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EFFECT ON MAST CELL HISTAMINE OF INHIBITING HISTAMINE FORMATION *IN VIVO* WITH α-FLUOROMETHYLHISTIDINE

DAVID LAGUNOFF*, ALPANA RAY and ALICE RICKARD
Department of Pathology, St. Louis University School of Medicine, St. Louis, MO 63104, U.S.A.

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Abstract—An irreversible inhibitor of histidine decarboxylase, α-fluoromethylhistidine (FMH), was used to inhibit histamine formation by mast cells *in vivo*. Even at doses of FMH sufficient to reduce histamine formation more than 95%, the ability of mast cells to synthesize histamine recovered rapidly. It was possible, however, to sustain levels of histamine-forming activity below 10% of normal with continuous administration of FHM from subcutaneously implanted osmotic pumps. Administration of FHM under these conditions did not deplete significantly mast cell histamine but did prevent the increase in total mast cell histamine that occurs over 14 days and also prevented the reconstitution of mast cell histamine stores after depletion by treatment with polymyxin B.

Histidine decarboxylase is responsible for the generation of the mast cell's complement of histamine [1]. The use of α-fluoromethylhistidine (FMH†), a potent, specific, irreversible inhibitor of the enzyme [2], has made it possible to examine several aspects of mast cell histamine metabolism. Previous work from this laboratory has shown that inactivation of mast cell histidine decarboxylase *in vitro* indirectly inhibits uptake of precursor histidine [3]. In this paper we demonstrate that FHM effectively inhibits mast cell decarboxylation of histidine *in vivo*. Mast cell decarboxylase activity rapidly recovered after a single dose of FHM, but with continuous administration of FHM it was possible to maintain low levels of activity for prolonged periods. Further, we examined the effect of prolonged suppression of histidine decarboxylase on mast cell histamine stores.

MATERIALS AND METHODS

Histidine HCl, histamine HCl, polymyxin B sulfate, and *o*-phthalaldehyde were Sigma products. L-[ring 2, side chain 2,3-^{3}H]Histidine (13.8 Ci/mmole) was purchased from New England Nuclear. α-Fluoromethylhistidine was provided by J. Kollonitsch of Merck, Sharp & Dohme Research Institute. Osmotic pumps were purchased (Alza Corp., Palo Alto, CA) and used according to instructions provided. The 2001 pump delivers 1 μl/hr for 7 days and the 2ML2 pump delivers 5 μl/hr for 14 days. Pumps were implanted subcutaneously in the rat dorsum using a clean, but not sterile, surgical technique under ether anesthesia. Healthy, adult, 6 to 7-week-old male Sprague–Dawley rats free of evidence of pulmonary infection were purchased from Sasco (Omaha, NE) and used in most experiments. Older rats, 6 months to 1 year of age, were used in the experiments on the acute effects of a single injection of FMH.

Cells were collected from the peritoneal cavities of rats after decapitation and exsanguination under ether anesthesia as previously described. Mast cells were isolated using Percoll [3].

FMH was injected into a tail vein or subcutaneously in a volume of 0.30 ml of saline. Polymyxin B in saline was administered either intraperitoneally or via tail vein. Histamine was extracted from peritoneal cells with 2.5% TCA. Extracts of the cells were assayed directly by the OPT method [3]. The significance of differences in histamine content of the total peritoneal cell population was evaluated by Student's *t*-test on the means of groups.

For measurement of histidine decarboxylation, 0.5×10^6 mast cells isolated on Percoll were incubated at 37° in a final volume of 75 μl of BSSA buffer, pH 7.2 (154 mM NaCl, 2.7 mM KCl, 0.68 mM $CaCl_2$, 0.01 M PO_4^{-3} M, 0.5% bovine serum albumin), in the presence of 5 μCi of [^{3}H]histidine for 15 min. Parallel control incubations were performed at 4°. After incubation, the cells were centrifuged and washed three times with 2 ml of BSSA buffer. The resulting cell pellets were extracted with 25 μl of 2.5% TCA. Since mast cells could not be isolated following degranulation in experiments in which polymyxin B was used, total peritoneal cells were used in the assay of histamine formation. We have shown previously that cells other than mast cells make a negligible contribution to the formation of histamine [3].

