Effects of histidine and N-acetylcysteine on diclofenac-induced anti-inflammatory response in acute inflammation in rats

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Received 16 February 2010; revised 21 June 2010

The intra-plantar injection of carrageenan elicited an inflammatory response characterized by increase of the paw thickness and infiltration of neutrophils in paw tissues. Histidine, n-acetylcysteine and diclofenac decreased paw thickness, and neutrophil infiltration in the paw tissues. The anti-inflammatory effect induced by co-administration of histidine and n-acetylcysteine with diclofenac, was more than that obtained from histidine and n-acetylcysteine administered alone. The results suggested that histidine, n-acetylcysteine and diclofenac produced anti-inflammatory activities by reducing paw edema and neutrophil infiltration induced by carrageenan. Inhibition of cyclooxygenase products such as prostaglandins may be involved in the anti-inflammatory effects induced by histidine and n-acetylcysteine.

Keywords: Carrageenan, Histidine, N-acetylcysteine, Neutrophil infiltration, Paw edema

Several experimental models of acute inflammation have been described after sub-plantar injection of inflammatory agents. The intra-plantar injections of histamine, serotonin, prostaglandin E2 and formalin produced paw edema and local neutrophil infiltration in rats14. Carrageenan, as an inflammatory agent(162,907),(839,995), have been frequently used for induction of local inflammation for identifying the mechanisms involved in the local inflammatory responses including vascular permeability, paw edema, neutrophil infiltration and production of free radicals, cytokines and prostaglandins as well as to explore the mechanism of anti-inflammatory agents5,7.

The role of some amino acid including histidine, leucine, isoleucine lysine, threonine, glutamine, phenylalanine, tryptophane and valine in the inflammatory reactions induced by colitis, acute and chronic infections, burn, trauma and sepsis is well established8-10. The amino acids such as tryptophane, phenylalanine, alanine, cysteine, hydroxyproline and tyrosine reduced gelatin-induced inflammation in rats11. The anti-inflammatory effects of aspartic acid, alanine, glycine and methylguanidine in the carrageenan-induced paw inflammation have been reported in rats12,13.

Histidine is one of the most common natural amino acids, and has many biological functions. Intraperitoneal injection and intestinal luminal administration of histidine produced anti-secretory activity by reducing the amount of fluid accumulating in the intestinal lumen14. Histidine also induced an anti-inflammatory activity by protecting the intestinal tissue from Salmonella typhimurium-induced damage15. Histidine attenuated the histopathological changes in the colon of acetic acid-induced acute colitis in rats8. N-acetylcysteine, the acetylated variant of the amino acid L-cysteine, exerts many functions in the body such as anti-oxidant activity15. N-acetylcysteine reduced leukocyte infiltration induced by carrageenan in the air pouch model of inflammation in rats16.

The cyclooxygenase products such as prostaglandins have many roles in the carrageenan-induced inflammation17,18. Diclofenac, as a non-steroidal anti-inflammatory drug, inhibits cyclooxygenase and is commonly used in inflammatory models as a reference drug19,20.

In the present study, the effects of intraperitoneal injection of histidine and n-acetylcysteine have been investigated on the paw edema and neutrophil infiltration in the paw tissues induced by intra-plantar injection of carrageenan in rats. Moreover, the effects of histidine and n-acetylcysteine on the anti-inflammatory response induced by diclofenac, as a reference drug, are also examined.
Materials and Methods

Animals—Healthy adult male Wistar rats, weighing 230–250 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 (23º ± 0.5°C) and under a 12:12 h light-dark cycle (lights on from 07:00 h). Six rats were used in each experiment. The experimental protocol was approved by the Veterinary Ethics Committee of Faculty of Veterinary Medicine of Urmia University.

Drugs—The following drugs were administered: carrageenan, histidine monohydrochloride, n-acetylcysteine and diclofenac sodium. The drugs were purchased from Merck Chemical Company, Darmstadt, Germany. All drugs were dissolved and diluted in normal saline.

