J. Pharm. Pharmacol. 1990, 42: 857–860
Received March 31, 1990

The Disposition of a Histidine Decarboxylase Inhibitor (*S*)-α-Fluoromethylhistidine in Rats

EIICHI SAKURAI, HIROSHI NIWA, SEIJI YAMASAKI†, 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 Seiryo-machi, Aoba-ku, Sendai 980, Japan*

Abstract—An amino acid analyser method using ninhydrin was developed for (*S*)-α-fluoromethylhistidine (FMH) with a minimum quantitation limit of 0·2 μg mL^{-1}. The assay was used to study the kinetics of FMH in rat. After bolus intravenous administration of FMH hydrochloride hemihydrate (50 mg kg^{-1}), plasma concentration decreased biexponentially with half-lives of 4·4 and 32·7 min. The distribution volumes of the central and peripheral compartments were 127·4 and 166·3 mL kg^{-1}, respectively. The tissue concentration of FMH was highest in the kidney and also decreased biphasically. The FMH concentrations in other tissues were lower, but their tissue/plasma ratios of FMH increased continuously after FMH injection, indicating that FMH partitioned into these tissues and was lost from them very slowly.

(*S*)-α-Fluoromethylhistidine (FMH) is a specific inhibitor of L-histidine decarboxylase, a histamine-forming enzyme, and strongly inhibits histamine formation in-vitro and in-vivo (Kollonitsch et al 1978; Garbarg et al 1980; Maeyama et al 1982; Kubota et al 1984). Thus, it has been used pharmacologically to deplete tissues of histamine (Bartholeyns & Bouclier 1982; Bouclier et al 1983a, b, c; Maeyama et al 1983; Slotkin et al 1983a, b; Duggan et al 1984; Nishibori et al 1984; Tung et al 1985) and as a drug in pathological conditions in which excess histamine is assumed to be involved, such as allergic diseases and mastocytosis (Granerus et al 1985; Olafsson 1985; Pipkorn et al 1987; Neittaanmäcki et al 1989). Those studies have suggested considerable variation in the disposition of the drug, but there is only one report of pharmacokinetic studies on FMH, which indicated little biotransformation in man (August et al 1985).

In the present study we established an amino acid analyser method for FMH and used it to determine plasma and tissue concentrations in rats to elucidate the pharmacokinetics of the drug in this species.

Materials and Methods

Materials

FMH was supplied by Dr J. Kollonitsch, Merck Sharp & Dohme Research Laboratories, Rahway, NJ, USA. All other reagents were of the highest grade available.

Animal experiments

Male Wistar rats (Shizuoka Experimental Animal Centre, Hamamatsu, Japan) were fasted for 18 h before experiments and anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.p.). FMH hydrochloride hemihydrate in 0·9% NaCl (saline) was injected rapidly into the femoral vein at a dose of 50·0 mg kg^{-1} (40·3 mg kg^{-1} of free base equivalent), the dosage that has been shown to be of use pharmacologically. Blood samples were collected by cardiocentesis into heparinized Vacutainers at intervals after drug administration. The plasma was promptly separated by centrifugation at 3000 rev min^{-1} for 10 min. After collection of the blood, liver, stomach, proximal small intestine, adrenal glands, kidneys and brain were quickly removed and chilled. The liver and kidneys were freed from blood by infusion of ice-cold saline, and the mucosae were scraped off the stomach and small intestine. The brain was placed on ice and separated into cerebellum, hippocampus, striatum, amygdala, cerebral cortex, midbrain, thalamus and hypothalamus by the method of Glowinski & Iversen (1966) with slight modification. The plasma and tissues were sonicated for 10 s in a sonicator (Sonifier 450, Branson, USA) in an ice bath in 2 and 9 volumes, respectively, of ice-cold 0·4 M perchloric acid.

