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Percutaneous absorption of betahistine through rat skin and pharmacokinetic analysis of the plasma concentration

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Summary

The percutaneous absorption of betahistine (BH) through rat skin was investigated by in vitro and in vivo studies. Additionally, to describe the plasma BH profile observed, a simple pharmacokinetic model, the two-compartment model including a zero-order absorption process, was presented. BH penetrated well from gel or adhesive formulations in the presence of an absorption enhancer (laurocapram and lauric acid) through rat skin, except for the formulation containing a lipid disperse system of BH, from which the in vitro penetration rate was the lowest of all formulations tested. In vivo, the drug was also absorbed through the skin from the formulations with enhancer. The gel formulation containing the lipid disperse system gave the highest and sustained plasma levels of BH, in contrast to the in vitro data, suggesting that this system would provide a drug delivery system capable of maintaining an effective plasma concentration for a prolonged time. The model successfully described the plasma levels of BH resulting from the rapid absorption during the initial time stage and the subsequent sustained absorption.

Introduction

Betahistine (BH) is a drug known to act as a diamine oxidase inhibitor and has properties similar to those of histamine (H-receptor effects) when given orally. It has been used in the treatment of diseases accompanied by impaired peripheral circulation, e.g., Meniére's syndrome (Elia, 1966; Esser and Reis, 1968). However, in most patients, this drug must be given three times a day, probably due to rapid elimination. In addition, the elimination kinetics of the drug have not been elucidated, although some investigators have reported the metabolic pathways (Bowman et al., 1972; Sternson et al., 1974).

There is considerable interest in the topical application of the drug intended for a systemic effect. It may be particularly useful for short-acting drugs since percutaneous absorption tends to be slow, and prolonged effects may be realized. The development of a percutaneous dosage

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form of BH will offer obvious advantages; more optimal therapy, patient convenience and improved patient compliance.

The present studies were undertaken to evaluate the percutaneous absorption of BH in the presence of absorption promoters and to develop a convenient formulation which can be applied to patients easily and has prolonged action. In addition, a trial for describing the plasma levels after dosing was tested by using a simple pharmacokinetic model, the two-compartment model with a zero-order absorption process.

Materials and Methods

Materials

BH was obtained from Aldrich Chemical Co. (Milwaukee, WI). BH mesylate was a generous gift from Nippon Zoki Pharmaceutical Co. (Osaka). 2,4,6-Trimethylaniline, an internal standard for GLC, and Hiviswako 104, a gel base, were purchased from Wako Pure Chemical Industries (Osaka). Sucrose ester of fatty acid (DK-ester) F-50, ethylhexyl acrylate suspension (7927/80) and laurocapram were generous gifts from Daiichi Industry Co. (Kyoto), Röhm Pharma (GmbH, Weiterstadt) and Nelson Research and Development Co. (Irvine, CA), respectively. Egg yolk lecithin (> 95% pure, PC) was obtained from Nichiyu Liposome Co. (Tokyo). All other chemicals and solvents used were of reagent grade or HPLC quality. Male Wistar rats, weighing 230–280 g, were used throughout this experiment. The animals had free access to MF diet (Oriental Yeast, Tokyo) for 3–4 days prior to and during experiments.

Preparation of gel and adhesive formulations and their transdermal systems

BH dissolved in a propylene glycol and ethanol mixture with or without absorption enhancer was mixed with a gel base (Hiviswako 104 or DK-ester F-50 and hydroxethyl cellulose) containing water. A BH lipid disperse system was prepared by mixing BH (unit weight) with a mixture (5 vols) of PC, cholesterol and dicetyl phosphate (10:1:1, mol/mol), followed by sonication for 2 min on ice after the addition of 5% mannitol solution (1 vol.). The lipid disperse system was mixed with the gel base. For formulation 5, DK-ester was mixed with hydroxyethyl cellulose swollen with water, and BH dissolved in solvents was mixed with the base. For an adhesive formulation, ethylhexyl acrylate suspension was heated at 120°C for 30 min and after cooling BH and absorption enhancer were mixed with the ethylhexyl acrylate-based adhesive (adhesive). Details of the formulations are listed in Table 1. The transdermal systems, containing 0.4 g (BH, 12 mg or 24 mg) of formulation and having an absorption area of 3.14 cm^2, were prepared using a corresponding gel formulation or adhesive described in Table 1, as reported previously (Ogiso et al., 1991).

