

Original Article

**Effects of Histidine and Dexamethasone on the Local Inflammation  
Induced by Histamine in Rats**

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**Abstract**

In this study, the effects of separate and combined intraperitoneal (IP) injections of histidine and dexamethasone were investigated on local inflammation in rats. Local inflammation was induced by subcutaneous (SC) injection of histamine (100  $\mu$ l, 0.1%) in ventral surface of right hind paw. The thickness of paw was measured at 30 min before and 30, 60, 90, 120, 150 and 180 min after injection of histamine, using a fine caliper. The number of neutrophils in paw tissue sections was counted 3 h after intraplantar (IPL) injection of histamine. The IPL injected histamine elicited an inflammatory response that was characterized by increase of paw thickness and by infiltration of neutrophils in paw tissues. IP injections of histidine at doses of 200 and 400 mg kg<sup>-1</sup> and dexamethasone at a dose of 1 mg kg<sup>-1</sup> significantly ( $P < 0.05$ ) decreased both paw thickness and infiltration of neutrophils in paw tissues. In combined treatment, IP injection of histidine (200 mg kg<sup>-1</sup>) with dexamethasone (1 mg kg<sup>-1</sup>) produced a more documented response in comparison with histidine and dexamethasone used alone. The results suggested that histidine and dexamethasone have anti-inflammatory activities. Histidine potentiated the anti-inflammatory effect of dexamethasone in histamine-induced local inflammation.

**Key words:** Histidine, Dexamethasone, Histamine, Inflammation, Rats

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## Introduction

Histamine is a biogenic amine and through its specific membrane receptors, H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub> and H<sub>4</sub>, plays an important role in physiological and pathological processes such as gastric acid secretion, smooth muscle contraction, neurotransmission, immunomodulation, angiogenesis and allergic disorders.<sup>1</sup> Histamine involves in the local inflammatory responses by activation of vasodilation, vascular permeability, edema formation, polymorphonuclear leukocyte infiltration and cytokine release.<sup>2-5</sup>

Some amino acids including leucine, isoleucine, lysine, threonine, glutamine, phenylalanine, tryptophane, valine, glycine, arginine, cysteine and taurine have been reported to have beneficial effects in treatments of acute and chronic infections, burn, trauma, sepsis, colitis and inflammatory bowel diseases.<sup>6-11</sup> Histidine, as a semiessential amino acid, has many biological functions. Histidine diminished the activity and mRNA expression of malic enzyme and fatty acid synthase and lowered body weight and hepatic triglyceride and cholesterol levels in mice consumed high saturated fat diet.<sup>12</sup> In addition, the acetic acid-induced edema and leukocyte infiltration in the colon of rats was attenuated by intraperitoneal injection of histidine.<sup>6</sup> Moreover, Farshid *et al.*<sup>7</sup> reported an anti-inflammatory effect of histidine in the carrageenan-induced paw inflammation in rats.

Dexamethasone, a well known steroidal anti-inflammatory drug, influences some functions of histamine including inhibition of histamine synthesis and release in nasal mucosa and lungs in rats.<sup>13,14</sup>

The present study was aimed to evaluate the effects of histidine and dexamethasone in separate and combined treatments on the local inflammation induced by SC injection of histamine in the ventral surface of the hind paw in rats.

## Materials and Methods

**Animals.** Healthy adult male Wistar rats, weighing 220–250 g were used in this study. Rats were maintained in polyethylene cages with food and water available ad libitum, in a laboratory with controlled ambient temperature ( $23 \pm 0.5^\circ$  C) and under a 12 h light-dark cycle (lights on from 07:00am). Six rats were used in each experiment. The experimental protocol was approved by the Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University.

**Drugs.** Drugs used in the present study included histamine dihydrochloride, histidine monohydrochloride (Merck Chemical Company, Darmstadt, Germany) and dexamethasone (Daroopaksh Co. Ltd, Iran). All drugs were dissolved in sterile normal saline 30 min before injection.

