Pharmacokinetics and First-Pass Effect of Bromhexine in Rats

Masahiro IWAKI, Taro OGISO,* and Yoshimasa ITO

Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, 577, Japan

(Received February 6, 1990)

The pharmacokinetics and first-pass effect of bromhexine (BH) were studied in rats. Upon i.v. administration of 0.3 and 1 mg/kg of BH hydrochloride to normal rats, the plasma concentrations followed a biexponential curve, with slower terminal elimination ($t_{1/2\beta} = 8.9\text{—}11$ h). In bile duct cannulated rats, the plasma concentration-time profile was similar to that of normal rats and the bile excreted within 30 h contained only $1.5 \pm 1.2\%$ of the dose as intact and conjugated BH. These results suggest that a slower terminal elimination of BH after i.v. injection is due to a relatively small plasma clearance and large distribution volume rather than the enterohepatic recycling of the drug. Renal clearance of BH was negligible since urinary excretion of intact BH for 24 h after i.v. injection did not exceed 1% of the dose. After oral administration of BH, the systemic availability ranged only 1.8—3.9% based on i.v. and oral data, showing the poor bioavailability of oral BH. The first-pass effect of BH was measured by comparing the area under plasma concentration-time curve (*AUC*) after i.v., oral or hepatic portal (h.p.v.) administration of the drug. The *AUC* following h.p.v. dosing was only one-tenth of that obtained following i.v. administration and the *AUC* after oral dose was a quarter of that after h.p.v. administration. The hepatic extraction ratio was estimated to be 0.92. A low bioavailability after oral BH was explained by both hepatic and intestinal first-pass clearance, but mainly due to hepatic extraction.

Keywords — bromhexine; pharmacokinetics; biliary excretion; bioavailability; first-pass effect; rat

Introduction

Bromhexine (BH) hydrochloride [*N*-cyclohexyl-*N*-methyl-2-(2-amino-3, 5-dibromobenzylammonium chloride, Bisolvon®)] is an expectorant drug, promoting bronchial secretion and having mucolytic properties. Its low drug level in circulating blood (ng/ml) is sufficient for pharmacological effectiveness. Pharmacokinetic studies of BH include a report of absorption, distribution and excretion patterns in the rat,¹⁾ a study of excretion and metabolism in the rabbit,²⁾ two studies of distribution in rats and mice,³⁾ and the species differences in metabolism and excretion.⁴⁾ However, for those experiments the isotope-labeled BH had been usually used because it was technically impossible to determine the clinical drug levels in blood and urine by chemical analytical method. Jauch and Hankwitz⁵⁾ showed that radioactivities found in urine and faeces after both intravenous (i.v.) and oral administrations were almost the same, and Nilsson *et al.*⁶⁾ found that total radi-

oactivity in the urine amounted to *ca.* 95% of the dose when administered as elixir and capsule formulation. Although these reports indicate good absorption of the drug from the gastrointestinal tract, the absolute bioavailability of BH is rather low (mean 26% in man⁷⁾ and 6% in dogs⁸⁾) probably due to a first-pass effect. A study of the disposition of non-isotopic BH in dogs has been reported using sensitive gas-liquid chromatographic (GLC) assay with electron-capture detection.⁸⁾ However information concerning the pharmacokinetic profile and first-pass metabolism of BH is still limited.

The present study was undertaken to investigate the plasma disposition of BH and to estimate the relative importance of the gut wall and the liver in the first-pass effect, if the liver and/or intestinal extraction contributes to the low bioavailability. We report here the pharmacokinetic and availability data obtained on rats after i.v., oral and hepatic portal venous (h.p.v.) administration of non-isotopic BH.

* To whom correspondence should be addressed.

Materials and Methods

Materials — BH hydrochloride and its reference metabolites (M IIIa, M V and M VIII, see reference of Schraven *et al.*²⁾) were kindly supplied by Boehringer Ingelheim Japan (Hyogo, Japan). Trifluoroacetic anhydride (TFAA), obtained in 1 ml sealed glass ampules, and liquid chromatographic reagent grade acetonitrile were purchased from Wako Pure Chemicals Ind., Ltd. *n*-Hexane (pesticide residual analysis grade) was obtained from Nacalai Tesque. Nicotinamide adenine dinucleotide phosphate (NADP) was purchased from Sigma Chemical Co. Glucose-6-phosphate (G-6-P) and G-6-P dehydrogenase were obtained from Oriental Yeast Co., Ltd. All other chemicals used were commercial products of analytical grade.