Analytical methods

The concentrations of FMH in the plasma and tissues were determined in an amino acid analyser as follows. The sonicated sample was centrifuged at 10 000 rev min^{-1} for 10 min at 4°C, and the supernatant filtered through an Ultrafree C3GC filter (Millipore, Tokyo, Japan) and stored at −20°C until assayed. For analysis, a Hitachi Model 835 amino acid analyser equipped with a detector and a stainless steel column (150 × 4·0 mm i.d.) packed with a Hitachi custom ion-exchange resin (#2619, 5 μm particle size) was used. Fifty μL of clear supernatant was injected into the column and material was eluted with 26·3, 50·0 and 90·0 mM citrate buffer (pH 3·2, 4·3 and 4·9, respectively) at a flow rate of 0·225 mL min^{-1}. The eluate was mixed at 53°C with ninhydrin solution flowing at a rate of 0·300 mL min^{-1}, and the absorbance of the mixture at 570 nm was measured. The retention time of FMH was 49·1 min (between phenylalanine and lysine), and the ninhydrin colour intensity of authentic FMH was 95% of that of leucine.

Data analysis

Plasma concentration-time curves were analysed by a two-compartment model according to the least-squares regression analysis program MULTI for biexponential decline

† Present address: Department of Pharmacology I, Tohoku University School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan.

Correspondence to: E. Sakurai, Department of Pharmaceutics I, Tohoku College of Pharmacy, 4-1 Komatsushima 4-chome, Aoba-ku, Sendai 980, Japan.

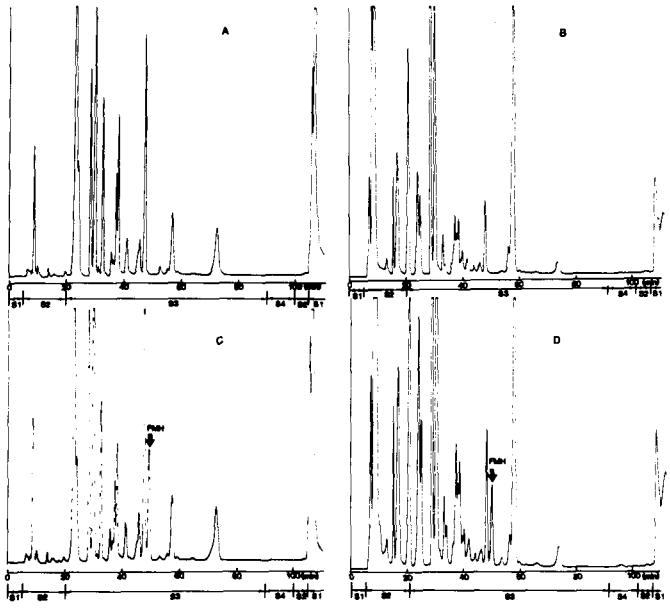

FIG. 1. Chromatograms of deproteinized samples of plasma and kidney of rats after i.v. injection of FMH. FMH (40·3 mg kg^{-1} as the free base) in saline (500 μL kg^{-1}) was injected i.v. and 15 min later the rats were killed, and the blood and kidney were obtained. Control rats were treated with saline only. Plasma and kidney were deproteinized with 0·4 M perchloric acid, and FMH in the extracts was determined in an amino acid analyser as described in Materials and Methods. A, B, Control samples; C, D, FMH-treated samples; A, C, plasma; B, D, kidney. S_1, S_2 and S_3, 26·3, 50·0 and 90·7 mM sodium citrate buffer of pH 3·2, 4·3 and 4·9, respectively; S_4, 0·2 M NaOH.

(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 are presented as mean ± s.e. of means for n experiments and analysed by Student's *t*-test.

Results

Determination of FMH in plasma and tissues

Fig. 1 shows chromatograms of deproteinized samples of the plasma (A, C) and kidney (B, D) obtained 15 min after rapid intravenous injection of saline (A, B) or FMH (C, D). There are no interfering peaks in the region with a retention time corresponding to that of FMH (49·1 min) in the chromatogram: the FMH peak is symmetrical and well separated from other peaks. The calibration curve was linear over the range 0·2–270 μg mL^{-1} with a correlation coefficient of more than 0·98 (n = 8) (data not shown), and the lower limit for quantitation was 0·2 μg mL^{-1}.

Time course of decrease in plasma concentration of FMH

Fig. 2 shows the time course of changes in plasma concentration of FMH after rapid intravenous injection of its hydrochloride hemihydrate (50 mg kg^{-1}). The pharmacokinetic parameters of FMH are listed in Table 1. Plasma FMH concentrations declined biphasically with half-lives of 4·4 ($t_{\frac{1}{2}}\alpha$) and 32·7 ($t_{\frac{1}{2}}\beta$) min, respectively. In the two compartment model the distribution volumes of the central and peripheral compartments (V_1 & V_2) were 127·4 and 166·3 mL kg^{-1}, respectively. The total clearance of FMH from rat plasma (CL) was 9·38 mL min^{-1} kg^{-1}, and the area under the plasma concentration-time curve (AUC) of FMH was estimated to be 4037 μg min mL^{-1}.