[^{3}H]Histamine was separated from [^{3}H]histidine in the TCA extracts using either thin-layer chromatography [3] or Biorex 70 (200–400 mesh, Biorad Laboratories, Richmond, CA) columns essentially as described by Lewis and Fennessy [4]. The Biorex 70 was washed several times with distilled water to remove fine particles. It was then suspended in 5 vol.

* Address all correspondence to: David Lagunoff, M.D., Department of Pathology, St. Louis University School of Medicine, 1402 South Grand Blvd., St. Louis, MO 63104, U.S.A.

† Abbreviations: BSS, balanced salt solution; BSSA, BSS with albumin; FMH, α-fluoromethylhistidine; OPT, *o*-phthalaldehyde; and TCA, trichloracetic.

of 1 N NaOH for 2 hr; the pH of the supernatant at this point was 9–10. Following distilled water washes sufficient to lower the pH of the supernatant to 5–6, the resin was suspended in 5 vol. of 1 N HCl for 1 hr. This step was followed by further distilled water washes until the pH of the supernatant again returned to 5–6. The resin was then equilibrated with 0.5 M phosphate buffer, pH 7.5, 0.1% EDTA, and stored at room temperature away from light. Each batch of resin was stored for a maximum of 2 weeks. Glass columns, prepared from short-tipped pasteur pipettes, 0.5 cm internal diameter, were packed with resin to a height of 3.0 cm. Aliquots (10 μl) of TCA extract were mixed with 50 μl of 0.5 M phosphate buffer, pH 7.5, and 1.0 ml containing 1 mg/ml each of histidine and histamine was added. This solution was added to a Biorex 70 column, and the column sequentially eluted with 1 ml × 4 of 0.5 M phosphate buffer, pH 7.5, 1 ml of distilled water, 1 ml of 1 N HCl, and another ml of 1 N HCl. Columns were centrifuged at 600 rpm for 2 min to collect each of the eluates. Histidine appeared in the phosphate buffer wash, whereas histamine was eluted in the HCl. The HCl eluates were combined, and a 250-μl sample was neutralized and counted in 10 ml of Bray's solution or 3a70 (Research Products Inc.). Values for cells incubated at 4° were subtracted from values obtained with the cells maintained at 37°.

RESULTS

The effect on peritoneal mast cell histidine decarboxylation of varying the dose of intravenously administered FMH was assessed. The results of administered doses of FMH from 0.2 to 1.0 mg per 100 g body weight are shown in Fig. 1. The 50% inhibitory dose calculated from the logit plot by linear regression using a least-squares fit is 0.07 mg/100 g. No obvious untoward effects of FMH on the rats were noted in this experiment.

Histidine decarboxylase is irreversibly inhibited by FMH [2], but the activity in a surviving cell can recover by synthesis of new enzyme. Since one objective of the investigation was the modification of histamine stores by the prolonged inhibition of mast cell decarboxylase activity, it was necessary to examine the recovery of enzyme activity in mast cells following extensive inactivation of histidine decarboxylase. An experiment using 0.5 mg FMH/100 g indicated rapid recovery of decarboxylase (Fig. 2). Larger doses resulted in prolonged inhibition, but it was clear that frequently repeated doses of FMH would be necessary for maintaining low levels of the enzyme for a period of weeks. We therefore turned to a method of continuous administration.

Continuous administration of FMH was accomplished with an implanted Alza 2001 osmotic pump [5]. The pumps were charged with 200 mg/ml FMH. Since these pumps release 1 μl/hr, the rate of release calculated for a 450 g rat is 1.07 mg/100 g per day. Prolonged suppression of mast cell histamine formation was achieved with this method (Fig. 3).

The effect of continuous infusion of FMH for 14 days on mast cell histamine was evaluated at a rate of either 0.9 mg/100 g per day or 1.8 mg/100 g per day in experiments using the larger Alza 2ML2 pumps after a priming i.v. dose of 2 mg/100 g. Histamine-forming activity was measured at 14 days (Table 1). Histamine in the total peritoneal cell population was measured at the time FMH was begun and 14 days later. With 1.8 mg/100 g/day of FMH there was a small decrease from the initial control level of mast cell histamine not achieving statistical significance at $P = 0.05$ (Table 2); with 0.9 mg/100 g/day there was a similar small but not significant increase (Table 2). The total cell histamine increase in the peritoneal cavity over 14 days was prevented by both doses of FMH (Table 2).

