J. Pharm. Pharmacol. 1994, 46: 591–595
Received May 4, 1993
Accepted December 3, 1993

Enantioselective Pharmacokinetics of Homochlorcyclizine: Disposition of (+)- and (−)-Homochlorcyclizine after Intravenous and Oral Administration of Racemic Homochlorcyclizine to Rats

MAYUMI NISHIKATA, AKI NAKAI, HITOMI FUSHIDA, KEISHIRO MIYAKE, TAKAICHI ARITA, KEN ISEKI* AND KATSUMI MIYAZAKI*

*Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 9-Bancho, Koshien, Nishinomiya 663, and *Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Nishi-5-chome, Kita-14-jo, Kita-ku, Sapporo 060, Japan*

Abstract—Concentrations of homochlorcyclizine enantiomers in blood, urine, and tissues of the liver, lung, kidney, brain, heart, spleen, intestine and stomach of rats after drug administration were determined by high-performance liquid chromatography on a chiral stationary phase. After intravenous administration (10 mg kg^{-1}), homochlorcyclizine was rapidly distributed in many tissues, with the highest concentration in lung. No differences were found between enantiomers in blood concentrations. After oral administration (50 mg kg^{-1}), the concentrations of the (+)-isomer in nearly all tissues were higher than those of the (−)-isomer. The $AUC_{0-\infty}$ values of the (+)- and (−)-isomers differed significantly. The absorption of racemic homochlorcyclizine from rat small intestine was not enantioselective. These results suggested that the different concentrations between enantiomers after oral administration were not caused by enantioselective absorption or distribution but rather by preferential first-pass metabolism of the (−)-isomer in the liver. The enantioselectivity of metabolism was also demonstrated by in-vitro experiments.

Homochlorcyclizine hydrochloride is a potent antihistamine used as a racemate. The antihistamine activity of the (−)-isomer is about 100 times that of the (+)-isomer in guinea-pig isolated ileum tests (Nishikata et al 1992a). Moreover, there are significant differences between enantiomers in the pharmacokinetics after oral administration of either one to rats (Nishikata et al 1992a) and man (Nishikata et al 1992b).

To study the enantioselective disposition of racemic homochlorcyclizine, an assay method was developed for the simultaneous determination of enantiomers in biological materials by high-performance liquid chromatography (HPLC) on a chiral stationary phase of ovomucoid-bonded silica (Nishikata et al 1993). The enantioselective pharmacokinetics after oral administration of racemic homochlorcyclizine hydrochloride has been reported for man, but not for rats.

As homochlorcyclizine is a highly lipophilic drug (Ogiso et al 1983), it is expected to accumulate in tissues. However, no detailed study has been reported on the tissue distribution of racemic homochlorcyclizine.

This paper describes the enantioselective disposition, particularly the tissue distribution of homochlorcyclizine after intravenous and oral administration of the racemic mixture to rats. Several in-vitro studies were also carried out, and the mechanism of enantioselective pharmacokinetics is discussed.

Correspondence: T. Arita, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 9-Bancho, Koshien, Nishinomiya 663, Japan.

Materials and Methods

Materials

Racemic homochlorcyclizine hydrochloride and diphenhydramine hydrochloride were purchased from Nippon Bulk Yakuhin Co., Ltd (Osaka, Japan). Both (+)- and (−)-homochlorcyclizine hydrochloride were obtained as described earlier (Nishikata et al 1992a). All other chemicals were of analytical reagent grade.

Animal experiments

Male Sprague-Dawley rats, 7–9 weeks old, were used, 7-week-old rats were used for in-vitro metabolic studies.

In-vivo pharmacokinetic studies

The jugular vein was cannulated (Upton 1975). Rats were fasted overnight before use and were not allowed food or water after drug administration. Rats were given 10 mL kg^{-1} racemic homochlorcyclizine solution via a stomach tube for oral administration (50 mg kg^{-1} as the hydrochloride salt) or 1 mL kg^{-1} body weight by injection into the jugular vein for intravenous administration (10 mg kg^{-1} as the hydrochloride salt). Rats were housed in individual metabolic cages after drug administration, and urine was collected up to 1 or 5 h. Blood was collected from the jugular vein at set times after administration. After exsanguination under diethyl ether anaesthesia, the liver, lung, kidney, brain, heart, spleen, intestine and stomach were quickly removed, weighed, chopped into small pieces, and then homogenized separately. All samples were analysed by a chiral HPLC method.

