J. Pharm. Pharmacol. 1994, 46: 209–212
Received March 11, 1993

The Disposition of Thioperamide, a Histamine H_3-Receptor Antagonist, in Rats

EIICHI SAKURAI, ERI GUNJI, YUKISUMI IIZUKA, NOBORU HIKICHI, KAZUTAKA MAEYAMA* AND TAKEHIKO WATANABE*

*Department of Pharmaceutics I, Tohoku College of Pharmacy, 4-1 Komatsushima 4-Chome, Aoba-ku, Sendai 981, and *Department of Pharmacology I, Tohoku University School of Medicine, 2-1 Seiryomachi, Aoba-ku, Sendai 980, Japan*

Abstract—An HPLC method using an ovomucoid-conjugated column has been developed for measurement of thioperamide, a histamine H_3 antagonist, with a minimum quantitation limit of 0·05 μg mL^{-1}. The assay was used to study the disposition of thioperamide in rats. After bolus intravenous administration of thioperamide (10 mg kg^{-1}), the plasma concentration decreased monoexponentially with a half-life of 26·9 min. The apparent total body clearance of thioperamide from rat plasma was 74·6 mL min^{-1} kg^{-1}. Although thioperamide was quickly transferred to various tissues, its concentrations in peripheral tissues were higher than that in the brain. However, the brain regional tissue/plasma ratios of thioperamide increased continuously after its injection.

Histamine plays an undisputed role in allergy and inflammation (via H_1 receptors) and in gastric secretion (via H_2 receptors) (Beaven 1978), which are blocked by H_1- and H_2-receptor antagonists, respectively. More recently, in addition to these two postsynaptic receptor subtypes, presynaptic H_3 receptors have been identified in the brain, regulating the release and synthesis of histamine (Arrang et al 1983). H_3 receptors are also present in a variety of peripheral sites such as the cardiovascular, respiratory and gastrointestinal systems (Trzeciakowski 1987; Ishikawa & Sperelakis 1987; Ea-Kim & Oudart 1988; Ichinose et al 1989; Andjelkovic et al 1990).

Thioperamide is a specific antagonist of this receptor (Arrang et al 1987). Garbarg et al (1989) and Oishi et al (1989) reported that thioperamide increased histamine turnover rate, measured by changes in the content of histamine and *N*-methylhistamine in the cortex of rats. Using a brain microdialysis technique, Mochizuki et al (1991) also demonstrated a 3-fold increase in the extracellular level of endogenous histamine in the anterior hypothalamic area after intraperitoneal injection of thioperamide (5 mg kg^{-1}) to urethane-anaesthetized rats. A decrease in brain histamine content of a mast-cell-deficient mouse (W/W^v) due to histamine release after intraperitoneal injection of thioperamide was also observed by Sakai et al (1991). After administration of thioperamide (10 mg kg^{-1}, i.p.) to rats, no increase in the histamine content of blood was detected by in-vivo microdialysis (Sakurai et al 1993). These varied results may be explained by variations in the disposition of the drug.

In the present study, we established an HPLC method for measurement of thioperamide using an ovomucoid-conjugated column and applied it to the determination of the plasma and tissue concentrations of the drug in rats to elucidate its pharmacokinetics.

Correspondence: E. Sakurai, Department of Pharmaceutics I, Tohoku College of Pharmacy, 4-1 Komatsushima 4-Chome, Aoba-ku, Sendai 981, Japan.

Materials and Methods

Materials

All reagents were of the highest grade available. Thioperamide was kindly supplied by Sumitomo Pharmac. Ind., Osaka, Japan.

Animal experiments

Male Wistar rats, 250–300 g (Japan SLC Inc., Hamamatsu, Japan), were starved for 18 h before the experiment and were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.). Thioperamide (10 mg kg^{-1}) was rapidly injected into the femoral vein. Blood samples were collected by cardiocentesis into heparinized Vacutainers at intervals after drug administration. The plasma was promptly separated by centrifugation at 800 *g* for 10 min. After collection of the blood, the liver, stomach, proximal small intestine, kidneys and brain were quickly removed and chilled. The liver and kidneys were freed from blood by infusion of ice-cold saline, and the mucosa was scraped off from the stomach and small intestine. The brain was placed on ice and separated into the cerebellum, hioppocampus, striatum, amygdala, cerebral cortex, midbrain, thalamus and hypothalamus by the method of Glowinski & Iversen (1966) with slight modifications (Onodera et al 1988). The plasma and tissues were sonicated for 10 s (Sonifier 450, Branson, USA) in an ice-bath in 2 and 9 vol, respectively, of ice-cold 0·4 M perchloric acid. The sonicated samples were centrifuged at 9000 *g* for 10 min at 4°C, and each supernatant was stored at −80°C until assayed.