We also investigated the effect of FMH treatment on the reconstitution of mast cell histamine following secretory depletion of the amine with polymyxin B.

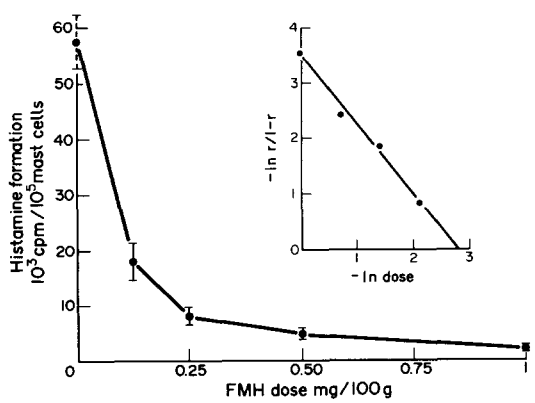

Fig. 1. Effect of FMH on histidine decarboxylation activity to form histamine. FMH was given as a single intravenous dose, and histamine formation was assayed on isolated mast cells after 2 hr. The values are means of four or more separate determinations. Bars represent ± S.E. Insert: Logit plot of data; r = fraction of decarboxylase activity remaining. The 50% inhibitory dose is determined from the intercept of the plotted line with the X axis.

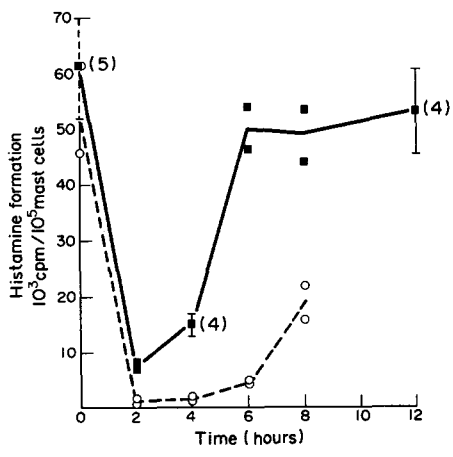

Fig. 2. Time course of inhibition of mast cell histidine decarboxylation by FMH. FMH was administered to rats, and mast cells were isolated at intervals thereafter and assayed for histamine formation. Error bars indicate ± S.E. in those instances for which three or more determinations were made. Key: 0.5 mg FMH/100 g administered intravenously (■—■), and 2.0 mg FMH/100 g administered subcutaneously (○—○).

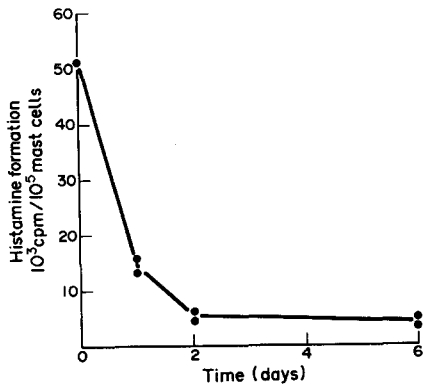

Fig. 3. Time course of inhibition of mast cell histidine decarboxylation by FMH administered continuously. On day 0, continuous administration was begun at a rate of 1.07 mg/100 g/day. Histamine formation was assayed on mast cells isolated from two rats each at 1, 2 and 6 days.

Polymyxin B was administered intraperitoneally in a dose of 200 μg/100 g, at a concentration of 2 mg/ml, 24 hr before starting the continuous infusion of FMH. Only animals that exhibited obvious systemic signs of massive histamine release in the form of transitory acral cyanosis and reversible prostration were used. Histamine content of peritoneal cells was measured 24 hr after polymyxin B treatment and again 14 days later. Reconstitution of histamine to initial levels occurred during this period in animals with intact histamine synthetic activity. In the animals subjected to a priming dose of FMH 24 hr after polymyxin B and continuous infusion for 14 days, the histidine decarboxylase inhibitor effectively suppressed the reconstitution of mast cell histamine (Table 2). Animals treated with FMH for 2 weeks had weight gains comparable to those of untreated animals (Table 3).