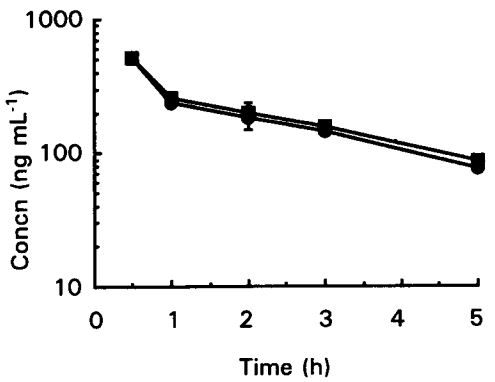

FIG. 1. Blood concentrations of (+)- and (−)-isomer after intravenous administration of 10 mg kg^{-1} racemic homochlorcyclizine hydrochloride to rats. Each point represents the mean ± s.e.m. of four rats. ● (+)-Isomer; ■ (−)-isomer.

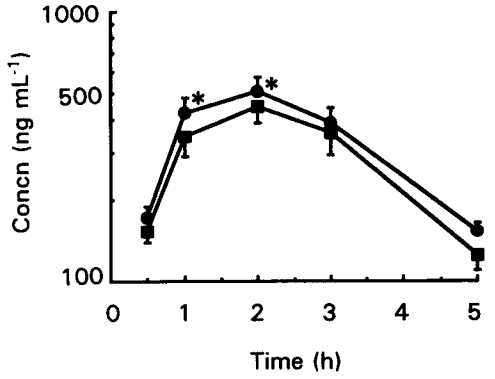

FIG. 2. Blood concentrations of (+)- and (−)-isomer after oral administration of 50 mg kg^{-1} racemic homochlorcyclizine hydrochloride to rats. Each point represents the mean ± s.e.m. of three rats. *$P < 0.05$ compared with the (−)-isomer by Student's t-test. ● (+)-Isomer; ■ (−)-isomer.

Table 1. Tissue ($\mu g\ g^{-1}$), blood ($\mu g\ mL^{-1}$) and urine ($\mu g\ mL^{-1}$) distribution of (+)- and (−)-homochlorcyclizine after intravenous administration of 10 mg kg^{-1} racemic homochlorcyclizine hydrochloride to rats.

	(+)		(−)	
Sample	1 h	5 h	1 h	5 h
Liver	0·42 ± 0·07	0·97 ± 0·15	0·50 ± 0·19	1·19 ± 0·08
Lung	41·32 ± 9·12	17·38 ± 4·77	40·19 ± 9·11	19·20 ± 6·43
Kidney	6·63 ± 0·29	2·74 ± 0·65	6·90 ± 0·16	2·78 ± 0·64
Brain	6·72 ± 1·00	1·96 ± 0·58	6·59 ± 0·82	1·94 ± 0·61
Heart	2·60 ± 0·06	0·96 ± 0·36	2·55 ± 0·06	0·99 ± 0·37
Spleen	3·61 ± 0·64	2·76 ± 1·15	3·48 ± 0·60	2·96 ± 1·24
Intestine	6·67 ± 0·68	2·10 ± 0·31	6·51 ± 0·54	2·15 ± 0·38
Stomach	3·83 ± 1·84	1·74 ± 0·13	3·85 ± 1·86	1·98 ± 0·31
Blood	0·24 ± 0·02	0·08 ± 0·01	0·26 ± 0·02	0·09 ± 0·01
Urine	0·06 ± 0·10	0·22 ± 0·13	0·03 ± 0·04	0·16 ± 0·10

Each value represents the mean ± s.d. of four experiments.