Analytical methods

The concentrations of thioperamide in the plasma and tissues were determined by separation on an HPLC system with detection at 250 nm as follows: the supernatant (1 mL) was mixed with 1 mL 5 M sodium hydroxide and 7 mL benzene in a centrifuge tube. The mixture was vigorously shaken for 10 min and centrifuged for 10 min at 800 *g*. Five millilitres of the organic layer was evaporated to dryness

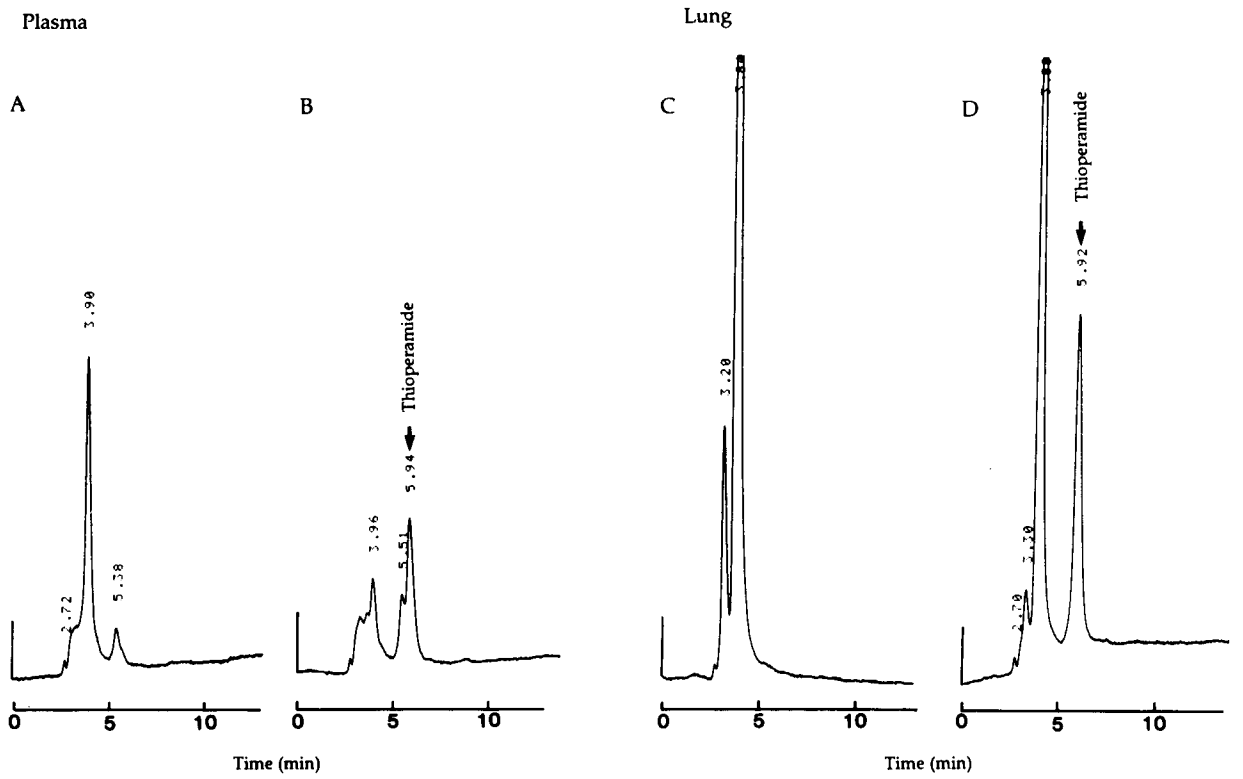

FIG. 1. Chromatograms of deproteinized samples of plasma and lung of anaesthetized (pentobarbitone sodium, 40 mg kg^{-1}, i.p.) rats after injection of thioperamide. Thirty minutes after thioperamide (10 mg kg^{-1}, i.v.), the rats were killed and the blood and lung were obtained. Control rats were treated with saline only. Plasma and lung were deproteinized with 0·4 M perchloric acid, and thioperamide in the extract was determined by HPLC as described in Materials and Methods. A, C, Control samples; B, D, thioperamide-treated samples; A, B, plasma; C, D, lung.