Animals and Treatment — Male Wistar rats (195–260 g) were used throughout this experiment. Under pentobarbital anesthesia (30 mg/kg, intraperitoneally), the right jugular vein was cannulated with microbore silicone tubing (Phicon tube® SH No.00, Fuji systems, Tokyo, Japan) to collect blood samples by a modified method of Upton.⁹⁾ Animals were allowed to recover overnight before drug administration. For intraportal administration, other animals, that were planted with the jugular vein cannulation, were cannulated into the ileocolic vein with PE-10 polyethylene tubing (Becton Dickinson Co., U.S.A.) under light ether anesthesia. For the biliary and urinary excretion studies, the common bile fistula (PE 10 polyethylene tubing) and urinary bladder cannula (PE 50 polyethylene tubing) were used to collect bile and urine samples, respectively. BH hydrochloride was dissolved in distilled water. For i.v. bolus administration, the drug solution was injected (1.0 or 0.3 mg of BH hydrochloride/kg) into the jugular vein cannula, which was then flushed with 0.2 ml of heparin-saline (50 IU/ml). After administration of BH to rats with or without biliary fistulization, blood samples (0.22 ml) were drawn from the jugular vein cannula. For oral and h.p.v. administrations, animals received the drug solution at a dose of 10 or 25 mg/kg without fasting. The blood specimens were immediately centrifuged at 4 °C for 3 min to ob-

tain the plasma.

The biliary and urinary excretions of BH and hydroxy-BH isomers were estimated simultaneously in rats with bile and urinary cannulation. The animals were kept in restraining cages with free access to water during the experiment. BH hydrochloride (1 mg/kg) was administered soon after recovery from the ethylether anesthesia for operative surgery, and bile and urine were collected until 30 and 24 h, respectively, following drug administration. All plasma, urine, and bile samples were stored at –20 °C until the assay.

Measurement of Plasma BH Level —

Plasma, 0.1 ml, was placed in a 15 ml Teflon liner screwcapped test tube. The following were added by pipet: 0.1 ml of internal standard solution (0.1 ng of santonin/ml in methanol), 0.5 ml of borate buffer (0.1 N sodium borate : 0.36 N NaOH = 2 : 1, pH 10.5) and 2.0 ml of *n*-hexane. The tube contents were mixed on a mixer for 20 min. After centrifugation, the organic layer was evaporated in a 5 ml glass stoppered conical vial at 40 °C *in vacuo*. The residue was dissolved in 100 µl of ethyl acetate. A slight excess TFAA was added and mixed well. After 5 min, superfluous TFAA was removed at 40 °C. The residue was dissolved in 50 µl of methanol, and *ca.* 5 µl was injected into the GLC column.

GLC was conducted on a gas chromatograph (Hitachi 163 type) equipped with a ⁶³Ni-electron capture detector (pulsed, variable frequency type). The liquid phase, 10% OV-101, was coated on 80–100 mesh Chromosorb W-AW DMCS (Gasukuro Kogyo Inc., Tokyo Japan) and packed into a 2 m × 3 mm i.d. spiral silanized glass column. The temperatures were: oven, 240 °C and injection port and detector, 280 °C. Nitrogen was used as the carrier gas at a flow rate of 40 ml/min.

A plot of the response ratios of BH-trifluoroacetic acid (TFA) to santonin vs. plasma concentration of BH in the 20–400 ng/ml range demonstrated a linear relationship (regression line: $y = 0.0235x$). A detection limit of *ca.* 5 ng of BH/ml was obtained using 0.1 ml of plasma for the analysis. The mean within-run precision of the overall procedure (CV) of 7.4%, as expressed as relative standard deviation, was obtained on analyzing each 4–5 samples.

Measurement of Biliary and Urinary BH and Hydroxy-BH — Unchanged and hydroxy-BH in bile or urine samples were analyzed by high performance liquid chromatography (HPLC). Samples of 200 μ l for bile and urine were used for determination. The extraction procedure of BH and hydroxy-BH (M IIIa and M V) was performed according to the method described by Matsumura *et al.*¹⁰⁾ For measurement of their conjugates, the specimens were hydrolyzed for 20 min at 95 °C with sulfuric acid (final concentration = 4 N) before the extraction.