DISCUSSION

Kollonitsch and co-workers [2] synthesized FMH as a suicide inhibitor of histidine decarboxylase. Garbarg *et al.* [6] subsequently found it to be an effective inhibitor of brain and stomach histidine decarboxylase *in vivo*. Bauza and Lagunoff [3] showed that FMH effectively inhibits rat mast cell histidine decarboxylase *in vitro*. Maeyama *et al.* [7] tested FMH in mice and found extensive inhibition of skin histidine decarboxylase with an approximate 50% reduction of enzyme activity at a dose of 5 mg/kg; when they administered 25 mg/kg intraperitoneally, skin histidine decarboxylase activity was reduced 90% at 1 hr and did not begin to return until after 8 hr. A single injection had no apparent effect on skin histamine content, in contrast to the significant decrease in histamine in brains and stomachs of the same mice. These results were interpreted as indicating that stable skin mast cell histamine levels were not affected by FMH inhibition of histidine decarboxylase, whereas more rapidly turning over pools of non-mast cell histamine in stomach and brain could be depleted by a single injection of FMH.

When efficacy is compared in terms of 50% inhibition, we find intravenous FMH in rats to be seven times more effective than Maeyama *et al.* found it to be when administered intraperitoneally to mice [7]. We measured activity in isolated peritoneal mast cells; they used skin homogenates. Recovery of activity seemed only slightly faster at comparable doses in rat peritoneal mast cells than in mouse skin. Our experiments using prolonged continuous infusion of FMH for 14 days extend the conclusion of Maeyama that inhibition of mast cell histidine decarboxylase has little effect on mast cell histamine stores. Our experiments indicate essentially no decrease in levels of mast cell histamine from the zero time controls. FMH does, however, prevent the increase in peritoneal cell histamine content that occurs in growing animals [8]. The failure to significantly deplete peritoneal mast cell histamine under conditions of prolonged 90% reduction of histamine formation is consistent with the evidence for slow turnover of rat peritoneal mast cell histamine found by Wingren *et al.* [9]. In their experiments, mast cells were labeled with [^{3}H]histidine and a half-life for the resulting [^{3}H]histamine of 23 days was

Table 1. Effect of 14 days of continuous FMH on histamine formation*

Expt.	Polymyxin B (μg/100 g)	FMH (mg/100 g/day)	Histamine formation (10^3 cpm)
1	0	0	54.8 ± 16 (4)
	200	0	61.2 ± 5.2 (5)
	0	0.9	11.2 ± 2.4 (4)
	200	0.9	9.6 ± 1.6 (4)
2	0	0	42.2 ± 4.4 (6)
	200	0	46.0 ± 2.8 (16)
	0	1.8	3.2 ± 1.2 (7)
	200	1.8	4.4 ± 0.8 (7)

* Polymyxin B was administered intraperitoneally 1 day prior to initiation of FMH treatment. Control rats received BSS. A primary dose of 2 mg/100 g of FMH was given at the time the osmotic pumps were implanted. Fourteen days later total peritoneal cell population was isolated and assayed for the formation of histamine from histidine. Values are given ± S.E. activity.

† Numbers in parentheses indicate the number of individual animals used.

Table 2. Effect of FMH on peritoneal cell histamine*

Day	FMH (mg/100 g/day)	Peritoneal cell histamine (μg) Control	Difference	Poly B	Difference
Expt. 1					
0		13.8 ± 1.6 (4)†		2.7 ± 0.7 (7)	
14	0	26.5 ± 3.0 (5)	12.7	9.1 ± 0.7 (16)	6.4
14	1.8	13.2 ± 1.6 (8)	[−0.6]	2.4 ± 0.4 (8)	[−0.3]
Expt. 2					
0		12.0 ± 1.1 (8)		3.2 ± 0.3 (8)	
14	0	21.5 ± 2.4 (7)	9.5	12.1 ± 0.6 (9)	9.1
14	0.9	14.4 ± 2.5 (7)	2.4	4.6 ± 0.6 (7)	1.4

* Polymyxin B (200 μg/100 g) in BSS was administered intraperitoneally 1 day before FMH treatment was begun. Control animals received BSS. On day 0 and day 14, peritoneal cells were collected and assayed for histamine. Values are means of μg per animal ± S.E. Differences are the mean values at day 14 minus the comparable mean values on day 0. Mean values for animals treated with FMH at 14 days were not significantly different from those for animals assayed on day 0, $P = 0.05$. Mean values for animals on day 14 not treated with FMH were increased significantly over mean values on day 0, $P < 0.05$.