Absorption studies by an in-situ loop method

The absorption experiment was carried out using an in-situ loop method by Levine & Pelikan (1961). Racemic homochlorcyclizine hydrochloride was dissolved in isotonic phosphate buffer solution (pH 6·4) (Koizumi et al 1964). Rats were fasted overnight before use and were anaesthetized by intraperitoneal injection of sodium pentobarbitone (30 mg kg^{-1}). The abdomen was then opened along the midline. A ligated intestinal loop (10 cm) was prepared at about 10 cm below the pylorus and the bile duct was ligated, and then 0·1 mL cm^{-1} of drug solution warmed at 37°C was injected into the loop. The body temperature of the rats was kept at 37°C using a heat lamp. After 15 min, the loop was removed and the contents were washed out with 10 mL buffer solution. The amounts of (+)- and (−)-homochlorcyclizine remaining in the lumen were determined by the chiral HPLC method.

In-vivo bile excretion studies

The rats were anaesthetized by intraperitoneal injection of sodium pentobarbitone, and the femoral vein was cannulated immediately before use. The bile duct was isolated by abdominal midline incision and cannulated with polyethylene tubing (PE 10). After recovery from the anaesthesia, the rats were placed in the metabolic cages and given 1 mL kg^{-1} racemic homochlorcyclizine in saline (5 mg kg^{-1} as the hydrochloride salt) by injection through the cannula. Bile was collected from 0 to 1, 1 to 2, 2 to 3, 3 to 5, 5 to 8, and 8 to 24 h, and its volume measured gravimetrically, assuming a density of 1·0 for bile. Bile was then analysed by HPLC using a chiral or nonchiral column.

In-vitro plasma-erythrocyte cell partition coefficient and plasma protein binding

Heparinized fresh blood obtained from the rats was used. To blood (1 mL) was added 10 μL homochlorcyclizine solution to make blood concentrations of 5, 10 and 50 $\mu g\ mL^{-1}$ of (+)- and (−)-homochlorcyclizine hydrochloride, respectively, and each sample was incubated at 37°C for 10 min. After centrifugation, the plasma was removed to another tube immediately. An aliquot of the plasma (200 μL) was used to determine the total concentration of homochlorcyclizine, and 150 μL plasma was used to estimate the plasma protein binding. The binding of homochlorcyclizine to plasma protein was determined by an ultrafiltration method using lower adsorptive Ultrafree Tube (UFC3LGCOO, Millipore, Japan). The filtrate was injected directly into the HPLC system (nonchiral) for determination of the free homochlorcyclizine concentration in the plasma.

In-vitro transfer of homochlorcyclizine to erythrocytes

The fresh blood obtained from other rats was centrifuged and washed five times with saline buffered with 10 mM phosphate (pH 7·4). The erythrocytes were suspended in buffer solutions with 5, 10 or 50 $\mu g\ mL^{-1}$ racemic homochlorcyclizine hydrochloride. The mixtures were incubated as above. After centrifugation, the concentration of (+)- and (−)-homochlorcyclizine was determined by the chiral HPLC method.

In-vitro metabolic studies

Rats were fasted for 24 h before the experiments and killed by decapitation. The liver, lung, kidney and intestine were quickly removed. After perfusion with ice-cold 1·15% KCl-phosphate buffer (pH 7·4), the tissues were homogenized with 4 vols buffer. The incubation mixture consisted of 1 mL

Table 2. Tissue ($\mu g\ g^{-1}$), blood ($\mu g\ mL^{-1}$) and urine ($\mu g\ mL^{-1}$) distribution of (+)- and (−)-homochlorcyclizine after oral administration of 50 mg kg^{-1} racemic homochlorcyclizine hydrochloride to rats.