The chromatographic system consisted of a solvent delivery system (LC-3A, Shimadzu Co., Kyoto, Japan), a C₈ column (LichroCART® RP-Select B, 5 μ m, 250 $\times$ 4 mm i.d., Cica-MERCK, Tokyo, Japan) with a protection column (LiChrospher® 100, RP-18 encapped, 5 μ m), an ultraviolet (UV) detector (SPD-2A, Shimadzu) and a data processor (C-R6A). The mobile phase consisted of methanol, acetonitrile and 0.01 M phosphate buffer, pH 7.0 (40 : 40 : 20, v/v). The mobile phase was degassed ultrasonically. The flow rate was 1.0 ml/min and the chromatography was performed at 35 °C. The detection set was 254 nm.

The analytical procedure for both bile and urine was linear in the 50—1500 ng/ml range. The regression line, however, for BH in bile had an intercept of *ca.* 25 ng/ml. A detection limit of *ca.* 50 ng of BH/ml and *ca.* 20 ng of hydroxy-BH/ml was observed. The mean precision was 9.4% and 4.3% for BH and hydroxy-BH, respectively.

In Vitro Biotransformation Study — The rat liver was perfused *via* the portal vein with ice-cold saline, rapidly excised, blotted dry, and weighed. The liver was cut into small pieces and homogenized in 3 volumes of ice-cold 1.15% KCl using a motor-driven Teflon pestle and glass homogenizer. For the preparation of intestinal mucous membrane homogenate, the duodeno-jejunal portion, about 30 cm below the pylorus, was used. The intestinal mucous membrane was scraped off by a glass plate and homogenized in 2 volumes of ice-cold 1.15% KCl using Physcotron® (Nichion Irika Kikai, Co.).

In vitro metabolism study was performed in a 1 ml incubation mixture system containing 0.8

mM NADP, 8 mM G-6-P, 5 mM MgCl₂, 10 mM nicotinamide, 1.4 units G-6-P dehydrogenase, tissue homogenate, and 4 mM BH. Protein content in the mixture was determined according to the method of Lowry *et al.*¹¹⁾ The concentrations of protein used were 13.2 mg protein/ml for liver homogenate and 12.3 mg protein/ml for intestinal mucous membrane homogenate, respectively. Incubation was conducted in air at 37 °C with shaking for 1 h. After incubation, the mixture was extracted with 2 ml of *n*-butanol. The extract was concentrated to dryness. The residues were dissolved in ether and spotted on a thin layer chromatography (TLC) plate precoated with silica gel 60 F₂₅₄ (Merck, 0.25 mm). The solvent system containing chloroform : methanol = 200 : 1 (v/v) was used for the development. The chromatogram was viewed under a UV-lamp at 254 nm.

Pharmacokinetic Analysis — Plasma BH concentrations after i.v. administration were fitted to a biexponential equation with a weighted iterative nonlinear least-squares regression analysis MULTI¹²⁾ on a personal computer (PC-286V, Seiko Epson Co., Tokyo, Japan). Each residual error was weighted by a factor equal to the reciprocal of squares of plasma concentration. The coefficients (*A*, *B*, α and β) from the fitted function were used to calculate the specific rate constants as well as volume parameters from the usual equations.¹³⁾

The area under the plasma concentration-time curve (*AUC*) from 0 to the last observed data point was calculated using the linear trapezoidal method. The residual area beyond the last sampling time was estimated as C'/k_z , where C' is the last observed concentration and k_z is the slope of the terminal log-linear phase. Mean residence time (MRT), total plasma clearance (CL_{tot}) and volume of distribution at steady state (V_{dss}) were calculated by means of moment analysis.¹⁴⁾

The parameters for availability were determined as follows:

$$F = (AUC_{p.o.}/D_{p.o.})/(AUC_{i.v.}/D_{i.v.}) \quad (1)$$

$$F_H = (AUC_{h.p.v.}/D_{h.p.v.})/(AUC_{i.v.}/D_{i.v.}) \quad (2)$$

$$f_a F_G = (AUC_{p.o.}/D_{p.o.})/(AUC_{h.p.v.}/D_{h.p.v.}) \quad (3)$$

where F is the oral bioavailability, f_a is the fraction of the administered dose absorbed into the gut wall, and F_G and F_H are the fractions of the absorbed dose escaping metabolism in the gut wall and liver, respectively. The subscripts $p.o.$, $i.v.$, and $h.p.v.$ refer to drug administration by the oral, $i.v.$, and hepatic portal venous routes, respectively, and D refers to the dose administered.