**Treatment groups.** Rats were divided into 6 groups of 6 animals in each group. Group I received IP injection of normal saline followed by IPL injection of histamine (100  $\mu$ l, 0.1 %). This group was served as the control group. In groups II, III and IV, IPL injection of histamine was performed after IP injections of histidine at doses of 100, 200 and 400 mg kg<sup>-1</sup>, respectively. Group V received IP injection of dexamethasone at a dose of 1 mg kg<sup>-1</sup> before IPL injection of histamine. Group VI treated with histidine (200 mg kg<sup>-1</sup>, IP) plus dexamethasone (1 mg kg<sup>-1</sup>, IP) before IPL injection of histamine. Histidine and dexamethasone were IP injected 45 and 30 min before IPL injection of histamine, respectively. The drug doses used here were selected according to our previous experiments and works done by other investigators in which the doses of histidine and dexamethasone were 50-200 mg kg<sup>-1</sup> and 0.5-3 mg kg<sup>-1</sup>, respectively.<sup>6,7,15,16</sup>

**Induction of paw inflammation.** Paw inflammation was induced by SC injection of 100  $\mu$ l histamine 0.1 % in the ventral surface of the right hind paw using a 29-gauge injection needle.<sup>2,4,16</sup> The magnitude

of paw edema was assessed by measuring dorsal-plantar paw thickness of the histamine injected paw with a fine caliper,<sup>7,17,18</sup> at 30 min before and 30, 60, 90, 120, 150 and 180 min after IPL injection of histamine. Percent change in paw thickness was then calculated using the following formula: [(thickness of the paw after injection – thickness of the paw before injection)/ thickness of the paw before injection] 100×.<sup>18</sup>

**Tissue collection and histopathology.** For histopathological evaluation of paw tissues, 3 h after IPL injection of histamine the animals were euthanized with a high dose of ether and then decapitated. Their paw tissues were collected for histopathological investigation. The specimens were fixed in 10 % buffer formal saline and were routinely processed for paraffin embedding. For each sample, 4-5 µm thick sections were cut and stained with hematoxylin-eosin to evaluate the acute inflammation. Neutrophils were counted by special morphometric lens device in 0.25 mm<sup>2</sup> microscopic field, from 10 different areas of the sections and the mean values were calculated. The final number of neutrophils was expressed as the mean of the number counted in six animals per group.

**Statistical analysis.** Paw thickness changes were analyzed by factorial

analysis of variance (ANOVA) followed by Duncan’s test. Statistical analysis of the number of neutrophils was performed by one-way analysis of variance (ANOVA) and Duncan’s test. Data are expressed as mean ± SEM. The probability of *P* < 0.05 was considered to show significant differences for all comparisons.

**Results**

The SC injection of histamine (control) into the plantar surface of the right hind paw evoked a local edema with maximal rate detected within 30 and 60 min after injection and thereafter declined to the end of the experiment. Histidine (100 mg kg<sup>-1</sup>, IP) was without significant effect, whereas at doses of 200 and 400 mg kg<sup>-1</sup>, histidine significantly (*P* < 0.05) decreased post-injection paw thickness induced by histamine (Table 1).

The IP injection of dexamethasone at a dose of 1 mg kg<sup>-1</sup> significantly (*P* < 0.05) reduced paw edema induced by histamine. The suppressive effect induced by co-administration of histidine (200 mg kg<sup>-1</sup>, IP) with dexamethasone (1 mg kg<sup>-1</sup>, IP) on paw edema were significantly (*P* < 0.05) more than those obtained from histidine (200 mg kg<sup>-1</sup>, IP) and dexamethasone (1 mg kg<sup>-1</sup>, IP) used alone (Table 1).

**Table 1.** Effects of histidine and dexamethasone on percent changes of paw thickness induced by intraplantar injection of histamine in rats (Mean ± SEM).

Treatments	30min	60min	90min	120min	150min	180min
Control (histamine, 0.1%, IPL)	37.4 ± 2.8	32.8 ± 3.1	24.1 ± 2.4	20.9 ± 1.9	20.2 ± 3.0	18.0 ± 3.2
Histidine (100mg kg <sup>-1</sup> , IP)	32.7 ± 2.6	26.4 ± 4.3	20.7 ± 3.0	15.5 ± 3.5	14.0 ± 2.7	15.2 ± 2.1
Histidine (200mg kg <sup>-1</sup> , IP)	34.2 ± 4.0	24.4 ± 2.2*	15.9 ± 2.4*	13.8 ± 2.7*	9.1 ± 2.3*	7.5 ± 1.7*
Histidine (400mg kg <sup>-1</sup> , IP)	25.4 ± 4.1*	18.9 ± 1.5*	14.4 ± 2.4*	11.5 ± 3.0*	6.9 ± 2.0*	5.9 ± 1.9*
Dexamethasone (1 mg kg <sup>-1</sup> , IP)	24.7 ± 2.2*	16.2 ± 1.3*	13.4 ± 2.1*	8.7 ± 1.1*	6.6 ± 1.6*	6.5 ± 1.3*
Histidine (200 mg kg <sup>-1</sup> , IP) + Dexamethasone (1mg kg <sup>-1</sup> , IP)	21.9 ± 2.7*	10.6 ± 2.3*†	8.0 ± 2.6*†	5.1 ± 1.2*†	2.2 ± 1.4*†	2.2 ± 1.5*†