	(+)		(−)	
Sample	1 h	5 h	1 h	5 h
Liver	24·79 ± 2·33*	8·65 ± 1·96	21·22 ± 3·10	7·09 ± 1·36
Lung	42·40 ± 15.92*	14·81 ± 4·67	32·52 ± 14·05	11·94 ± 3·21
Kidney	14·28 ± 4·56*	12·82 ± 4·27*	11·96 ± 3·67	8·62 ± 2·41
Brain	4·01 ± 1·44*	6·47 ± 0·41*	2·88 ± 1·10	5·13 ± 0·48
Heart	3·74 ± 0·81*	2·29 ± 0·32*	2·93 ± 0·88	1·84 ± 0·11
Spleen	7·99 ± 4·26*	4·52 ± 0·40*	6·71 ± 3·93	3·56 ± 0·20
Intestine	277·18 ± 10·10	9·86 ± 2·92*	265·30 ± 10·94	8·21 ± 2·69
Stomach	643·88 ± 74·14	9·80 ± 3·37	613·88 ± 86·93	9·91 ± 3·10
Blood	0·42 ± 0·12*	0·15 ± 0·02	0·34 ± 0·09	0·13 ± 0·03
Urine	0·06 ± 0·07	0·26 ± 0·12*	0·06 ± 0·06	0·23 ± 0·14

Each value represents the mean ± s.d. of four experiments. *$P < 0.05$ compared with the (−)-isomer by Student's t-test.

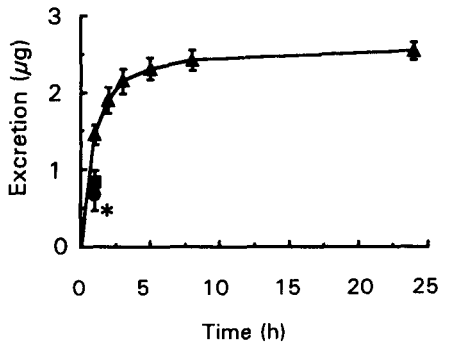

FIG. 3. Cumulative biliary excretion of homochlorcyclizine after intravenous administration of 5 mg kg^{-1} racemic homochlorcyclizine hydrochloride to rats. Each point represents the mean ± s.e.m. of four rats. ▲ Sum of (+)- and (−)-homochlorcyclizine; ● (+)-isomer; ■ (−)-isomer. *$P < 0.05$ compared with the (−)-isomer by Student's t-test.

of 9000 g supernatant (200 mg liver), 4 or 20 μM racemic homochlorcyclizine, 90 μM NADP, 4 mM glucose-6-phosphate, 10 mM $MgCl_2$, and 1·5 mM nicotinamide in a total volume made to 5 mL with 0·1 M phosphate buffer (pH 7·4). The incubation was carried out at 37°C at set times. The amount of unchanged homochlorcyclizine was determined by the chiral HPLC method.

Sample preparations for HPLC

To 0·5 mL of biological samples (urine, blood, plasma and various organ homogenates), was added 0·2 mL internal standard solution (diphenhydramine hydrochloride, 3 $\mu g\ mL^{-1}$), 3 mL purified water and 0·2 mL 4 M sodium hydroxide with vigorous mixing. The drug was extracted with 5 mL n-hexane. The extract was evaporated to dryness at 40°C and then dissolved in the mobile phase (120 μL), and 100 μL of this was injected into the HPLC.

HPLC methods

Homochlorcyclizine in the samples was determined by chiral and nonchiral HPLC methods described earlier (Nishikata et al 1993). An OVM column (Ultron ES-OVM, 150 mm × 4·6 mm i.d.) (Shinwa Chemical Industries, Kyoto, Japan) and acetate buffer (pH 4·7)-methanol (75:25, v/v) were used for the chiral HPLC method. A Shim-pack CLC-CN column (150 mm × 6 mm i.d.) (Shimadzu, Kyoto, Japan) and phosphate buffer (pH 3·0)-acetonitrile (67:33, v/v) were used for the nonchiral HPLC method.

Data analysis

Enantiomeric differences were analysed for their significance by Student's t-test.

Results

Disposition after intravenous administration

Fig. 1 shows the time course of changes in blood concentration of each enantiomer after intravenous administration of 10 mg kg^{-1} racemic homochlorcyclizine hydrochloride to rats. The elimination half-lives of (+)- and (−)-homochlorcyclizine were about the same (1·14 ± 0·13 and 1·32 ± 0·20 h, respectively).