Statistical Analysis — All the results were expressed as the mean $\pm$ S.D. The statistical significance of difference between two groups was tested by using Student's t -test. Differences were considered significant when p was less than 5%.

Results and Discussion

Pharmacokinetic Study Following Intravenous Administration

The plasma disposition of BH after single $i.v.$ administration of 0.3 or 1 mg/kg in normal rats is shown in Fig. 1. The decline in plasma concentration followed a biexponential curve. The initial distribution phase lasted about 2 h, followed by a slower terminal disposition [the ap-

Fig. 1. Time Courses of Plasma Concentrations of BH in Rats Following Intravenous Administration with or without Bile-Fistulization

Each point represents the mean $\pm$ S.D. of 7—9 animals. Solid lines indicate the predicted curves obtained by a computer fitting technique: ●, 1 mg/kg in normal rats; ■, 0.3 mg/kg in normal rats; ○, 1 mg/kg in bile-cannulated rats.

parent terminal elimination half-life ($t_{1/2\beta}$) ranged 7.3—17.0 h]. Table I shows the pharmacokinetic constants derived from model-dependent or independent analyses after $i.v.$ administration. There were no significant differences between the α , β , CL_{tot} and MRT values

TABLE I. Pharmacokinetic Parameters of BH after $i.v.$ Administration with or without Bile-Fistulization^{a)}

Parameter	Intact rats		Bile-cannulated rats
	0.3 mg/kg ($n=8$)	1.0 mg/kg ($n=9$)	1.0 mg/kg ($n=7$)
A (ng/ml)	172 $\pm$ 81	339 $\pm$ 191	287 $\pm$ 149
α (h^{-1})	3.49 $\pm$ 1.30	3.90 $\pm$ 2.64	3.22 $\pm$ 1.51
B (ng/ml)	56.8 $\pm$ 25.4	121 $\pm$ 41	105 $\pm$ 36
β (h^{-1})	0.0775 $\pm$ 0.0181	0.0607 $\pm$ 0.0221	0.0723 $\pm$ 0.0225
$t_{1/2\alpha}$ (h)	0.222 $\pm$ 0.076	0.276 $\pm$ 0.195	0.254 $\pm$ 0.105
$t_{1/2\beta}$ (h)	9.41 $\pm$ 2.26	12.8 $\pm$ 4.51	10.8 $\pm$ 4.19
k_{12} (h^{-1})	2.34 $\pm$ 1.12	2.61 $\pm$ 2.07	2.07 $\pm$ 1.13
k_{21} (h^{-1})	0.929 $\pm$ 0.391	1.128 $\pm$ 0.824	0.975 $\pm$ 0.506
k_{10} (h^{-1})	0.300 $\pm$ 0.093	0.223 $\pm$ 0.117	0.247 $\pm$ 0.091
$AUC_{0-\infty}$ (ng·h/ml)	798 $\pm$ 295	2181 $\pm$ 425	1599 $\pm$ 419
V_1 (l/kg)	1.48 $\pm$ 0.62	2.45 $\pm$ 0.76	3.03 $\pm$ 1.48
V_2 (l/kg)	3.72 $\pm$ 1.38	5.51 $\pm$ 1.60	5.12 $\pm$ 0.98
V_{dss} (l/kg)	4.53 $\pm$ 1.42 ^{b)}	7.50 $\pm$ 1.87	9.31 $\pm$ 4.00
CL_{tot} (l/kg/h)	0.359 $\pm$ 0.115	0.474 $\pm$ 0.096	0.680 $\pm$ 0.260
MRT (h)	11.6 $\pm$ 2.6	16.7 $\pm$ 6.3	14.3 $\pm$ 6.1

a) Values expressed as mean $\pm$ S.D. for number (n) of animals. b) $p < 0.05$ compared with that after 1.0 mg/kg dose.

Fig. 2. Time Courses of Cumulative Biliary and Urinary Excretion Profile of BH and Hydroxy-BH's

Data were expressed as percentages of sum of free and conjugated forms extracted by ethylether after hydrolysis with sulfuric acid. Each point represent the mean $\pm$ S.D. of 4–5 animals. ●, biliary BH; ○, biliary hydroxy-BH's; ■, urinary BH.

in two dosage levels, however V_{dss} after the 1 mg/kg dose was significantly larger ($p < 0.05$) than after the 0.3 mg/kg dose. The reason for the difference in V_{dss} is unclear. The initial rapid distribution phase was observed, with mean distribution half-life values ($t_{1/2\alpha}$) of 0.18–0.30 h and the V_{dss} was extremely larger than body weight. These results suggest the rapid and extensive extravascular distribution and the high tissue binding of the drug, being in agreement with previous reports in the literature of dogs⁸) and man.⁵) This is explained by the high lipophilic properties of free BH base.