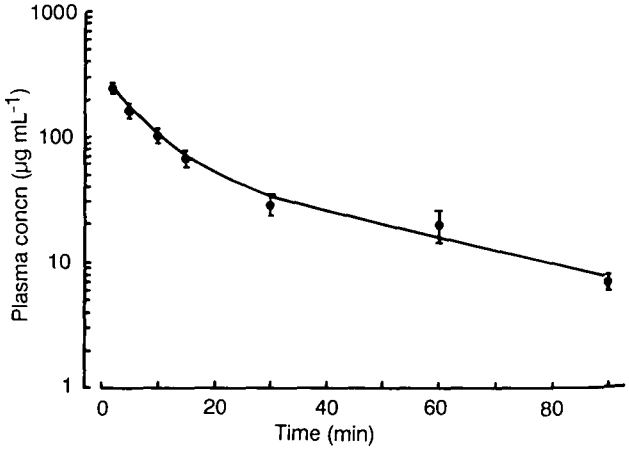

FIG. 2. Plasma concentration of FMH after i.v. injection into rats. Conditions were as for Fig. 1 except that blood was obtained at the indicated times after injection of FMH. Each point is the mean of 4–5 experiments.

Distribution of FMH to tissues

Fig. 3A shows the time courses of changes in concentration of FMH in the gastric and small intestinal mucosae, liver, adrenal gland, kidney and brain after its rapid i.v. injection. The concentration of FMH in the kidney was much higher than that in other tissues and decreased biphasically. The FMH concentration in the brain was lower than those in other tissues, and was higher in the cerebellum, cerebral

Table 1. Pharmacokinetic parameters of FMH after rapid intravenous administration of its hydrochloride to rats.

Parameter	Meaning	Value	Unit
A	Defined in the equation below	258·7 ± 13·5	μg mL^{-1}
B	Defined in the equation below	58·6 ± 1·1	μg mL^{-1}
α	Defined in the equation below	0·161 ± 0·012	min^{-1}
β	Defined in the equation below	0·022 ± 0·003	min^{-1}
k_{12}	Transfer rate constant from C* to P*	0·062 ± 0·002	min^{-1}
k_{21}	Transfer rate constant from P* to C*	0·048 ± 0·006	min^{-1}
k_{el}	Elimination rate constant from C*	0·073 ± 0·007	min^{-1}
V_1	Volume of distribution of C*	127·4 ± 5·6	mL kg^{-1}
V_2	Volume of distribution of P*	166·3 ± 6·1	mL kg^{-1}
V_{ss}	Steady state volume of distribution ($V_1 + V_2$)	293·7 ± 3·1	mL kg^{-1}
$t_{\frac{1}{2}}\alpha$	Half-time of distribution phase	4·35 ± 0·31	min
$t_{\frac{1}{2}}\beta$	Half-time of elimination phase	32·72 ± 4·29	min
AUC	Area under the plasma concentration time curve	4036·9 ± 430·6	μg min mL^{-1}
CL	Total body clearance ($k_{el} \cdot V_1$)	9·38 ± 1·27	mL min^{-1} kg^{-1}
MRT	Mean residence time	27·3 ± 0·23	min

A bolus dose of FMH hydrochloride was administered intravenously and the plasma FMH concentration was measured for 90 min at the times shown in Fig. 2. The data were fitted to the equation $C_t = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$ for the plasma concentration, C_t, at time t, and pharmacokinetic parameters (average of 4–5 experiments) were calculated as described in the Method. C* and P* represent the central and peripheral compartment, respectively.

cortex and midbrain than in the other regions. The tissue-to-plasma concentration ratios (T/P ratios) of FMH determined at various times after FMH administration are shown in Fig. 3B. The value in the kidney reached an equilibrium at about 5, 30 min after administration. The T/P ratios in all other tissues were small, but continued to rise for at least 90 min after FMH administration.