Table 1 depicts concentrations of (+)- and (−)-isomers in blood, urine and tissues of the liver, lung, kidney, brain, heart, spleen, intestine and stomach after intravenous administration of racemic homochlorcyclizine hydrochloride (10 mg kg^{-1}). No differences were found between enantiomers. The highest tissue concentration was found in the lung. Relatively low concentrations were found in blood and urine.

Disposition after oral administration

Fig. 2 shows the mean concentration in blood profiles of each enantiomer after oral administration of 50 mg kg^{-1} racemic homochlorcyclizine hydrochloride to rats. The tissue distributions of (+)- and (−)-isomer after oral administration of racemic homochlorcyclizine hydrochloride (50 mg kg^{-1}) are presented in Table 2. The concentrations of (+)-isomer in all tissues except for the stomach and intestine were significantly higher than those of the (−)-isomer, the ratio of (+)-form to (−)-form being about 1·25. The $AUC_{0-\infty}$ value of the (+)-isomer (2·72 ± 0·13 $\mu g\ h\ mL^{-1}$) exceeded that of the (−)-isomer (1·96 ± 0·09 $\mu g\ h\ mL^{-1}$) ($P < 0.05$). These findings suggest that the enantioselective pharmacokinetics after oral administration result from enantioselectivity of either the absorption or of the first-pass metabolism process.

Absorption studies by in-situ loop method

Approximately 25% of both (+)- and (−)-isomer remained

Table 3. Partition of homochlorcyclizine between blood cells and buffer or plasma and the plasma protein binding in rat blood.

Sample	Concn in blood ($\mu g\ mL^{-1}$)	Partition coefficient (blood cells:buffer)	Partition coefficient (blood cells:plasma)	Plasma protein binding (%)	Free concn in plasma ($\mu g\ mL^{-1}$)
(+)-Isomer	5	19·56±4·06	4·49±0·35**	93·39±0·23*	0·14±0·01
	10	19·58±2·53	4·04±0·15**	94·15±1·13	0·26±0·05
	50	18·48±1·07	3·24±0·32	93·17±0·49*	1·79±0·10
(−)-Isomer	5	18·61±3·32	3·35±0·29	95·96±1·13	0·10±0·03
	10	19·97±1·36	3·17±0·16	94·81±0·33	0·28±0·02
	50	18·11±0·35	2·79±0·30	94·13±0·40	1·70±0·04

Each value represents the mean±s.d. of 3–5 experiments. *$P<0·05$, **$P<0·01$ compared with the (−)-isomer by Student's t-test.

Table 4. Percentage of remaining (+)- and (−)-isomers after incubation of racemic homochlorcyclizine with tissue 9000 g supernatant fraction from rats.

Tissue	Concn (μM)	Incubation time (min)	% Remaining	
			(+)-Isomer	(−)-Isomer
Kidney	4	30	101·41±1·95	99·03±2·92
	20	60	101·12±3·36	98·88±3·05
Lung	4	30	99·48±8·84	102·48±9·73
	20	60	98·82±1·65	101·19±1·63
Intestine	4	30	99·17±0·06	99·68±0·67
	20	60	100·36±6·59	99·64±6·48
Liver	4	30	1·05±0·17	0·56±0·79
	20	60	0·99±0·63	0·96±0·74

Each value represents the mean±s.d. of 3–4 experiments.

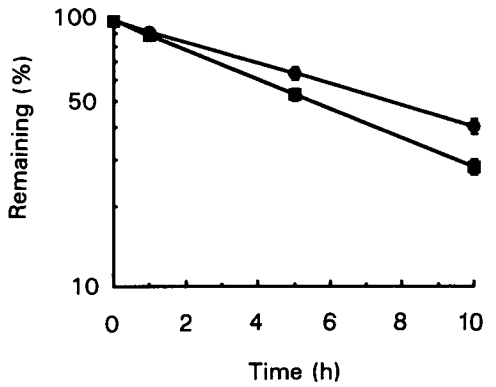

FIG. 4. Percentage of remaining (+)- and (−)-isomer after incubation of racemic homochlorcyclizine hydrochloride (20 μM) with liver 9000 g supernatant fraction. Each point represents the mean±s.e.m. of three experiments. ● (+)-Isomer; ■ (−)-isomer.