Treatment groups—Rats were divided into following 12 groups of 6 animals each. Group I treated with ip injection of normal saline followed by intra-plantar injection of 2% carrageenan. In groups II and III, intra-plantar injection of carrageenan was performed 30 min after ip injection of histidine at doses of 100 and 200 mg/kg, Groups IV and V treated with ip injection of n-acetylcysteine at doses of 100 and 200 mg/kg 30 min before intra-plantar injection of carrageenan. Groups VI, VII and VIII received ip injection of diclofenac at doses of 5, 10 and 20 mg/kg 20 min before intra-plantar injection of carrageenan. In groups IX and X, intra-plantar injection of carrageenan was performed 30 min after ip injection of histidine and n-acetylcysteine at the same dose of 100 mg/kg and 20 min after ip injection of diclofenac (5 mg/kg). Groups XI and XII received ip injection of histidine and n-acetylcysteine at the same dose of 200 mg/kg plus ip injection of diclofenac at a dose of 10 mg/kg, 30 and 20 min before intra-plantar injection of carrageenan. The drug doses used here were selected according to our previous experiments and were closer to other investigations in which the used doses of histidine, n-acetylcysteine and diclofenac were 50-200 mg/kg, 50-200 mg/kg and 5-25 mg/kg, respectively.

Induction of paw edema—For induction of paw edema, each rat was subcutaneously injected with 100 µl carrageenan (2%) in the ventral surface of the right hind paw using a 26-gauge injection needle and was then returned to its cage. The magnitude of paw edema was assessed by measuring the dorsal-plantar paw thickness with a fine caliper, at 1 h before and 1, 2, 3, 4 and 5 h after carrageenan injection. Edema was expressed as the increase in paw thickness (mm) after carrageenan injection relative to the pre-injection value for each animal.

Histopathological evaluation—For histopathological evaluation of paw tissues, the animals were euthanized by decapitation 5 h after carrageenan injection, and their paw tissues were collected for histopathological investigation. The specimens were fixed in 10% buffer formal saline and 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 mm² 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 6 animals per group.

Statistical analysis—Values reported are the mean±SE. Statistical analysis was performed by repeated measure analysis of variance (ANOVA) and Duncan’s test for the data obtained from the paw edema. Data obtained from the neutrophil infiltration were analyzed by one-way analysis of variance (ANOVA) followed by Duncan’s test. The significance level was expressed as P<0.05.

Results

Intra-plantar injection of normal saline produced no edema in the paw. Injection of carrageenan into the plantar surface of hind paw evoked a local edema with maximal rate detected within 2 h after injection and thereafter declined to the end of the experiment. Histidine and n-acetylcysteine at the same dose of 100 mg/kg had no effect, whereas at the same dose of 200 mg/kg, histidine and n-acetylcysteine significantly (P<0.05) decreased 2, 3, 4 and 5 h of paw thickness induced by carrageenan. N-acetylcysteine (200 mg/kg) also significantly (P<0.05) suppressed 1 h post-carrageenan-induced inflammation (Fig. 1).

Diclofenac at a dose of 5 mg/kg produced no significant effect. Two and 4 h after carrageenan-induced paw edema was significantly (P<0.05) suppressed with 10 mg/kg of diclofenac, and at a dose of 20 mg/kg diclofenac significantly (P<0.05) decreased the paw thickness induced by carrageenan on 1, 2, 3, 4 and 5 h following the induction of inflammation (Fig. 2).
Co-administration of histidine and n-acetylcysteine at the same dose of 100 mg/kg with diclofenac (5 mg/kg) significantly ($P < 0.05$) decreased the paw thickness induced by carrageenan on 2 and 3 h following the induction of inflammation when compared with both control and diclofenac (5 mg/kg) used alone (Fig. 3). Co-administration of histidine and n-acetylcysteine at the same dose of 200 mg/kg with diclofenac (10 mg/kg) significantly ($P < 0.05$) decreased paw thickness when compared with diclofenac (10 mg/kg) used alone (Fig. 4).

