HISTOPATHOLOGICAL COMPARISON OF THE EFFECTS OF HISTIDINE AND KETOTIFEN IN A RAT MODEL OF COLITIS

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Abstract

The effects of histidine and ketotifen in separate and combined treatments were histopathologically investigated in a rat model of acute colitis. Acute colitis was induced by the intracolonic instillation of 1 ml of 4% acetic acid. Histopathological changes of the colon were scored based on the severity of inflammatory and degenerative changes. Compared to the intact group, there was congestion, oedema, haemorrhages, leukocyte infiltration, and degeneration in the acetic-acid-induced colitis group. In the separate treatments with intraperitoneal (i.p.) injection of histidine at doses of 40 and 200 mg/kg and with oral administration (p.o.) of ketotifen (0.4 and 2 mg/kg) applied 1 and 24 h after induction of colitis, the inflammatory and degenerative changes were significantly (P<0.05) reduced. In combined treatment, co-administration of histidine (200 mg/kg, i.p.) with ketotifen (2 mg/kg, p.o.) produced a more documented response in comparison with the histidine (200 mg/kg, i.p.) and ketotifen (2 mg/kg, p.o.) used alone. The results suggested that intracolonic instillation of acetic acid produced acute colitis. Both histidine and ketotifen exerted anti-inflammatory effects on acetic-acid-induced colitis. In addition, ketotifen potentiated the anti-inflammatory effect of histidine.

Key words: rats, histidine, ketotifen, colitis, histopathology.

Ulcerative colitis, together with Crohn’s disease, as the primary constituent of inflammatory bowel disease, is characterised by intestinal inflammation, frequently relapsing with clinical manifestations including diarrhoea, blood in the stool, abdominal pain, and weight loss (12). Although several factors are reportedly involved in the development of inflammatory bowel disease, including immune dysfunction, genetic susceptibility, and bacterial flora within the intestinal environment, the underlying pathogenic mechanism has not yet been identified (13). Within the past two decades, several laboratory animal models of colitis using dextran sodium sulfate, 2,4,6-trinitrobenzene sulfonic acid, oxazolone, indomethacin, and acetic acid have been reported that demonstrate various pathophysiological and therapeutic effects on human inflammatory bowel disease (13, 19). There are several categories of treatments relevant to inflammatory bowel disease, including aminosalicylates, corticosteroids, immunosuppressants, anti-tumour necrosis factor-α antibodies, recombinant cytokines, growth factors, gene therapy, antibiotics, prebiotics, probiotics, and symbiotics (6, 13, 34).

Some amino acids have recently been reported to contribute to the modulation of gut inflammation in colitis models in rats (7, 10, 30). Histidine is a conditionally-essential amino acid, known as an efficient scavenger, both of hydroxyl radicals and non-radical toxic oxygen species, and singlet oxygen (20, 31). Intraperitoneal injection and intra-intestinal administration of histidine produced anti-secretory activity by reducing the amount of fluid accumulating in the intestinal lumen. Histidine also induced an anti-inflammatory activity by the protection of the intestinal tissue from Salmonella Typhimurium-induced damage (25). Recently, it has been reported that dietary histidine ameliorates colitis by the inhibition of the production of tumour necrosis factor-α and interleukine-6 in mice (1).

Ketotifen is a selective stabiliser of mast cells and an orally-active prophylactic agent for the management of bronchial asthma and allergic disorders (11). In both trinitrobenzene sulfonic acid- and acetic acid-induced colitis, a reducing effect of ketotifen on the mucosal damage was reported (3). Treatment with ketotifen increased the survival rates in the intestinal ischaemia reperfusion injury in rats (35).

The present study was conducted to evaluate and to compare the effects of histidine and ketotifen in separate and combined treatments on the histopathological changes in the colon induced by the intracolonic instillation of acetic acid in rats.
Material and Methods

Animals. After ethical approval obtained from the Laboratory Animal Care and Use Centre of the Faculty, 42 male Wistar rats (230–270 g) were housed in groups of six animals per cage, with free access to feed and water, and keeping to the 12 h light-dark cycle.

Drugs. The following drugs were used in the study: L-histidine monohydrochloride (Merck Chemical Company, Germany) and ketotifen (Sigma-Aldrich Chemical Company, USA). Histidine was dissolved in normal saline and was injected intraperitoneally in a volume of 1 ml/kg b.w. Ketotifen suspension was prepared in normal saline and was orally administered in a constant volume of 0.3 ml for each rat.

Induction of colitis. Colitis was induced by intracolonic instillation of 4% acetic acid. After a 24 h fasting, each rat was slightly anaesthetised with intraperitoneal injection of a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). A polyethylene catheter, with inner diameter of 1 mm, was lubricated with liquid paraffin and was then inserted into the lumen of the colon via the anus. The catheter was advanced so that the tip was 8 cm proximal to the anus. Initially, each rat received 1 ml of normal saline flush followed by manual palpation of the abdomen to remove any faeces. Then 1 ml of 4% acetic acid (v/v in normal saline) was slowly infused into the colon and the rat was then maintained in a head-down position for 30 s to limit expulsion of the solution. Finally, each rat received 1 ml of colonic wash containing normal saline. Control rats were treated identically but instead of 4% acetic acid, they received a 1-ml normal saline infusion.