† Numbers in parentheses indicate the number of animals in each group.

found. If a similar exponential loss of histamine occurred in the presence of FMH, we would expect a 35% decrease in histamine in 14 days. Two possibilities could account for our failure to observe a decrease of such magnitude: (1) the low levels of remaining histidine decarboxylase were sufficient to replace the loss of histamine, or (2) the continuing synthesis of histamine contributed to the turnover of histamine in the animals with normal levels of the decarboxylase so that inhibition of histidine decarboxylase extended the half-life of mast cell histamine. The latter proposal is testable by measuring the half-life of histamine under conditions of histidine decarboxylase inhibition by FMH. Consistent with the latter suggestion is the observation from Enerback's laboratory that a forced increase in serotonin uptake into the granule causes a corresponding reduction in granule histamine [9], perhaps by displacement of histamine.

Based on morphological studies of the restoration of mast cells after polymyxin B induced degranulation, Kruger and Lagunoff [10] have proposed that resident peritoneal mast cells which had released their granules were responsible for the regeneration of a normally granulated population of mast cells. The control level of mast cell numbers was restored 2 weeks after depletion with polymyxin B, but the histamine content of the cells at that time had only returned to 50% of normal. In the present experiment, we have used a single intraperitoneal dose of polymyxin B rather than the 3 doses used in the earlier experiments [10] and achieved a reduction of 75% or more of the histamine content. In the absence of inhibitor, the original levels of histamine were restored in 14 days; however, because of continued synthesis in inhibited cells, histamine in the polymyxin B treated animals did not reach the levels found in the control rats at this time. Continuous infusion of FMH largely prevented the restoration of histamine levels, consistent with the effectiveness of FMH in preventing any increase of histamine content of normal mast cells over the same period.

Bouclier *et al.* [11] have administered 12.5 mg/100 g/day of FMH for 21 days with osmotic pumps. They found significant decreases in histamine content in all organs they examined: hypothalmus, gastric mucosa, spleen, lung, thymus, liver and heart. The decrease in tissue histamine was greatest in the hypothalmus (94%), and least in the heart (38%). Their studies did not include any estimation of the extent

Table 3. Effect of FMH on weight gain on rats*

Treatment	Rat weight increment over 14 days (g) FMH (0.9 mg/100 g/day)	FMH (1.8 mg/100 g/day)
BSS	98 ± 3.7 (7)	74 ± 6.2 (5)
FMH	100 ± 3.2 (6)	77 ± 9.6 (8)
Poly B	74 ± 5.8 (9)	75 ± 4.2 (13)
Poly B + FMH	86 ± 6.1 (7)	66 ± 7.1 (9)

* Each rat was weighed prior to any treatment and again after 14 days. The values in parentheses are the number of rats. The other values are the means of the weight gains ± S.E. for each group. None of the differences between the means for the weight changes for the groups with and without FMH (BSS vs FMH; Poly B vs Poly B + FMH) were significant at $P = 0.10$ by Student's two-tailed *t*-test.

of increase in histamine in the various tissues over the time course of their experiment in the absence of FMH.

Our results indicate that FMH under conditions of continuous infusion can effectively inhibit histamine synthesis by rat peritoneal mast cells. Depletion of mast cell histamine stores with subsequent inhibition of new synthesis provides a potentially useful method for studying the role of mast cell histamine in rats under a variety of circumstances. It is also interesting to speculate on the potential usefulness of FMH as a drug in the treatment of histamine-dependent hypersensitivities. Our results suggest that, while it may not be feasible to deplete histamine stores with inhibition of histidine decarboxylase prior to the release of histamine, FMH might be useful in preventing the resynthesis of normal histamine stores in those circumstances in which patients release substantial amounts of histamine.

With respect to possible deleterious effects of FMH on CNS function or gastric secretion, neither Bouclier *et al.* [11] nor we observed any obvious deleterious effects of prolonged continuous infusion of FMH, and the rats gained weight at a rate comparable to that of untreated controls.

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