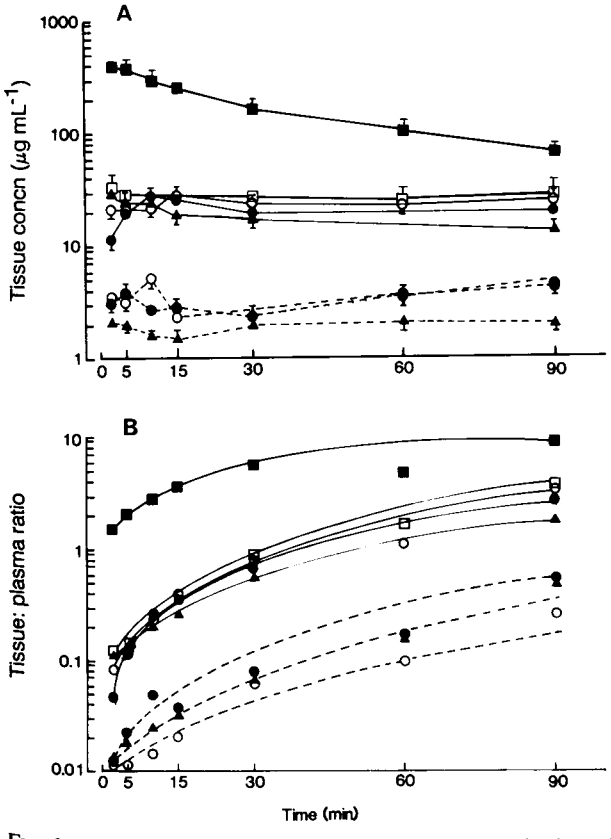

FIG. 3. Time courses of transfer of FMH to tissues. Injection of FMH and measurement of its concentrations in plasma and tissues were as described in the legends to Figs 1, 2. Each point represents the mean for 4–5 experiments. A, tissue FMH concentrations; B, tissue/plasma ratios. (—■—), kidney; (—□—), adrenal glands; (—○—), small intestinal mucosa; (—●—), liver; (—▲—), gastric mucosa; (- - -○- - -), midbrain; (- - -●- - -), cerebellum; (- - -▲- - -), cerebral cortex.

Discussion

Although FMH, a new specific inhibitor of L-histidine decarboxylase (HDC), strongly inhibits histamine formation in-vivo (see introduction), after a single administration of FMH, the decrease of histamine does not persist: the histamine level begins to increase again after 8–12 h and returns to the original level after 24 h (Garbarg et al 1980; Maeyama et al 1982). This is because HDC is induced and histamine is synthesized de-novo. The reason for this short duration of histamine depletion by FMH is not clear, but may be due to rapid clearance of FMH or its low transfer to tissues because it is an amino acid analogue. However, there has been no extensive study on the pharmacokinetics of FMH, and it is necessary to establish a suitable method for its administration to achieve prolonged histamine depletion. In this study we have established an amino acid analyser assay for FMH with a quantitation limit of 0·2 μg mL^{-1}. However, this value is equivalent to 20 μM FMH in tissues, far more than the concentration of FMH which inhibits HDC completely (Kubota et al 1984). To measure lower levels of FMH in tissues, particularly in the brain, a more sensitive method of FMH assay will be necessary.

The finding that the T/P ratio in the kidney reached equilibrium within 30 min after FMH administration and was greater than unity (Fig. 3) suggests that rat kidney has a specific transport mechanism for uptake of FMH.

The present results show that FMH activity is related to its ability to reach and maintain an effective concentration in the tissues. However, further investigations are necessary on the elimination of FMH to establish a suitable method for its administration which will inhibit HDC and deplete tissues of histamine continuously. It will also be interesting to compare the pharmacokinetic parameters of FMH with those of histidine and α-methylhistidine.

Acknowledgements

This work was partially supported by grants-in-aid from the Ministry of Education, Science and Culture of the Japanese Government (#61870012, #63065004).