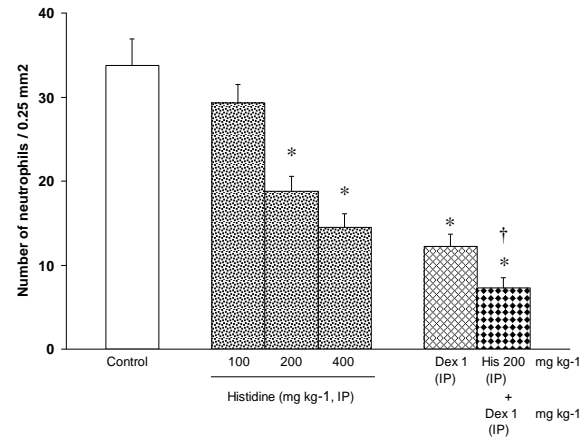
Values are expressed as mean ± SEM, \**P* < 0.05 significantly different from control (histamine 0.1 %) group, †*P* < 0.05 significantly different from histidine (200 mg kg<sup>-1</sup>) and dexamethasone (1 mg kg<sup>-1</sup>) groups, IPL: intraplantar, IP: intraperitoneal, n: 6 rats for each group. Percent change in paw thickness was calculated using the following formula: [(thickness of the paw after injection – thickness of the paw before injection)/ thickness of the paw before injection] 100×.<sup>18, 19</sup>

Histopathological leukocytic infiltration mainly neutrophils was observed in the inflamed area. As presented in figure 1 and showed in figure 2A, the number of neutrophils was highest ( $33.8 \pm 2.4$ ) in the IPL histamine injected (control) group. The IP injection of histidine at a dose of  $100 \text{ mg kg}^{-1}$  did not bring about significant change in the number of neutrophils in inflamed area (Figure 1 and figure 2B), whereas at doses of 200 and  $400 \text{ mg kg}^{-1}$ , histidine significantly ( $P < 0.05$ ) decreased neutrophil infiltration (Figure 1 and figures 2C and 2D). Dexamethasone ( $1 \text{ mg kg}^{-1}$ , IP) significantly ( $P < 0.05$ ) decreased neutrophil infiltration (Fig 1 and Fig 2E). The suppressive effect of histidine ( $200 \text{ mg kg}^{-1}$ , IP) plus dexamethasone ( $1 \text{ mg kg}^{-1}$ , IP) on neutrophil infiltration was significantly ( $P < 0.05$ ) more than those obtained from histidine ( $200 \text{ mg kg}^{-1}$ , IP) and dexamethasone ( $1 \text{ mg kg}^{-1}$ , IP) used alone (Fig. 1 and Fig. 2F).