Histopathologically, congestion, edema, haemorrhages and leukocytic infiltration, mainly neutrophils were observed in the inflammed area. As presented in figure 5 and showed in figure 7a, the number of neutrophils was the highest ($68.4 \pm 3.5$) in the intra-plantar carrageenan injected (control) group. Intrapertitoneal injection of histidine at a dose of 200 mg/kg, but not at a dose of 100 mg/kg, significantly ($P < 0.05$) decreased the number of neutrophils in the inflammed area (Fig. 5, Fig. 7b and Fig. 7c). N-acetylcysteine at a dose of 200 mg/kg, but not at a dose of 100 mg/kg, significantly ($P < 0.05$) decreased the neutrophil infiltration (Fig. 5, Fig 7d and Fig 7e). Diclofenac at a dose of 20 mg/kg, but not at doses of 5 and 10 mg/kg, significantly ($P < 0.05$) lowered the number of neutrophils (Fig. 5, Fig 7f, Fig. 7g and Fig. 7h). Co-administration of n-acetylcysteine, but not
histidine, at the same dose of 100 mg/kg with diclofenac (5 mg/kg) significantly \((P < 0.05)\) decreased the number of neutrophils in inflamed area when compared with control (Fig. 6, Fig 7i and Fig 7j). Co-administration of histidine and n-acetylcysteine at the same dose of 200 mg/kg with diclofenac (10 mg/kg) significantly \((P < 0.05)\) decreased the number of neutrophils in inflamed area when compared with diclofenac 10 mg/kg used alone (Fig. 6, Fig 7k and Fig 7l).

**Discussion**

In this study, intra-plantar injection of carrageenan (100 µl, 2%) produced a local edema initiated 1 h after injection, reached to its maximum rate at 2 h post injection and then declined to the end of the experiment. In addition, carrageenan caused neutrophil infiltration in the paw tissue. Intra-plantar injection of carrageenan produces a biphasic (an early phase: up to 1 h to 2 h and a late phase: 2 h to 6 h) pattern in the paw edema\(^5,19,25,26\). Carrageenan-induced paw inflammation is a useful model to assess the contribution of inflammatory mediators involved in vascular changes and neutrophil infiltration associated with an acute inflammatory response\(^{29}\). One of the consequences of acute inflammation is the leakage of plasma elements from blood vessels to the inflamed tissue and the infiltration of neutrophils\(^{3,4}\). Histamine, serotonin, bradykinin, prostaglandins, hydrogen sulfide and nitric oxide are involved in the carrageenan-induced local paw inflammatory reaction\(^{7,29-31}\).

In the present study, histidine and n-acetylcysteine reduced both paw edema and neutrophil infiltration in the paw tissues induced by carrageenan. L-histidine, but not D-histidine, attenuates brain edema induced by cryogenic surgery in rats\(^{32}\). In rats treated with histidine after induction of focal thrombotic cerebral ischemia, the brain water (brain edema) was decreased\(^{33}\). Intrapertoneal injection of histidine produced antinociception in an inflammatory model of pain in mice\(^{34}\). N-acetylcysteine attenuated both fluid accumulation in the pleural cavity and infiltration of neutrophils in lung tissues induced by injection of carrageenan into the pleural cavity\(^{35}\). In addition, n-acetylcysteine reduced leukocyte infiltration induced by carrageenan in the air pouch model of inflammation in rats\(^{16}\). However, in the present study histidine and n-acetylcysteine produced anti-inflammatory activities by reducing edema and neutrophil infiltration.

The results of the present study clearly indicate that diclofenac suppressed carrageenan-induced paw inflammation. In addition, both histidine and n-acetylcysteine increased the anti-inflammatory activity of diclofenac. Diclofenac and other anti-
inflammatory drugs such as indomethacin and celecoxib have abilities to inhibit cyclooxygenase, and were frequently used as reference drugs in studying the anti-inflammatory and analgesic mechanisms of drugs and plant extracts\textsuperscript{4,17-19,36}. The major products of cyclooxygenase are prostaglandins\textsuperscript{17}. Prostaglandins are important mediators of acute inflammation\textsuperscript{17}, and the involvement of prostaglandin E\textsubscript{2} has been reported in the carrageenan-induced inflammation\textsuperscript{36}. Administration of L-histidine reduced the net secretion of the small intestine of mice challenged with cholera toxin and reduced the capacity of prostaglandin E\textsubscript{2} to stimulate Na\textsuperscript{+} transport\textsuperscript{17}. The anti-rheumatic property of amino acid L-histidine at a high dose is related to its inhibitory effect on the synthesis of prostaglandins\textsuperscript{38}. On the other hand, N-acetylcysteine enhanced the action of diclofenac and rofecoxib in suppressing of prostaglandin E\textsubscript{2} formation in monocytes\textsuperscript{39}. Therefore, it seems that the enhanced effects of histidine and N-acetylcysteine on the anti-inflammatory action of diclofenac observed in the present study may be associated to the abilities of histidine and N-acetylcysteine in suppressing of prostaglandins production.