Figure 2 shows the cumulative amounts of BH excreted in bile for 30 h, as well as hydroxy-BH isomers which underwent hydroxylation of cyclohexyl ring in *m-trans*, *m-cis* and *p-trans* position, following i.v. administration. For BH in the rat, the biliary route constituted only a minor pathway of elimination. Only $1.5 \pm 1.2\%$ of administered dose was recovered in bile as unchanged and conjugated BH. The degree of the biliary BH conjugated was also investigated before and after hydrolysis with sulfuric acid. Only negligible amounts of unchanged BH were detected in bile; the percentage of intact BH to total (free and conjugated) BH was about 12% ($n = 2$ rats).

Total hydroxy-BH's excreted as free plus con-

jugated forms in bile amounted to $4.8 \pm 0.9\%$ of dose. The alkaline extract of bile additionally contained some unknown peaks eluted at early retention time. The retention time of one of these peaks was identical with an authentic metabolite M VIII, which is one of the hydroxylated and N-demethylated isomers. The area of this peak, however, was unambiguously much higher than those of BH and hydroxy-BH's.

As shown in Fig. 2, the cumulative urinary excretion of total BH (free + conjugates) for 24 h did not exceed 1% of the dose in any rats studied. This suggests that the renal clearance of BH is almost negligible. Kopitar *et al.*⁴) have shown that more than half of the extracts from rat urine were considerably polar metabolites, dibromoanthranilic acid and its glycine conjugate (commonly named as M XII and M XIIa).

In past excretion studies for BH, ^{14}C -labeled BH was administered to animals and total biliary radioactivity was measured. Beisenherz *et al.*¹) have reported that within 24 h of i.v. ^{14}C -labeled BH administration about 29% and 24% of the dose were excreted in the rats' urine and faeces, respectively. However, the contribution of individual excreted components is not clear. Based on the present result, it is likely that in rats BH is cleared extensively by metabolism, followed by excretion into urine and bile, and hence a large portion of biliary and urinary BH probably exist as more polar metabolites. To our knowledge there are no findings that these metabolites produce a stimulation of bronchial secretion, in spite of the fact that many metabolites have been identified.

Although the possibility of enterohepatic recirculation of BH has been suggested,⁵) there is little information available on the pharmacokinetics of BH in the biliary fistulization state. To investigate whether BH is subjected to the enterohepatic cycling or not, we also determined the plasma BH concentrations in bile-exteriorized rats. In the rats with bile fistulas, the plasma disappearance followed a biexponential curve and was similar to that in intact rats (Fig. 1). As shown in Table I, there were no significant differences between the parameters in intact and bile-exteriorized rats after 1 mg/kg dosing. Based on the fact that only small quantities of

Fig. 3. Time Courses of Plasma Concentrations of BH in Rats Following Oral Administration

Each point represents the mean $\pm$ S.D. of 4–6 animals.

●, 25 mg/kg; ○, 10 mg/kg.

free and conjugated BH were recovered in the bile (Fig. 2), these results suggest little or negligible enterohepatic circulation of BH. Therefore, the slower terminal elimination of BH after i.v. administration (Fig. 1) is due to a relatively small total plasma clearance, large distribution volume and prolonged storage of the drug in the tissues.

Bioavailability Study

Generally the absolute oral bioavailability of BH is rather low.^{7,8)} However, there are no other published studies reporting the role of the gut wall and the liver in the first-pass effect of BH. We have estimated the relative importance of the gut wall and the liver in the presystemic clearance, if these organs contribute to the low bioavailability. For oral study, the doses were in-

creased because of the analytical limitation in sensitivity. Schraven *et al.*²⁾ reported, even at a 1000 mg/kg does in rabbits, urine–faeces excretion rates similar to those with 0.7 mg/kg, suggesting linear pharmacokinetics of BH over an extensive dose range. Figure 3 depicts the mean plasma BH concentration–time course after oral administration of 10 or 25 mg/kg. In all cases peak plasma BH concentrations were reached within 1 h after the dosing with no apparent absorption lag time.