15 min after administration of 50 and 250 μg racemic homochlorcyclizine hydrochloride into the loop. Absorption of both (+)- and (−)-homochlorcyclizine was rapid, and no difference between the enantiomers was observed.

In-vivo bile excretion studies

The cumulative biliary excretion of homochlorcyclizine after intravenous administration of 5 mg kg^{-1} racemic homochlorcyclizine hydrochloride is shown in Fig. 3. The percentage of biliary recovery of the dose within 24 h was 0·15%. There was a significant difference between the concentrations of the enantiomers at 0–1 h after administration. However, the concentrations after 2 h were very low and could not be assayed by the chiral HPLC method.

In-vitro transfer of homochlorcyclizine to erythrocytes and plasma protein binding

Free drug concentrations in plasma could not be detected by the chiral HPLC method, due to the low concentrations. These studies were carried out using each enantiomer instead of racemic homochlorcyclizine hydrochloride, with the homochlorcyclizine being measured by the nonchiral HPLC method. The results of the partition and the binding to the plasma protein studies are summarized in Table 3. Although the partition coefficients between the blood cell and the isotonic phosphate buffer in (+)- and (−)-isomer were almost the same, the protein binding slightly differed between the enantiomers.

In-vitro metabolism studies

The results of metabolic experiments in kidney, lung, small intestine and liver are listed in Table 4. Homochlorcyclizine was metabolized only by the liver and at a rapid rate. Fig. 4 shows the concentrations of the (+)- and (−)-isomer profiles at different times after incubation of racemic homochlorcyclizine (20 μM) with liver 9000 g supernatant fraction. The metabolic initial rate of the (−)-isomer (30·55 nmol (g liver)$^{-1}$ min^{-1}) was higher than that of the (+)-isomer (24·54 nmol (g liver)$^{-1}$ min^{-1}). The ratio of the (+)-form to the (−)-form was about 0·81.

Discussion

Tissue distribution studies showed rapid and extensive uptake of both (+)- and (−)-homochlorcyclizine by many tissues, irrespective of the route of administration. In particular, the lung contained higher homochlorcyclizine concentrations than other tissues, a concentration that was approximately 160-fold that in the blood at 1 h after intravenous administration.

A preferential uptake into the lung has already been described for other basic drugs with high lipid solubility (Brown 1974; Bend et al 1985). Homochlorcyclizine is a weak base with pK_a of 8·5 and its partition coefficient is 16·65 in octanol-water (Ogiso et al 1983). Therefore, this extreme distribution of basic homochlorcyclizine in the lung can be assumed to be mainly due to its high lipid solubility.

The liver concentrations were relatively low after intravenous and oral administration, perhaps due to its rapid metabolism.

The concentrations in blood and tissues after oral administration were markedly lower than after intravenous administration, indicating a high presystemic clearance for homochlorcyclizine. Furthermore, the enantioselective disposition depended on the route of administration, that is, significant differences between the (+)- and the (−)-isomer were found after oral administration but not after intravenous administration. The absorption values of the isomers from the small intestine in rat were almost the same. These results suggest that first-pass metabolism plays a major role in the enantioselective pharmacokinetics after oral administration.

In man, one of the major urinary metabolites of homochlorcyclizine is a quarternary ammonium *N*-glucuronide (Nishikata et al 1992b). However, preliminary experiments revealed that its *N*-glucuronide was not present in rat urine. The formation of quarternary ammonium-linked glucuronide in the metabolic transformation of some tertiary amines is important only in man and other higher primates; it is negligible in other laboratory animals (Hucker et al 1974, 1978; Fischer et al 1980). Therefore, the cytochrome P450-dependent-oxidative metabolism of racemic homochlorcyclizine was examined in-vitro.

In rats, homochlorcyclizine was rapidly metabolized only by the liver, and the metabolic rate of the (−)-isomer was approximately 25% faster than that of the (+)-isomer in-vitro. This degree of difference agreed with that (about 25%) of the concentration in blood and tissues after oral administration, demonstrating that the significant difference in the pharmacokinetics of the enantiomers after oral administration was caused by the preferential first-pass metabolism of the (−)-isomer in the liver.