TABLE 1

Composition of betahistine gel formulation and adhesive

Material (g)	Rp. 1	Rp. 2	Rp. 3	Rp. 4	Rp. 5	Rp. 6	Rp. 7
Hiviswako 104	1.0	1.0	2.0	1.0	–	–	–
DK-ester F-50	–	–	–	–	3.0	3.0	–
Hydroxyethyl cellulose	–	–	–	–	6.0	6.0	–
Acrylate adhesive	–	–	–	–	–	–	92.0
Propylene glycol	20.0	20.0	20.0	20.0	30.0	30.0	–
Ethanol	30.0	30.0	30.0	15.0	–	–	–
Diisopropanolamine	1.1	1.1	2.2	1.1	–	–	–
Betahistine (BH)	3.0	3.0	3.0	3.0 [a]	3.0	3.0	6.0
Laurocapram	–	4.0	–	4.0	–	4.0	2.0
Lauric acid	–	–	4.4	–	–	–	–

The gel ointments (Rp. 1–6) were prepared by adding purified water to give a total weight of 100 g. [a] Lipid disperse system containing BH (3% w/w) was used for the formulation.

Intravenous (i.v.) administration

On the day before the experiment, the jugular vein of the rats was cannulated with silicon tubing (Upton, 1975; Baker and Niazi, 1983). On the next day, BH mesylate dissolved in saline was administered intravenously at a 5.0 mg (BH equivalent)/kg dose. After administration, blood samples were withdrawn from the cannulated jugular vein periodically into a heparinized syringe.

In vitro percutaneous penetration experiment

On the day before the experiment, the hair of the abdominal area of rats was removed with an electric clipper and an electric razor. On the next day, pieces (3 × 3 cm area) of full-thickness abdominal skin were excised from the rats. The adherent fat and other debris were removed from the under surface. The dermal side of the skin was soaked in a buffer solution (0.85% NaCl-10 mM phosphate buffer, pH 7.4) for 12 h at 5°C. Then, 0.1 g (betahistine 3 or 6 mg) of formulation was uniformly spread over the stratum corneum surface of the skin, 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. The diffusion cell was thermoregulated with a water jacket at 37°C. Gentamicin sulfate solution (10 mg/ml, Sigma Chemical Co. St., Lous, MO) was added to the receptor fluid in the ratio of 1:100. Aliquots (50 μl) of the receptor fluid (0.85% NaCl-10 mM phosphate buffer, pH 7.4) were withdrawn periodically for 24 h and stored frozen until assay.

In vivo percutaneous absorption experiment

On the day before the experiment, the jugular vein of the rats was cannulated with silicon tubing (Upton, 1975; Baker and Niazi, 1983) and the hair of the abdominal area was carefully removed with an electric clipper. On the next day, the transdermal system (0.4 g/3.14 cm^2) was applied to the abdomen of the rats. The system was fixed with an adhesive (Aronalpha, Konishi Co., Osaka) and immediately occluded with an adhesive tape. The system was applied for 48 h. Blood samples (0.2 ml) were collected periodically for 48 h after dosing. The plasma was separated immediately by centrifugation and stored frozen until assay.

Determination of BH

BH in plasma and sample solution was determined by GLC methods as follows. A 100 μl aliquot of plasma or 50 μl of sample solution was mixed with 0.1 ml of 10% (w/w) ammonium hydroxide. To the solution 2.5 ml of chloroform was added and then followed by shaking and centrifuging. The organic layer (2.0 ml) was evaporated under reduced pressure, and the residue was dissolved in 30 μl of ethanol containing 2,4,5-trimethylaniline (2 μg/ml). The solution was injected into a gas-liquid chromatograph (Hitachi, model 263–50, with a flame thermonic detector; Unisole 10T plus KOH on 80/100 mesh Uniport HP, 3 mm × 1 m). The instrument settings were: column temperature, 145°C; injection port and detector block temperature, 225°C. Gas flow rates were: helium, 30 ml/min; hydrogen, 3 ml/min; air, 100 ml/min.