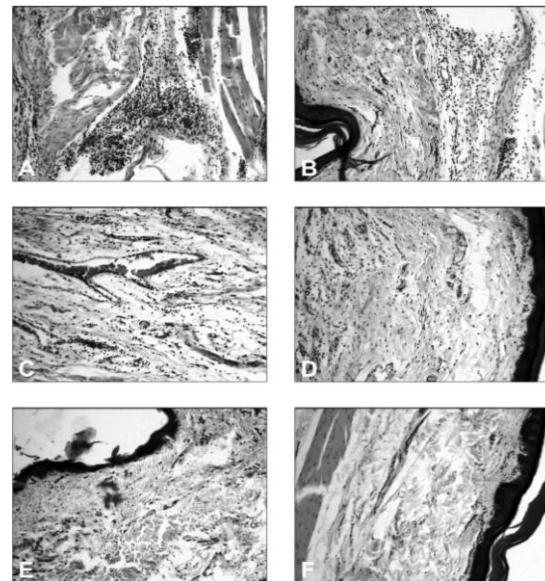
## Discussion

In this study, IPL injection of histamine produced paw edema as well as neutrophil infiltration in the paw tissue. Histamine-induced inflammation has been well established as a valid model to study paw oedema and neutrophil infiltration in paw tissue after inflammatory states.<sup>2,4</sup> It has been reported that transtympanic injection of histamine produces edema, vascular dilation and congestion, neutrophil infiltration and presence of eosinophils in the middle-ear mucosa in rabbits.<sup>3</sup> Several reports have confirmed that histamine alone and in participation with chemoattractants such as platelet activating factor, interleukin 8 and leukotriene B<sub>4</sub>, involves in the regulation of neutrophil recruitment.<sup>19,20</sup> The results of present study indicated that histidine showed an anti-inflammatory activity against development of edema and neutrophil infiltration induced by

histamine in the rat paw. Although histidine is a precursor of histamine,<sup>13</sup> in some biological events, histidine and histamine exert opposite effects.



**Fig 1.** Effects of intraperitoneal injections of histidine and dexamethasone on the number of neutrophils in the paw tissue 3h after intraplantar injection of histamine in rats. \* $P < 0.05$  as compared with control and histidine ( $100 \text{ mg kg}^{-1}$ ) groups, †  $P < 0.05$  as compared with histidine ( $200 \text{ mg kg}^{-1}$ ) and dexamethasone ( $1 \text{ mg kg}^{-1}$ ) groups, n: 6 rats for each group.



**Fig 2.** Histopathological localization of neutrophils in the rat paw. (A) Control group (only histamine, 0.1 %), this group showed significant neutrophil infiltration; (B) histidine  $100 \text{ mg kg}^{-1}$  + histamine 0.1 %; (C) histidine  $200 \text{ mg kg}^{-1}$  + histamine 0.1 %; (D) histidine  $400 \text{ mg kg}^{-1}$  + histamine 0.1%; (E) dexamethasone  $1 \text{ mg kg}^{-1}$  + histamine 0.1 % (F), histidine  $200 \text{ mg kg}^{-1}$  + dexamethasone  $1 \text{ mg kg}^{-1}$  + histamine 0.1 % (H&E 100×).

The edematogenic effect of histamine after IPL injection of the amine was reported in rats,<sup>2,4</sup> whereas brain edema induced by cryogenic surgery and acute liver failure were attenuated by L-histidine in rats.<sup>21,22</sup> Moreover, the role of tumor necrosis factor- $\alpha$  in histamine-induced paw edema was reported in rats.<sup>5</sup> On the other hand, histidine diminished acetaminophen-induced elevation of tumor necrosis factor- $\alpha$  in mice.<sup>23</sup> Histidine has a potent antioxidant property<sup>24</sup>, on the other hand, histamine and histamine-activated neutrophils can trigger the production of oxygen-derived free radicals.<sup>25</sup> In the present study, dexamethasone produced an anti-inflammatory effect by reducing histamine-induced paw edema and neutrophil infiltration in paw tissues. Histamine is produced by the decarboxylation of L-histidine via histidine decarboxylase.<sup>13,14</sup> It was reported that dexamethasone suppressed synthesis of histamine by repressing both transcription and activity of histidine decarboxylase in toluene diisocyanate-induced nasal allergy in rats.<sup>13,14</sup> Moreover, dexamethasone inhibited histamine-induced edema, vascular dilation and neutrophil infiltration in a rabbit model of middle-ear mucosal inflammation.<sup>3</sup> On the other hand, nontoxic natural antioxidants such as tannic acid, melatonin and ascorbic acid prolonged the suppressive effect of dexamethasone in both ischemic- and histamine-induced paw edema in mice.<sup>15</sup>

In the present study, a documented anti-inflammatory effect was observed when effective doses of histidine (200 mg kg<sup>-1</sup>) and dexamethasone (1 mg kg<sup>-1</sup>) were co-administered. This indicates that a potentiation effect may exist between histidine and dexamethasone in producing anti-inflammatory effects in histamine-induced paw inflammation in rats. There is not any report describing the direct effect of histidine on the anti-inflammatory property of dexamethasone. Therefore, the results presented here could be the first

report showing the effect of histidine on histamine-induced paw edema as well as the potentiation effect of histidine on the anti-inflammatory property of dexamethasone.

In conclusion, the present results showed that histidine and dexamethasone produced anti-inflammatory effects by reducing both edema and neutrophil infiltration in histamine-induced local paw inflammation. Moreover, histidine potentiated the anti-inflammatory effect of dexamethasone.

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