hair of the abdominal area of male Wistar rats was carefully removed with an electric razor 24 h prior to application of the formulation. Pieces (2 × 2 cm area) of full-thickness skin were freshly excised from the rats and the dermal

side of the skin was soaked in a buffer solution (0.9% NaCl–10 mM phosphate buffer, pH 7.4) for 12 h at 5 °C to equilibrate the skin.⁷⁾ 0.15 g of formulation was uniformly spread over the stratum corneum surface of the skin, which was then mounted in a Franz diffusion cell (reservoir volume 13.0 ml, a 1.0 cm i.d. O-ring flange), and occluded with a sheet of aluminum foil. A gentamycin solution (10 mg/ml, Boehringer Mannheim) was added to the receptor fluid in the ratio of 1:100. The cells were incubated for 12 h at 37 °C.

In Vivo Percutaneous Absorption Experiment The hair of the abdominal area of male Wistar rats was removed and the rat jugular vein was cannulated with silicone tubing (Phicon tube, SH No. 00, Fuji Systems) under pentobarbital anesthesia (50 mg/kg) 24 h prior to application of the formulation.^{8,9)} 0.6 g of a given formulation was uniformly spread over the shaved skin (3.8 cm², designated by attaching a silicone rubber sheet with a cut-out area, using α -cyanoacrylate) and immediately occluded with a sheet of aluminum foil and adhesive tape. The ointment remained in contact with the skin for 48 h. Blood samples (0.2 ml) were collected periodically after dosing through the tubing. The plasma was separated immediately by centrifugation and stored frozen until assay.

Determination of BH BH in the sample was determined by the method reported previously.¹⁾

Determination of BH in Ointment and Skin The remaining ointment after application for 18 h was collected. The skin was wiped off with gauze soaked in saline. Pieces (0.5 g) of skin were homogenized in 2 ml of saline using a micro homogenizer (Physcotron NS-10, Nichion). To 50 mg of ointment or skin homogenate, 0.75 ml of 6N NaOH and 0.1 ml of imipramine hydrochloride (4 mg/ml) solution was added, then the contents were extracted with 1.0 ml of ether. The extractions with 0.2N HCl and ether at alkaline pH were mutually repeated twice. The final ether layer

TABLE I. Composition of BH Ointments

Rp. 1	BH	2.0 g
	(Azone	3.0 g)
	Plastibase	98.0 g (95.0 g)
Rp. 2	Macrogol 4000	40.0 g
	Macrogol 400	30.0 g
	Carbitol	20.0 g
	BH	2.0 g
	(Azone	3.0 g)
	Purified water	ad 100.0 g
Rp. 3	DK-ester F-160	3.0 g
	Hydroxyethyl cellulose	7.0 g
	Carbitol	30.0 g
	BH	2.0 g
	(Azone ^{a)}	3.0 g)
	(Lauric acid ^{a)}	1.1 g)
	Purified water	ad 100.0 g

a) Azone or lauric acid was added to the ointment.

was evaporated. The residue dissolved in 100 μ l of a mobile phase (acetonitrile-methanol-0.01 N phosphate buffer, pH 7.0, 40:40:20, v/v) was filtrated through a Chromatodisc filter (0.45 μ m, Biofield Co.). Three μ l of the filtrate were injected into a column (4.6 mm $\times$ 25 cm) packed with LiChrospher 100 RP-8 (5 μ m, Kanto Chemical, Tokyo), using a Shimadzu LC-6A liquid chromatograph equipped with a SPD-6AV UV-VIS detector. The flow rate was 1.0 ml/min and detection was at 254 nm.

Analysis of Data The area under the plasma concentration-time curve (AUC) after topical administration of BH was calculated by the trapezoidal method, and the AUC beyond the last observed plasma concentration (C_T) was extrapolated according to C_T/k_e , where k_e is the elimination rate constant.

The absolute bioavailability was calculated by the following equation:

$$\text{bioavailability (\%)} = \frac{AUC_{p.c.} \cdot \text{dose}_{i.v.}}{AUC_{i.v.} \cdot \text{dose}_{p.c.}} \times 100$$

The $AUC_{i.v.}$ was adopted from the previous paper.¹¹

Plasma concentration-time curves obtained after administration and the *in vitro* penetration data were analyzed by the iterative nonlinear least-squares regression procedure, MULTI.¹⁰ The means of all data are presented with their standard deviation (S.D.). Statistical analysis was performed using the non-paired Student's *t*-test, and a *p*-value of 0.05 or less was considered to be significant.

Results and Discussion

In Vitro Percutaneous Penetration of BH Hydrochloride and BH Base The penetration of BH hydrochloride and the free base through rat skin was compared using a macrogol formulation with or without Azone (Rp. 2). The results are shown in Fig. 1. The hydrochloride from the formulation without Azone barely penetrated, while the free base penetrated appreciably. This result agreed with that in the case of propranolol absorption.¹¹ The addition of Azone to the formulations considerably enhanced the permeation. In view of the results, the free base of BH was used for subsequent experiments. However, the free base may have a low thermodynamic activity, as suggested by its high melting point (dec. 237.5–238 °C) and low solubility.¹² Since percutaneous absorption of drugs is affected by their physicochemical properties,¹³ BH may be not absorbed as much as expected.