The administrations of the high dose of non-steroidal anti-inflammatory drugs produce several adverse reactions, primarily gastrointestinal toxicity such as hemorrhages and ulcerations\textsuperscript{40}. One might expect to eliminate this toxicity by a strategy, which provides an effective treatment with a dose of non-steroidal anti-inflammatory drugs as low as possible. In the present study, ineffective dose (100 mg/kg) of

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**Fig. 7**—Effects of histidine, N-acetylcysteine and diclofenac on neutrophil infiltration induced by carrageenan in rat paw tissues. Animals were treated with: (a) normal saline + carrageenan, (b) histidine (100 mg/kg) + carrageenan, (c) histidine (200 mg/kg) + carrageenan, (d) N-acetylcysteine (100 mg/kg) + carrageenan, (e) N-acetylcysteine (200 mg/kg) + carrageenan, (f) diclofenac (5 mg/kg) + carrageenan, (g) diclofenac (10 mg/kg) + carrageenan, (h) diclofenac (20 mg/kg) + carrageenan, (i) histidine (100 mg/kg) + diclofenac (5 mg/kg) + carrageenan, (j) N-acetylcysteine (100 mg/kg) + diclofenac (5 mg/kg) + carrageenan, (k) histidine (200 mg/kg) + diclofenac (10 mg/kg) + carrageenan, (l) N-acetylcysteine (200 mg/kg) + diclofenac (10 mg/kg) + carrageenan. Extensive neutrophil infiltrations are seen in a, b, d, f and i. Moderate neutrophil infiltrations are seen in c, e and j. Mild neutrophil infiltrations are seen in g, h, k and l (H&E X 100)
n-acetylcysteine, but not histidine, produced an anti-edematogenic activity when used with ineffective dose (5 mg/kg) of diclofenac. On the other hand, effective dose (200 mg/kg) of both histidine and n-acetylcysteine increased the anti-inflammatory activity of 10 mg/kg diclofenac. In other words, the anti-inflammatory effects produced by histidine and especially n-acetylcysteine with diclofenac in combined treatments were more documented than those obtained from separate treatments with histidine, n-acetylcysteine and diclofenac. Moreover, n-acetylcysteine reached the anti-inflammatory effect of diclofenac 10 mg/kg to that obtained from 20 mg/kg diclofenac. These indicate that the synergistic and additive effects may exist between histidine and n-acetylcysteine with diclofenac in producing anti-inflammatory effects in carrageenan-induced paw inflammation in rats.

In conclusion, both histidine and n-acetylcysteine either macroscopically or histopathologically produced anti-inflammatory effects in the carrageenan-induced acute inflammation. In addition, histidine and especially n-acetylcysteine enhanced the anti-inflammatory activity of diclofenac in carrageenan-induced acute inflammation. The inhibition of cyclooxygenase may be involved in the anti-inflammatory effects of histidine and n-acetylcysteine.

References
6 Kumar P P & Kutan G, *Vernonia cinerea* L. scavenges free radicals and regulates nitric oxide and proinflammatory cytokines profile in carrageenan-induced paw edema model, **Immunopharmacol Immunotoxicol**, 31 (2009) 94.
11 Meyers B E, Moonka D K & Davis R H, The effect of selected amino acids on gelatin-induced inflammation in adult male mice, **Inflammation**, 3 (1979) 225.


27 Cannon K E, Leurs R & Hough L B, Activation of peripheral and spinal histamine H3 receptors inhibits formalin-induced inflammation and nociception, respectively, Pharmacol Biochem Behav, 88 (2007) 122.


33 Li S Q, Han H & He J, Histidine ameliorated brain edema and cardiac dysfunction during local thrombotic cerebral ischemia in rats, Zhongguo Yao Li Xue Bao, 16 (1995) 156.