under reduced pressure. The residue was redissolved in 100 μL acetonitrile–0·05 M sodium phosphate buffer (pH 5·6) (9:1, v/v) and a 20 μL sample was injected onto the HPLC column. HPLC was carried out with an L-5000 (Yanagimoto, Kyoto, Japan) apparatus equipped with a UV detector (UV-8 model II, Tosoh) and an ovomucoid-conjugated column (Ultron ES-OVM, 150 × 4·0 mm i.d., Shinwa Kako, Kyoto, Japan), which was considered good for the analysis of basic compounds, amongst the best commercially available columns. Material was eluted with acetonitrile–0·05 M sodium phosphate buffer (pH 5·6) (9:1, v/v) at a flow rate of 1·0 mL min^{-1} at 30°C, and the absorbance at 250 nm was measured. The relative standard deviations (n = 10) for retention time and peak height of thioperamide were 0·6 and 0·8%, respectively. Determination of thioperamide was effected by the external standard method. The recovery of thioperamide with benzene was estimated to be about 85·1 ± 6·3% (n = 5).

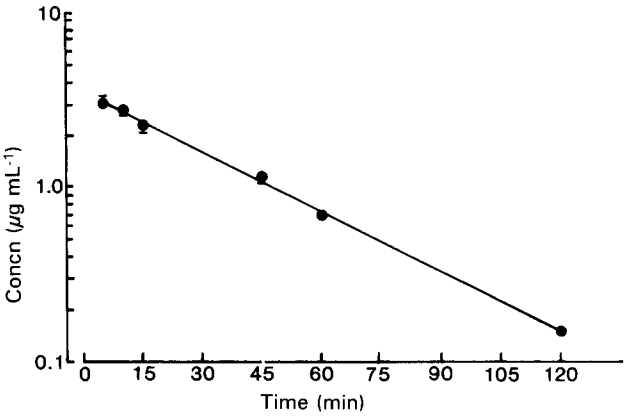

FIG. 2. Plasma concentration of thioperamide after intravenous injection into rats. Conditions were as for Fig. 1 except that blood was obtained at the indicated times after injection of thioperamide. Each point is the mean ± s.e. of four experiments.

Data analysis

Plasma concentration-time curves were analysed by a one-compartment model according to the nonlinear least-squares regression analysis program MULTI for monoexponential decline (Yamaoka et al 1981). The area under the plasma concentration-time curve (AUC) and the mean residence time (MRT) were calculated by standard linear trapezoidal integration with extrapolation to infinite time. The tissue-to-plasma concentration ratios (T/P ratios) were estimated at various times after drug administration. Values corrected for recovery are presented as means ± s.e.m. for n experiments and analysed by Student's *t*-test.

Results

Determination of thioperamide in plasma and tissues

Fig. 1 shows chromatograms of deproteinized samples of the plasma (A, B) and lung (C, D) obtained 15 min after rapid intravenous injection of either saline (A, C) or thioperamide

Table 1. Plasma pharmacokinetic parameters after rapid intravenous administration of thioperamide (10 mg kg^{-1}) to rats.

Parameter	Value	Unit
C_0	3·50±0·15	$\mu g\ mL^{-1}$
k_{el}	0·0262±0·002	min^{-1}
$t_{\frac{1}{2}}$	26·9±2·2	min
Vd	2869±123	$mL\ kg^{-1}$
CL	74·6±3·6	$mL\ min^{-1}\ kg^{-1}$
AUC	129·0±7·8	$\mu g\ min\ mL^{-1}$
MRT	39·8±3·0	min

A bolus dose of thioperamide was administered intravenously and the plasma thioperamide concentration was measured for 120 min at the times shown in Fig. 2. The equation fitted to the data was $Ct = C_0\ e^{-k_{el}t}$ for the plasma concentration, Ct, at time t. Pharmacokinetic parameters (average of four experiments) were calculated as described in Materials and Methods.