Analysis of data

The in vitro percutaneous parameters were calculated from the penetration data by using the following equations (Chow et al., 1984):

$$D = \delta^2/6\tau$$

$$J_s = \frac{D \cdot K_m \cdot C_s}{\delta} = K_p \cdot C_s$$

where J_s is the penetration rate, D denotes the diffusion constant within skin, K_m is the skin/vehicle partition coefficient of drug, τ represents the lag time calculated from the intercept of the flux with the time axis, and δ is the thickness (assumed to be 0.002 cm for rat stratum corneum) of the stratum corneum, K_p denotes the permeability coefficient through the corneum, and C_s is the drug concentration in the formulation.

Kinetic parameters were calculated by using the least-squares fit program, PCNONLIN (SCI Software, Lexington, KY). The plasma concentration data after i.v. administration were fitted to the equation:

$$C_t = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$$

where C_t is the drug concentration at time, t and A, α, B and β are the biexponential equation constants. The half-life ($t_{1/2}$) of the terminal phase was calculated as $t_{1/2,\beta} = 0.693/\beta$. The area under the plasma concentration-time curve (AUC), the area under the first moment curve (AUMC) and the mean residence time (MRT) were calculated by means of the following equations:

$$\text{AUC} = A/\alpha + B/\beta$$

$$\text{AUMC} = A/\alpha^2 + B/\beta^2$$

$$\text{MRT} = \text{AUMC}/\text{AUC}$$

The total clearance (Cl_{tot}) was estimated according to the following equation:

$$\text{Cl}_{\text{tot}} = X_0/\text{AUC}$$

where X_0 is the dose.

The AUC_{0-48} after percutaneous administration was determined by the trapezoidal method. The absolute bioavailability was calculated using the AUC values.

To analysis the plasma concentration profiles after percutaneous application, the two-compartment model includes a zero-order absorption process and one first-order elimination process. Differential equations required in the absorption model are follows:

$$dX_0/dt = -k_0 \quad (1)$$

$$dC_1 dt = k_0/V_1 - (k_{12} + k_{10})C_1 + k_{21}X_2/V_1 \quad (2)$$

$$dX_2/dt = k_{12}X_1 - k_{21}X_2 \quad (3)$$

$$X_0 = \text{dose}, C_1 = 0 \text{ and } X_2 = 0 \text{ at } t = 0 \quad (4)$$

where C_1 is the plasma concentration, X_0 and X_2 are the amount of drug in the absorption and peripheral compartments, respectively, k_0 denotes the zero-order absorption rate, k_{10} is the elimination rate constant from the plama compartment, k_{12} and k_{21} represent the transfer rate constants between plasma and peripheral compartments, and V_1 is the distribution volume of plasma compartment.

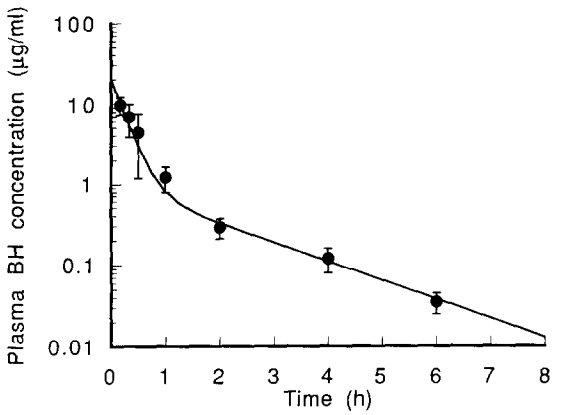

Fig. 1. Plasma concentration of betahistine after a single i.v. administration. Each point represents the mean ± SD ($n = 4$). The solid line represents the simulation curve. The dose was 5.0 mg/kg.