References

August, T. F., Musson, D. G., Hwang, S. S., Duggan, D. E., Hoodke, K. F., Roman, I. J., Ferguson, R. J., Bayne, W. F. (1985) Bioanalysis and disposition of α-fluoromethylhistidine, a new histidine decarboxylase inhibitor. J. Pharm. Sci. 74: 871–875

Bartholeyns, J., Bouclier, M. (1982) Effect of α-monofluoromethylhistidine, an irreversible inhibitor of histidine decarboxylase, on gestation in mice. Contraception 26: 535–542

Bouclier, M., Jung, M., Gerhart, F. (1983a) Inhibition of histamine biosynthesis and gastric function in the rat: effect on pentagastrin-induced gastric acid secretion. Agents Actions 13: 241–246

Bouclier, M., Jung, M. J., Gerhart, F. (1983b) Effect of prolonged inhibition of histidine decarboxylase on tissue histamine concentrations. Experientia 39: 1303–1305

Bouclier, M., Jung, M. J., Gerhart, F. (1983c) α-Fluoromethylhistidine inhibition of histidine decarboxylase in pylorus ligated rat. Biochem. Pharmacol. 32: 1553–1556

Duggan, D. E., Hooke, K. F., Maycock, A. L. (1984) Inhibition of histamine synthesis in vitro and in vivo by S- α-fluoromethylhistidine. Ibid. 33: 4003–4009

Garbarg, M., Barbin, G., Rodergas, E., Schwartz, J. C. (1980) Inhibition of histamine synthesis in brain by α-fluoromethylhistidine, a new irreversible inhibitor: in vitro and in vivo studies. J. Neurochem. 35: 1045–1052

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. Ibid. 13: 655–669

Granerus, G., Olafsson, J. H., Roupe, G. (1985) Treatment of two mastocytosis patients with a histidine decarboxylase inhibitor. Agents Actions 16: 244–248

Kollonitsch, J., Patchett, A. A., Marburg, S., Maycock, A. L., Perkins, L. M., Douldouras, G. A., Duggan, D. E., Aster, S. D. (1978) Selective inhibitors of biosynthesis of aminergic neurotransmitters. Nature 274: 906–908

Kubota, H., Watanabe, T., Taguchi, Y., Hayashi, H., Wada, H. (1984) The mechanism of the inhibition of mammalian histidine decarboxylase by α-fluoromethylhistidine. Biochem. Pharmacol. 33: 983–990

Maeyama, K., Watanabe, T., Taguchi, Y., Yamatodani, A., Wada, H. (1982) Effect of α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, on histamine levels in mouse tissues. Ibid. 31: 2367–2370

Maeyama, K., Watanabe, T., Yamatodani, A., Taguchi, Y., Kambe, H., Wada, H. (1983) Effect of α-fluoromethylhistidine on the histamine content of the brain of W/W^v mice devoid of mast cells; turnover of brain histamine. J. Neurochem. 41: 128–134

Neittaanmäki, H., Fraki, J. E., Harvima, R. J., Forstrom, L. (1989) α-Fluoromethylhistidine in the treatment of idiopathic cold urticaria. Arch. Dermatol. Res. 281: 99–104

Nishibori, M., Oishi, R., Saeki, K. (1984) Histamine turnover in the brain of different mammalian species. Implications for neuronal histamine half-life. J. Neurochem. 43: 1544–1549

Olafsson, J. H. (1985) Cutaneous and systemic mastocytosis in adults. A clinical, histopathological evaluation in relation to histamine metabolism. Acta Dermato-Venereologica 115 (Suppl.): 1–43

Pipkorn, U., Granerus, G., Proud, D., Kagey-Sobotka, A., Norman, P. S., Lichtenstein, L. M., Naclerio, R. M. (1987) The effect of a histamine synthesis inhibitor on the immediate nasal allergic reaction. Allergy (Copenhagen) 42: 496–501

Slotkin, T. A., Slepetis, R. J., Weigel, S. J., Whitmore, W. L. (1983a) Effects of α-fluoromethylhistidine (FMH), an irreversible inhibitor of histidine decarboxylase, on development of brain histamine and catecholamine system in the neonatal rat. Life Sciences 32: 2897–2903

Slotkin, T. A., Bartolome, J., Whitmore, W. L. (1983b) Developmental effects of α-fluoromethylhistidine, an irreversible inhibitor of histidine decarboxylase, on growth and on levels and turnover of catecholamine. Ibid. 33: 2137–2145

Tung, A. S., Blake, J. T., Roman, I. J., Vlasses, P. H., Ferguson, R. K., Zweerink, H. J. (1985) In vivo and in vitro inhibition of human histidine decarboxylase by (S)- α-fluoromethylhistidine. Biochem. Pharmacol. 34: 3509–3515.

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