Table II shows some pharmacokinetic parameters after oral administration. Systemic availability of BH, calculated by comparing AUC with that following 1 mg/kg of i.v. BH administration, ranged only 1.8–3.9% (mean 2.4%), indicating the poor bioavailability in rats. These data are in agreement with the results of Vandecasteele-Thienpont *et al.*⁸⁾ using dogs (the average bioavailability was $5.8 \pm 0.5\%$). Jauch and Hankwitz⁵⁾ reported that a total plasma radioactivity 30 min after oral administration of ¹⁴C-BH in man was considerably higher than that after i.v. dose and that total excreted radioactivity in urine and faeces after oral and i.v. dose were almost the same. Additionally, Nilsson *et al.*⁶⁾ found that total radioactivity in the urine amounted to *ca.* 95% of the dose after oral ¹⁴C-BH. These reports suggest that the absorption of BH from the gastro-intestinal tract is almost complete. Therefore, it is likely that the

TABLE II. Pharmacokinetic Parameters of BH Following *p.o.* Administration in Intact Rat^{a)}

Parameter	Dose	
	10 mg/kg (<i>n</i> = 4)	25 mg/kg (<i>n</i> = 6)
$k_z^{b)}$ (h^{-1})	0.0581 ± 0.0162	0.0381 ± 0.0121
AUC (ng·h/ml)	535 ± 57	1315 ± 394
MRT (h)	17.0 ± 4.6	21.5 ± 4.4
BA ^{c)} (%)	2.5 ± 0.3	2.4 ± 0.7

a) Values expressed as mean $\pm$ S.D. for number (*n*) of animals. b) Apparent elimination rate constant for terminal phase. c) Bioavailability.

Fig. 4. Time Courses of Plasma Concentrations of BH in Rats Following Hepatic Portal Vein Administration

Each point represents the mean $\pm$ S.D. of 4–5 animals.

●, 25 mg/kg; ○, 10 mg/kg.

TABLE III. Oral Bioavailability Parameters of BH^{a)}

Parameter	Dose	
	10 mg/kg	25 mg/kg
Bioavailability	0.025	0.024
Fraction of absorbed dose escaping gut wall extraction (F_G)	0.29	0.21
Fraction of absorbed dose escaping liver extraction (F_H)	0.085	0.11
Gut extraction ratio ($E_G = 1 - F_G$)	0.71	0.79
Hepatic extraction ratio ($E_H = 1 - F_H$)	0.92	0.89

a) These parameters were estimated by using the mean *AUC* values from Eqs. 1–3, provided that f_a equaled 1.

low bioavailability is due to the first-pass effect, as speculated by Vandecasteele-Thienpont *et al.*⁸⁾

The two processes responsible for the presystemic clearance are gastrointestinal and hepatic extraction and/or metabolism. To estimate the hepatic first-pass effect, we have determined the *AUC* following h.p.v. administration. Figure 4 depicts the plasma BH concentration–time course after h.p.v. administration of 10 or 25 mg/kg of BH. Reduced systemic availability was observed following h.p.v. administration, indicating that BH is subjected to the extensive hepatic first-pass metabolism and/or extraction. The bioavailability parameters are summarized in Table III along with the fraction of gastrointestinal and hepatic extraction ratio, assuming

that the absorption of BH is complete as mentioned above. The hepatic extraction ratio (E_H) was larger than the gut extraction (E_G), suggesting that the liver plays a greater role in the first-pass effect of orally administered BH, though the later was still relatively high. With the reservation that BH is completely absorbed as discussed above, the extremely low systemic bioavailability after oral BH is explained by the first-pass metabolism by both liver and gut wall (presumably in mucus-covered membrane or within epithelial cell) during absorption from the intestinal lumen.

In order to ascertain the presystemic metabolism by liver and gut wall, BH was incubated with rat liver and intestinal mucous membrane homogenates. The results of the *in vitro* biotransformation study are shown in Fig. 5. As shown the figure, the formation of metabolites from BH was observed in both liver and intestinal mucosa homogenates. Therefore, these support the concept that BH is subjected to the both intestinal and hepatic first-pass metabolism, although the liver plays a greater role in producing the first-pass clearance of orally administered BH than the gut wall in the dose range tested. In man the first-pass effect after oral administration is also approximated to about 75%.⁷⁾

Presystemic metabolism and/or extraction is clearly important in the disposition of BH. While there is species difference in the excretion and fraction of metabolites formed between humans and rats,⁴⁾ the present results lead us to be careful to that the bioavailability of BH after oral dosage would be affected by the change in physiological conditions (e.g. blood flow rate and intrinsic clearance) of intestinal gut and liver.