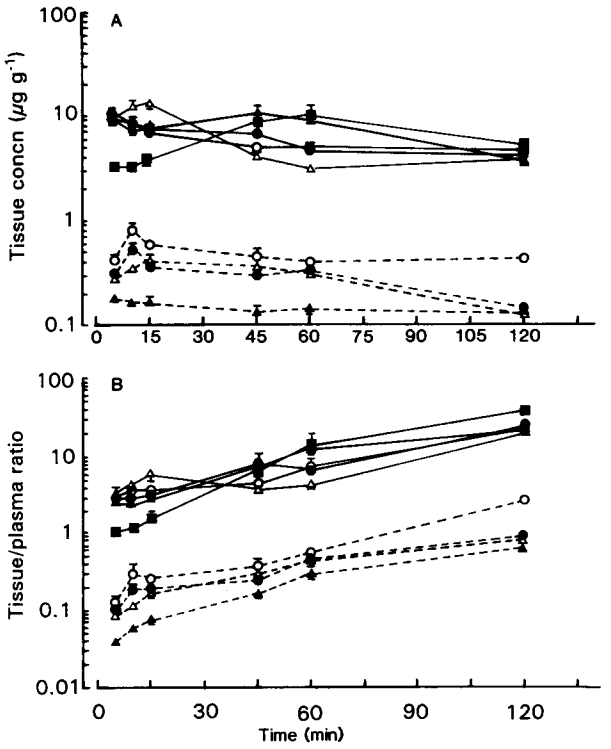

FIG. 3. Time course of transfer of thioperamide to tissues. Injection of thioperamide and measurement of its concentrations in plasma and tissues were as described in the legends to Figs 1, 2. Each point represents the mean±s.e. of four experiments. A, Tissue thioperamide concentrations; B, tissue/plasma ratios. —▲— Lung, —●— liver, —○—, kidney, —△— small intestinal mucosa, —■— gastric mucosa, - - - ○ - - - hypothalamus, - - - △ - - - thalamus, - - - ● - - - striatum, - - - ▲ - - - cerebral cortex.

(B, D). There were few interfering peaks in the region with a retention time corresponding to that of thioperamide (5·9 min) in the chromatogram. The calibration curve was linear over the range 0·05–5·0 $\mu g\ mL^{-1}$ with a correlation coefficient of more than 0·99 (n=8) (data not shown), and the lower limit for quantitation was 0·05 $\mu g\ mL^{-1}$.

Time-course of decrease in plasma concentration of thioperamide

Fig. 2 shows the time-course of changes in plasma concentration of thioperamide after rapid intravenous injection (10 mg kg^{-1}). Plasma thioperamide concentrations declined monophasically with a mean half-life ($t_{\frac{1}{2}}$, n=4) of 26·9 min. In the one-compartment model the distribution volume (Vd) was 2869 mL kg^{-1}. The total clearance (CL) of thioperamide from rat plasma was 74·6 mL $min^{-1}\ kg^{-1}$, and the area under the plasma concentration-time curve (AUC) of thioperamide was estimated to be 129·0 $\mu g\ min\ mL^{-1}$. The pharmacokinetic parameters of thioperamide are listed in Table 1.

Distribution of thioperamide to tissues

Fig. 3A shows the time-course of changes in concentrations of thioperamide in the gastric and small intestinal mucosae, liver, kidney and brain after its rapid intravenous injection. There were only relatively small differences between the concentrations of thioperamide in the peripheral tissues. However, thioperamide concentration in the brain was lower than those in other tissues. Within the brain, concentrations were higher in the hypothalamus, thalamus and striatum than in other regions. The tissue-to-plasma concentration ratios (T/P ratios) of thioperamide, determined at various times after its administration, are shown in Fig. 3B. The values in the peripheral tissues were greater than 1, whereas those in the various brain regions were smaller than 1.

Discussion

In this study, we established an HPLC method for measurement of thioperamide, an H_3-receptor antagonist, using an ovomucoid-conjugated column with a quantitation limit of 0·05 $\mu g\ mL^{-1}$. This enabled measurement of the pharmacokinetics of thioperamide and its distribution to tissues in rats.

The disappearance of thioperamide from rat plasma was very rapid and the drug was taken up readily by the tissues. In addition, the findings that the T/P ratios in various peripheral tissues continued to rise for at least 120 min after administration of thioperamide and was greater than 1·0, suggested that rat peripheral tissues may have an active transport mechanism for the uptake of this (imidazole-4-yl)piperidine derivative.

The concentration of thioperamide in the brain was lower than that in peripheral tissues. The level of drug in the hypothalamus and thalamus was higher than in the cerebral cortex. Larger numbers of H_3-receptor binding sites have been found in the cerebral cortex, striatum, olfactory tubercles, nucleus accumbens and substantia nigra, than in the hypothalamus, hippocampus and pons (West et al 1990). Our results, therefore, show that the regional distribution of thioperamide is not parallel to that of H_3 receptors in rat brain. However, the cell bodies of the histaminergic neurons are localized in the tuberomammillary nucleus in the posterior hypothalamic region (Watanabe et al 1984), while their fibres are found in almost all regions of the brain (Inagaki et al 1988). Since the transfer of thioperamide to the brain is the highest in the hypothalamus, it is conceivable that the regional distribution of this drug is in accord with that of histaminergic neurons.