The mean plasma concentration-time course data obtained after administration were fitted to multi-exponential equations by PCNONLIN. All computations were carried out using a personal computer (Sanyo MBC-18NVH4).

Statistical analysis was performed by using the unpaired Student's t-test, and the significance level adopted was $p < 0.05$. The means of data are presented with their standard deviation (mean ± SD).

Results and Discussion

Plasma concentration of BH after single i.v. administration

The plasma concentrations after a single i.v. administration of BH (5 mg/kg) are shown in Fig. 1. The plasma decay curve after dosing showed biexponential kinetics. The pharmacokinetic parameters calculated by using the two-compartment open model and moment analysis are listed in Table 2. The half-life ($t_{1/2,\beta}$, 0.536 h) of the β-phase and MRT (0.67 h) suggested the rapid elimination of the drug in rats. To date, since quantitative data have been not available concerning pharmacokinetic analysis, the present results may represent the first report on kinetics.

TABLE 2

Pharmacokinetic parameters of betahistine after intravenous administration

Parameter	Estimate
A (μg/ml)	19.70 ± 7.14
α (h^{-1})	4.34 ± 1.34
B (μg/ml)	0.95 ± 0.43
β (h^{-1})	0.54 ± 0.12
k_{12} (h^{-1})	0.97 ± 0.82
k_{21} (h^{-1})	0.74 ± 0.26
k_{10} (h^{-1})	3.17 ± 0.71
V_1 (l/kg)	1.34 ± 0.43
Cl_{tot} (l h^{-1} kg^{-1})	1.43 ± 0.36
$AUC_{0-\infty}$ (μg h ml^{-1}) [a]	7.54 ± 2.16
MRT (h)	0.67 ± 0.11

[a] Using the equation. Each value represents the mean ± SD ($n = 4$).

In vitro percutaneous penetration studies

In vitro percutaneous absorption was investigated in order to compare the penetration of BH from various formulations. The penetration profiles for representative formulations are shown as a function of time in Fig. 2, and the calculated penetration parameters, being apparent values, are presented in Table 3. The drug penetrated through the skin at a rate profile which could be described approximately by zero-order kinetics during the first several hours, except for formulation 2 which showed two distinct penetration profiles, namely, a rapid and a slow phase, as shown in Fig. 2b. The apparent penetration rates (J_s) of BH from formulations 2 and 6 were the greatest and the lag time (τ) for formulation 6

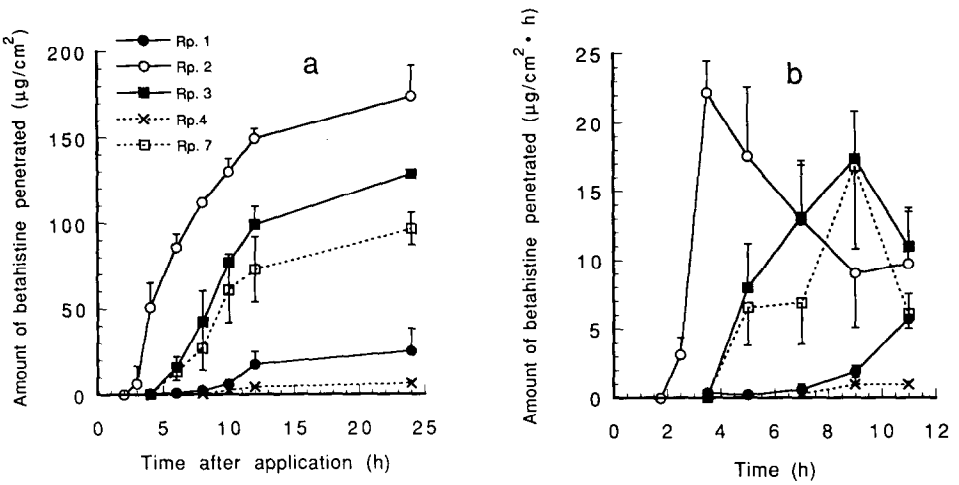

Fig. 2. Penetration profiles of betahistine through rat skin. Each point represents the mean ± SD ($n = 4$). Applied dose of formulation was 0.1 g/0.785 cm^2. (a) Cumulative amount; (b) amount penetrated per h.