Fig. 5. TLC of *in Vitro* BH Metabolic Study

a, liver; b, boiled liver; c, intestinal mucosa; d, boiled intestinal mucosa; e, authentic BH. Boiled homogenates were prepared by heating homogenates at 100 °C for 10 min.

Although the adequate oral dosage forms for human use can improve the bioavailability of BH, an upper limit for the bioavailability will be determined by the metabolic activity of the gut and the liver.

In conclusion, our results, showing the poor excretion of intact or conjugated BH into bile, indicate little or no entero-hepatic recycling of BH. Additionally, a poor bioavailability following oral administration is due to presystemic clearance by both intestinal mucosa and liver, although the latter is predominant.

Acknowledgment We are deeply grateful to Boehringer Ingelheim Japan for supply of bromhexine and its metabolites, and wish to thank MSc. Ken Tsunekawa and MSc. Hideyoshi Horibe for their technical assistance in this work.

References

- 1) G. Beisenherz, F. W. Koss, L. Klatt, und B. Binder: Über der Verteilung der Radioaktivität im Organismus und in den Ausscheidungen der Ratte nach Gabe von ^{14}C -Bisolvon, *Ther. Umsch.*, **22**, 322—326 (1965).
- 2) E. Schraven, F. W. Koss, J. Keck, and G. Beisenherz: Excretion, isolation and identification of the metabolites of Bisolvon®, *Eur. J. Pharmacol.*, **1**, 445—451 (1967).
- 3) Z. Kopitar, O. Leder, and V. Heinz: Autoradiographic studies on the distribution of ^{14}C -Bisolvon in rat and mice, *Arzneim.-Forsch.*, **21**, 914—918 (1971).
- 4) Z. Kopitar, R. Jauch, R. Hankwitz, and H. Pelzer: Species differences in metabolism and excretion of bromhexine in mice, rats, rabbits, dogs and man, *Eur. J. Pharmacol.*, **21**, 6—10 (1973).
- 5) R. Jauch and R. Hankwitz: The absorption, excretion and metabolic pattern of bromhexine in man after oral and i.v. administration, *Arzneim.-Forsch.*, **25**, 1954—1958 (1975).
- 6) H. T. Nilsson, G. G. A. Persson, and K. Tegnér: A preliminary study on the oral absorption of bromhexine and ephedrine administered as elixir and as chew-capsule, *Acta Pharm. Suec.*, **13**, 241—244 (1976).
- 7) E. Bechgaard and A. Nielsen: Bioavailability of bromhexine tablets and preliminary pharmacokinetics in humans, *Biopharm. Drug Dispos.*, **3**, 337—344 (1982).
- 8) L. M. R. Vandecasteele-Thienpont, F. M. Belpaire, R. A. Braeckman, and A. P. De Leenheer: Pharmacokinetics of bromhexine in the dog, *Arzneim.-Forsch.*, **30**, 1643—1645 (1980).
- 9) R. A. Upton: Simple and reliable methods for serial sampling of blood from rats, *J. Pharm. Sci.*, **64**, 112—114 (1975).
- 10) R. Matsumura, T. Yoshida, S. Kyui, S. Kobayashi, M. Toyoda, A. Nagakura, and H. Kohei: Studies on blood concentration of bromhexine hydrochloride (Bisolvon) in solution and syrup dosage form in rabbits, *Iyaku-hin Kenkyu*, **10**, 279—289 (1979).
- 11) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall: Protein measurement with Folin phenol reagent, *J. Biol. Chem.*, **193**, 265—275 (1951).
- 12) K. Yamaoka: Nonlinear Regression Program MULTI and Its Algorithm, "Analytical Method for Pharmacokinetics Using Computer," Nankodo, Tokyo, 1984, pp. 81—83.
- 13) M. Gibaldi and D. Perrier: "Pharmacokinetics," 2nd ed., Marcel Dekker, Inc., New York and Basel, 1982, pp. 84—111.
- 14) M. Gibaldi and D. Perrier: "Pharmacokinetics," 2nd ed., Marcel Dekker, Inc., New York and Basel, 1982, pp. 409—417.