References

Andjelkovic, I. Z., Collis, C. S., Rosic, M. A., Segal, M. B., Zlokovic, B. Z. (1990) Effects of *N*-alphamethylhistamine on the isolated

guinea pig heart. Evidence for H_3-histamine receptors. J. Physiol. 424: 58P

Arrang, J. M., Garbarg, M., Schwartz, J. C. (1983) Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. Nature 302: 832–837

Arrang, J. M., Garbarg, M., Lancelot, J. C., Lecome, J. M., Pollard, H., Robba, M., Schunack, W., Schwartz, J. C. (1987) Highly potent and selective ligands for histamine H_3-receptors. Nature 327: 117–123

Beaven, M. A. (1978) Histamine: its role in physiological and pathological processes. In: Dukor, P., Kallos, P., Trnka, Z., Waksman, B. H., de Weck, A. L. (eds) Monographs in Allergy. Vol. 13, S. Karger, Basel, pp 1–114

Ea-Kim, L., Oudart, N. (1988) A highly potent and selective H_3-agonist relaxes rabbit middle cerebral artery, in vitro. Eur. J. Pharmacol. 150: 393–396

Garbarg, M., Tuong, M. D., Gros, C., Schwartz, J. C. (1989) Effects of histamine H_3-receptor ligands on various biochemical indices of histaminergic neuron activity in rat brain. Eur. J. Pharmacol. 164: 1–11

Glowinski, J., Iversen, L. L. (1966) Regional studies of catecholamines in the rat brain. I. The disposition of (^{3}H)norepinephrine, (^{3}H)dopamine and (^{3}H)dopa in various regions of the brain. J. Neurochem. 13: 655–669

Ichinose, M., Stretton, C. D., Schwartz, J. C., Barnes, P. J. (1989) Histamine H_3 receptors inhibit cholinergic neurotransmission in guinea-pig airways. Br. J. Pharmacol. 97: 13–15

Inagaki, N., Yamatodani, A., Ando-Yamamoto, M., Tohyama, M., Watanabe, T., Wada, H. (1988) Organization of histaminergic fibers in the rat brain. J. Comp. Neurol. 273: 283–300

Ishikawa, S., Sperelakis, N. (1987) A novel class (H_3) of histamine receptors on perivascular nerve terminals. Nature 327: 158–160

Mochizuki, T., Yamatodani, A., Okamura, K., Takemura, M., Inagaki, N., Wada, H. (1991) In vivo release of neuronal histamine in the hypothalamus of rat measured by microdialysis. Naunyn Schmiedebergs Arch. Pharmacol. 343: 190–195

Oishi, R., Itoh, Y., Nishibori, M., Saeki, K. (1989) Effects of the histamine H_3-agonist (*R*)-α-methylhistamine and the antagonist thioperamide on histamine metabolism in the mouse and rat brain. J. Neurochem. 52: 1388–1392

Onodera, K., Maeyama, K., Watanabe, T. (1988) Regional changes in brain histamine levels following dietary-induced thiamine deficiency in rats. Jpn. J. Pharmacol. 47: 323–326

Sakai, N., Onodera, K., Kazutaka, M., Yanai, K., Watanabe, T. (1991) Effects of thioperamide, a histamine H_3 receptor antagonist, on locomotor activity and brain histamine content in mast cell-deficient W/W^V mice. Life Sci. 48: 2397–2404

Sakurai, E., Gunji, E., Iizuka, Y., Hikichi, N., Maeyama, K., Watanabe, T. (1993) In vivo microdialysis measurement of histamine in rat blood: effects of compound 48/80 and histamine antagonists. J. Pharmacol. Methods 29: 105–109

Trzeciakowski, J. P. (1987) Inhibition of guinea pig ileum contractions mediated by a class of receptor resembling the H_3 subtype. J. Pharmacol. Exp. Ther. 243: 874–880

Watanabe, T., Taguchi, Y., Shiosaka, S., Tanaka, J., Kubota, H., Terano, Y., Tohyama, M., Wada, H. (1984) Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. Brain Res. 295: 13–25

West, R. E., Zweig, A., Granzow, R. T., Siegel, M. I., Egan, R. W. (1990) Biexponential kinetics of (*R*)-α-[^{3}H] methylhistamine binding to the rat brain H_3 histamine receptor. J. Neurochem. 55: 1612–1616

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobiodyn. 4: 879–885