In Vitro Percutaneous Penetration of BH after Application of Various Formulations The penetration of BH through rat skin was compared using Plastibase, macrogol and DK-ester formulations. The results are shown in Fig. 2. The penetration of BH from Plastibase was extremely low, even if Azone was added in the base (Fig. 2). DK-ester for-

mulation showed the best penetration of BH among the three formulations. When an absorption enhancer, either Azone or lauric acid, was added to the formulation, the penetration of BH was significantly increased. The penetration profiles always consisted of a lag time followed by a linear rise. The lag time tended to decrease slightly in the formulation with Azone or lauric acid. The apparent penetration rate (slope of the linear portion of the profile) was enhanced 1.5–2.5 fold in the presence of the enhancers, except in the Plastibase formulation.

Fig. 2. Penetration Profiles of BH through Rat Skin after Application of Various Ointments

A, Plastibase; B, macrogol; C, DK-ester. Each point represents the mean $\pm$ S.D. ($n=3-5$). Applied dose was 0.15 g/0.785 cm². ●, without enhancer; ○, with Azone; △, with lauric acid.

Fig. 3. Plasma Concentrations of BH after Percutaneous Administration of DK-ester Ointment

Each point represents the mean $\pm$ S.D. ($n=3$). Applied dose was 0.6 g/3.8 cm². The ointment was removed after 48 h. ●, without enhancer; ○, with Azone; △, with lauric acid.

TABLE II. Model-Independent Pharmacokinetic Parameters of BH Following Percutaneous and Oral Administrations

Parameter	DK-ester formulation (BH 12 mg/rat)			Oral (10 mg/kg)
	Without enhancer	+ Azone	+ LA ^a	
$AUC_{0-\infty}$ (ng·h/ml)	1729 $\pm$ 242	2920 $\pm$ 680 ^b	2674 $\pm$ 665 ^b	535 $\pm$ 57
Bioavailability (%)	1.5 $\pm$ 0.2	2.5 $\pm$ 0.7	2.7 $\pm$ 0.6 ^b	2.5 $\pm$ 0.3

Each value represents the mean $\pm$ S.D. ($n=3$). a) Lauric acid. b) $p < 0.05$ compared with no enhancer.

Fig. 1. Penetration Profiles of BH through Rat Skin after Application of Macrogol Ointment

A, BH-HCl; B, BH base. Each point represents the mean $\pm$ S.D. ($n=3$). Applied dose was 0.15 g/0.785 cm². ●, without Azone; ○, with Azone.

Fig. 4. Amount of BH Remaining in DK-ester Ointment and Rat Abdominal Skin after Application of the Ointment

Each bar represents the mean $\pm$ S.D. ($n=3$). Application time of ointment was 18 h.

In Vivo Percutaneous Absorption of BH DK-ester formulations, which showed good penetration of BH in the *in vitro* experiment, were used for the *in vivo* study. The plasma concentrations of BH after a 48-h application are depicted in Fig. 3. The plasma levels of BH after application of the formulation with Azone or lauric acid were much higher than those after the formulation without each enhancer. The model-independent pharmacokinetic parameters calculated are shown in Table II together with the oral data obtained previously.¹⁾ The bioavailability was only 2.5 and 2.7% for the formulations with Azone or lauric acid, indicating low absorption. It is shown that the low drug level of BH in circulating blood (ng/ml) is sufficient for pharmacological effectiveness.⁴⁾ Therefore, the low plasma levels after percutaneous absorption would also be sufficient for pharmacological effect.

Amount of BH Remaining in Ointment and Skin after Application To confirm the low bioavailability and the cause, we determined the amount of BH remaining in ointment and the skin after an 18-h application. The results

are shown in Fig. 4. The amount of BH remaining in the ointment was $88.6 \pm 8.0\%$ for the formulation without Azone and $93.7 \pm 6.9\%$ for the formulation with Azone. As a result, about 11 and 6% of the drug was released from the bases in 18 h, respectively. The low values were probably due to the high drug retention of the ointment base used. On the other hand, the amount in the skin was low, 4.3 and 2.8%, respectively. These results indicate that BH may be slowly released from the DK-ester formulation, due to the high lipophilicity of BH (octanol/water distribution constant $10^{6.2-14}$). Therefore, these characteristics of BH may also be used for the sustained release of the drug.

In conclusion, therefore, the present results lead us to postulate that BH was absorbed through rat skin, but the amounts absorbed during 48 h was considerably low. The percutaneous DK-ester formulations gave constant plasma levels after application to rat abdomen. The release of BH from DK-ester formulations was very slow and BH was largely retarded in the base.

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