TABLE 3

Penetration parameters of betahistine through rat skin

Parameter	Rp. 1	Rp. 2	Rp. 3	Rp. 4	Rp. 5	Rp. 6	Rp. 7
Lag time (h)	5.31 ± 1.22	1.36 ± 0.44	4.94 ± 1.53	6.92 ± 1.47	2.40 ± 0.63	0.70 ± 0.30	4.39 ± 0.34
J_s (μg h^{-1} cm^{-2})	2.37 ± 0.97	15.21 ± 0.18	10.9 ± 2.06	0.95 ± 0.30	6.93 ± 1.38	15.02 ± 1.79	12.90 ± 0.72
D ($\times 10^{-7}$ cm^2/h)	1.26 ± 0.42	4.90 ± 0.52	1.35 ± 0.16	0.96 ± 0.27	2.78 ± 1.34	9.52 ± 6.72	1.52 ± 0.23
K_p ($\times 10^{-4}$ cm/h)	0.79 ± 0.31	5.06 ± 0.06	3.64 ± 0.68	0.32 ± 0.10	2.31 ± 0.34	5.01 ± 0.54	2.16 ± 0.16
K_m	1.26 ± 0.32	2.06 ± 0.29	5.40 ± 0.68	0.67 ± 0.21	1.66 ± 0.56	1.05 ± 0.47	1.42 ± 0.19

Each value represents the mean ± SD ($n = 4$–5).
Applied formation was 0.1 g/0.785 cm^2 (BH, 3 or 6 mg/0.785 cm^2).

was the smallest of all formulations. Drug penetration from the formulations with no enhancers (formulations 1 and 5) was only slight compared with those with enhancers, except for that from formulation 4. The J_s and other parameters for formulation 4 were extremely small, contrary to expectation. This may be due to the slow penetration of the lipid dispersions through the viable epidermis of skin or to the low extent of drug release from the lipid disperse system.

The in vitro study demonstrated that BH could penetrate across rat skin.

In vivo percutaneous absorption studies

From the results of the in vitro penetration study, the formulations with absorption enhancers (systems 2–4 and 7) were used for investigating in vivo absorption. The plasma BH concentrations during a single percutaneous application of these systems are shown in Fig. 3. The parameters obtained are presented in Table 4. In most cases, although the plasma levels of BH increased slightly during the initial stage after application of these systems, the levels generally remained constant for a prolonged time. The plasma levels of BH after application of system 2 (formulation 2) were relatively high (C_{max}, 0.284 $\pm$ 0.009 μg/ml), while system 3, which contained lauric acid as an enhancer, gave slightly lower plasma levels. The reason for the curious profile observed for system 2, of which the levels were enhanced 30–36 h after application, is unclear based on the data obtained. The AUC_{0-48} calculated for system 2 was 8.68 $\pm$ 1.58 μg h ml^{-1}, this value being much greater than that for systems 3 and 7. Rapid color development was observed for formulation 3, probably due to the degradation of BH. The relatively low absorption after application of system 3 may thus be partly ascribed to chemical changes in BH.

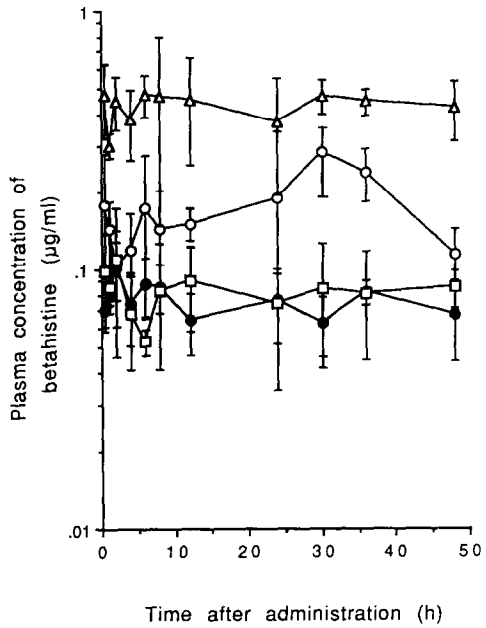

Fig. 3. Plasma concentration of betahistine after application of percutaneous absorption systems with enhancers. (○) System 2, (●) system 3, (△) system 4 and (□) system 7. Each point represents the mean $\pm$ SD ($n = 4$). Applied dose of formulation was 0.4 g/3.14 cm^2.