The binding of the (−)-isomer to plasma protein was slightly higher than that of the (+)-isomer in rats. However, the blood concentrations of the (−)-isomer after oral administration to rats were lower than those of the (+)-isomer. Moreover, there were no differences between the enantiomers in the distribution and elimination after intravenous administration to rats. These results indicate that the difference between the enantiomers in the plasma protein binding had little effect on the enantioselective disposition after oral administration to rats.

There was also a significant difference between the enantiomers in the bile excretion at 1 h after intravenous administration. However, as the percentage of biliary recovery of the dose was extremely low, the enantioselective excretion to the bile may be negligible.

Our investigations showed enantioselective pharmacokinetics after oral administration of racemic homochlorcyclizine hydrochloride to rats, because of the preferential first-pass metabolism of the (−)-isomer in the liver.

References

Bend, J. R., Serabjit-Singh, C. J., Philpot, R. M. (1985) The pulmonary uptake, accumulation, and metabolism of xenobiotics. Ann. Rev. Pharmacol. Toxicol. 25: 97–125

Brown, E. A. B. (1974) The localization, metabolism, and effects of drugs and toxicants in lung. Drug Metab. Rev. 3: 33–87

Fischer, L. J., Thies, R. L., Charkowski, D., Donham, K. J. (1980) Formation and urinary excretion of cyproheptadine glucuronide in monkeys, chimpanzees, and humans. Drug Metab. Dispos. 8: 422–424

Hucker, H. B., Balletto, A. J., Stauffer, S. C., Zacchei, A. G., Arison, B. H. (1974) Physiological disposition and urinary metabolites of cyproheptadine in the dog, rat, and cat. Drug Metab. Dispos. 2: 406–415

Hucker, H. B., Stauffer, S. C., Balletto, A. J., White, S. D., Zacchei, A. G., Arison, B. H. (1978) Physiological disposition and metabolism of cyclobenzaprine in the rat, dog, rhesus monkey, and man. Drug Metab. Dispos. 6: 659–672

Koizumi, T., Arita, T., Kakemi, K. (1964) Absorption and excretion of drugs XIX. Some pharmacokinetic aspects of absorption and excretion of sulfonamides. (1). Absorption from rat stomach. Chem. Pharm. Bull. 12: 413–420

Levine, R. R., Pelikan, E. W. (1961) The influence of experimental procedures and dose on the intestinal absorption of an ionium compounds, benzomethamine. J. Pharmacol. Exp. Ther. 131: 319–327

Nishikata, M., Nakai, A., Fushida, H., Miyake, K., Arita, T., Kitagawa, S., Kunitomo, M., Iseki, K., Miyazaki, K. (1992a) Method for optical resolution of racemic homochlorcyclizine and comparison of optical isomers in antihistamine activity and pharmacokinetics. Chem. Pharm. Bull. 40: 1341–1342

Nishikata, M., Nomura, A., Iseki, K., Miyazaki, K., Nakai, A., Fushida, H., Miyake, K., Arita, T. (1992b) Enantioselective pharmacokinetics of homochlorcyclizine II: disposition and metabolism of (+)-, (−)- and racemic homochlorcyclizine after oral administration to man. Eur. J. Clin. Pharmacol. 43: 533–538

Nishikata, M., Nakai, A., Fushida, H., Miyake, K., Arita, T., Iseki, K., Miyazaki, K., Nomura, A. (1993) Enantioselective pharmacokinetics of homochlorcyclizine III. Simultaneous determination of (+)- and (−)-homochlorcyclizine in human urine by high-performance liquid chromatography. J. Chromatogr. 612: 239–244

Ogiso, T., Iwaki, M., Kuranari, M. (1983) Relationship between hemolytic concentrations and physicochemical properties of basic drugs, and major factors inducing hemolysis. Chem. Pharm. Bull. 31: 4508–4516

Upton, R. A. (1975) Simple and reliable method for serial sampling of blood from rats. J. Pharm. Sci. 64: 112–114