The in vivo percutaneous absorption of the drug through the skin from system 4 was the largest of all systems, resulting in high and sustained plasma concentrations of BH. As a result,

TABLE 4

Pharmacokinetic parameters following application of percutaneous absorption systems

Parameter	System			
	2	3	4	7
$AUC_{0-48\,h}$ (μg h ml^{-1})	8.68 $\pm$ 1.58	3.49 $\pm$ 0.52 [a]	20.40 $\pm$ 3.70 [a]	3.88 $\pm$ 0.67 [a]
MRT (h)	25.02 $\pm$ 2.27	23.49 $\pm$ 1.55	24.47 $\pm$ 2.28	24.06 $\pm$ 3.20
Bioavailability (%)	12.00 $\pm$ 2.18	4.82 $\pm$ 0.71 [a]	28.19 $\pm$ 5.11 [a]	2.68 $\pm$ 0.47 [a]

Each value represents the mean $\pm$ SD ($n = 4$–5).
Applied dose of formation was 0.4 g/3.14 cm^2 (BH, 12 or 24 mg/3.14 cm^2).
[a] $p < 0.01$ compared with system 2.

the AUC value for BH in system 4 was the highest out of the systems tested, suggesting that this system would provide a drug delivery system capable of maintaining an effective plasma concentration for a considerable length of time. Our results demonstrate good agreement with published data reporting that lipid disperse systems or liposome systems enhance the penetration of many drugs through skin (Mezei and Gulasekharam, 1982; Kimura et al., 1989; Kurosaki et al., 1991).

It has been shown that the use of a lipid disperse system composed of PC and glycosyl ceramide enhances the skin penetration of flufenamic acid approx. 2.2-fold compared with that from the lipid-free suspension (Kimura et al., 1989; Kurosaki et al., 1991), and that liposomes can be used to deliver drugs into skin in greater quantities than conventional vehicles (Mezei and Gulasekharam, 1982; Mezei, 1988). In this study, BH absorption via the skin was significantly enhanced by the lipid disperse system. One of the possible mechanisms for the enhancing action of these lipids may be the increased diffusion of drug in the stratum corneum and the increased permeation of the lipid dispersions into the intercellular lipids, based on the cooperative effect of the lipids and enhancer.

With system (formulation) 4, the discrepancy between in vitro and in vivo penetration may be partly due to differences in the rate of penetration of the lipid disperse system through the whole skin and the stratum corneum plus viable epidermis. The dermis, which is hydrophilic, would retain the lipid dispersions to a great extent and result in the slow penetration of BH in the dispersions. Reinfenrath et al. (1991) reported that the dermis interacts with the penetrant during both in vivo and in vitro absorption and that the dermis may possess a depot for lipophilic compounds during in vitro percutaneous absorption that is not observed in vivo.

Analysis of plasma drug concentration data using the model

We have previously analyzed the plasma profile of indomethacin and valproic acid administered percutaneously according to a pharmacokinetic model which includes two parallel absorption processes (Ogiso et al., 1988, 1989). In this study, the plasma concentration-time profiles after application of the transdermal systems were analyzed using a two-compartment model with a zero-order absorption process. Fig. 4 depicts the plasma concentrations of BH observed after percutaneous application of the systems and the fitting curves calculated according to the model using the parameters estimated following i.v. administration and the zero-order absorption rate (71.2 ± 4.1 μg/h for Rp. 3, 391.8 ± 18.3 μg/h for Rp. 4, 76.8 ± 5.8 μg/h for Rp. 7), although there was considerable disagreement between the in

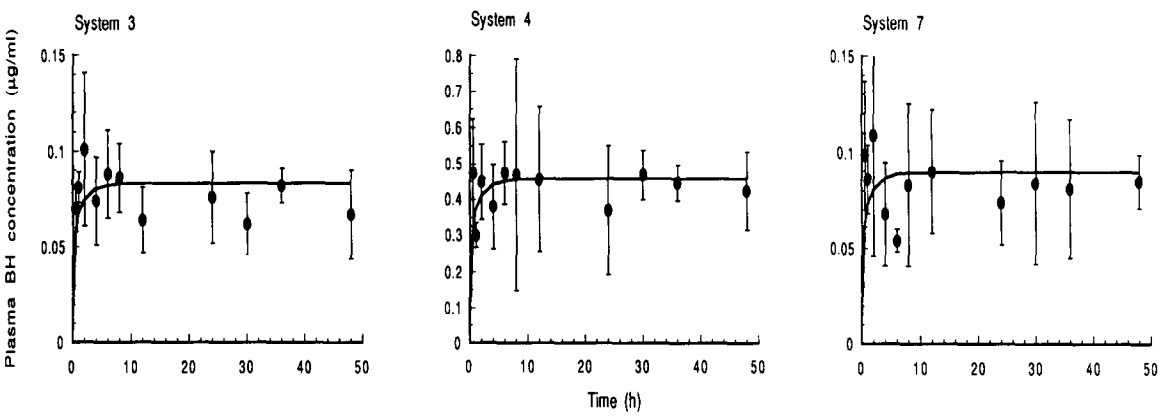

Fig. 4. Plasma concentration of betahistine after application of percutaneous absorption system and the simulation curves (solid line) calculated.

vitro and in vivo parameters, since rapid absorption of BH was observed in vivo. An satisfactory fit was obtained between the observed and calculated curves for the systems.

From the results of the in vivo absorption experiment (Fig. 4), it was clearly demonstrated that the transfer of BH into skin would be much more rapid than that predicted based on the in vitro penetration study and hence that drug molecules accumulated in the skin would slowly and sustainedly pass through the skin, probably via the intercellular and transcellular pathways, consequently resulting in sustained plasma concentraions of BH. It appears probable that the drug dissolved in solvents (ethanol and propylene glycol) would rapidly penetrate through the hair follicles of the rats during the initial time period. It has been reported that during the early period of the diffusion process, the flux through the hair follicles is much larger than that through the stratum corneum (Barry, 1983; Illel et al., 1991).

To the best of our knowledge, there are no determination methods available for the assay of plasma BH, except for radiolabeling method and mass fragmentography. An assay method for BH was first reported by Douglas and Hohing (1978), who used GLC equipped with a hydrogen flame ionization detector and determined concentrations ranging from 0.6 to 6.0 μg/ml. On the other hand, we could determine concentrations over the range 0.05–1 μg/ml, with a recovery of 98.5%.

Our goal in this study is to develop a transdermal therapeutic system of BH for treatment of impaired peripheral circulation such as Meniére's syndrome. The plasma concentrations of BH during application of system 4 were within the range 300–475 ng/ml for 48 h. The effective plasma concentration range of BH has not been evaluated in man. Judging from previous data indicating that the maximum plasma concentrations after oral dosings (50 mg/kg) to dogs are 2.6–4.8 ng/ml (Tanaka, 1971), the effective plasma concentration in man (dose, 6–12 mg once) would be at the ng/ml level. Thus, the transdermal systems, particularly system 4, prepared in this study may have the potential for development of an efficient controlled-release system for treatment of Meniére's syndrome, assuming that the penetration rate of BH through human skin is slower than that through rat skin. In addition, the negligible irritant effect of transdermal system 4 may make the system acceptable for clinical use.

In conclusion, therefore, the present results lead us to postulate that BH was absorbed through rat skin. The transdermal system (gel formulation and adhesive with laurocapram) gave comparatively high and constant plasma levels of BH after application to rats. The simple model presented in this paper gave an approximate description of the time course of plasma BH concentrations following application of the systems.

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