Treatment groups. The rats were divided into seven groups of six animals in each group. Group I, intact group, was administered intraperitoneally and orally with normal saline 1 and 24 h after intracolonic administration of normal saline. Group II, control group, received intraperitoneally and orally normal saline 1 and 24 h after intracolonic administration of acetic acid. Groups III and IV were injected intraperitoneally with histidine at doses of 40 and 200 mg/kg, respectively, 1 and 24 h after induction of colitis. Groups V and VI received orally 0.4 and 2 mg/kg of ketotifen, respectively, 1 and 24 h after induction of colitis. Group VII received intraperitoneal injection of histidine at a dose of 200 mg/kg plus oral administration of ketotifen at a dose of 2 mg/kg, 1 and 24 h after induction of colitis.

Tissue collection and histopathology. Forty-eight hours after the induction of colitis, animals were euthanised using diethyl ether. Necropsy of the animals was done and the colon was removed and fixed in 10% buffered formol saline. Paraffin sections (4-5 µm) were stained by haematoxylin and eosin and examined under a light microscope. The evaluation of the sections was based on the severity of the pathological changes. The following scores were given to lesions observed: 0 - none, 1 - minimal, 2 - mild, 3 - moderate, and 4 - severe.

Statistics. Score values were expressed as mean ± SEM. Differences among treated groups were analysed using one-way analysis of variance (ANOVA) followed by Duncan test. The statistical significance level was accepted for P<0.05.

Results

The intracolonic instillation of normal saline produced no detectable histopathological changes in the colon of group I (Table 1 and Fig. 1).

Table 1

The effects of separate and combined treatments with histidine and ketotifen on the histopathological changes induced by the intracolonic instillation of acetic acid in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Histopathological changes</th>
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<tbody>
<tr>
<td></td>
<td>Congestion</td>
</tr>
<tr>
<td>Intact (NS, i.c.)</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Control (AA, 4%, i.c.)</td>
<td>3.8 ± 0.2*</td>
</tr>
<tr>
<td>Histidine (40 mg/kg, i.p.)</td>
<td>1.4 ± 0.2†</td>
</tr>
<tr>
<td>Histidine (200 mg/kg, i.p.)</td>
<td>0.8 ± 0.2†</td>
</tr>
<tr>
<td>Ketotifen (0.4 mg/kg, p.o.)</td>
<td>0.6 ± 0.2†</td>
</tr>
<tr>
<td>Ketotifen (2 mg/kg, p.o.)</td>
<td>0.4 ± 0.2†</td>
</tr>
<tr>
<td>Histidine (200 mg/kg, i.p.)+</td>
<td>0.2 ± 0.2†</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.M. The severity of histopathological changes was scored according to the observation of lesions in five different microscopic areas as: 0 - none, 1 - minimal, 2 - mild, 3 - moderate, 4 - severe. * significant difference compared to intact group (P<0.05); † significant difference compared to control group (P<0.05); ‡ significant difference compared to histidine (200 mg/kg) group (P<0.05); NS – normal saline ; AA - acetic acid; i.c. – intracolonic; p.o. - per os; i.p. - intraperitoneal.
Fig. 1. Rat colon in intact group, no detectable changes are seen, HE, 100x.

Fig. 2. Rat colon affected with acetic-acid-induced colitis (control group): mucosa is necrotic and severely damaged (arrow head), and submucosal oedema, congestion, and leukocytic infiltration are seen (arrow), HE, 100x.

Fig. 3. Rat colon affected with colitis treated with 40 mg/kg of histidine: inflammatory cell infiltration and oedema are minimal (star), HE, 100x.

Fig. 4. Rat colon affected with colitis treated with 200 mg/kg of histidine: inflammatory cell infiltration and oedema decreased (star), HE, 100x.

Fig. 5. Rat colon affected with colitis treated with 0.4 mg/kg of ketotifen: marked reduction in inflammatory response (star), HE, 100x.

Fig. 6. Rat colon affected with colitis treated with 2 mg/kg of ketotifen: reduction in the inflammatory lesions (star), HE, 100x.

Fig. 7. Rat colon affected with colitis treated with 200 mg/kg histidine plus 2 mg/kg of ketotifen: the inflammatory reaction is very minimal (arrow head), HE, 100x.
In group II, the intracolonic instillation of acetic acid produced severe histopathological changes in the colon, including congestion, haemorrhages, oedema, leukocyte infiltration, and necrosis of the mucosa (Table 1 and Fig. 2). In groups III and IV, intraperitoneal injections of histidine at doses of 40 and 200 mg/kg significantly (P<0.05) decreased the intensity of congestion, haemorrhages, oedema, leukocyte infiltration, and necrosis. When the inhibitory effects of histidine on the histopathological changes were compared, a significant (P<0.05) difference was observed only on the congestion rate (Table 1, Fig. 3, and Fig. 4). In the groups V and VI, oral administration of ketotifen at doses of 0.4 and 2 mg/kg significantly (P<0.05) reduced both the inflammatory and degenerative changes. However, a significant (P<0.05) difference was observed only in the leukocyte infiltration when the effects of ketotifen on the histopathological changes were compared (Table 1, Fig. 5, and Fig. 6). When the results obtained after the treatment with histidine and ketotifen were compared, significant (P<0.05) differences were observed in all histopathological changes (Table 1). In group VII, the inhibitory effects produced by the combination of histidine (200 mg/kg, i.p.) and ketotifen (2 mg/kg, p.o.) on the oedema and leukocytic infiltration were significantly (P<0.05) more distinct than those obtained after treatment with histidine (200 mg/kg, i.p.) and ketotifen (2 mg/kg, p.o.) alone (Table 1 and Fig. 7).

Discussion

In the present study, the experimental induction of colitis with instillation of 1 ml of 4% acetic acid into the colon was successfully achieved, and confirmed by histopathological findings such as congestion, haemorrhages, oedema, leukocytic infiltration, and degenerative changes such as necrosis. It was found that intra-rectal administration of 3%-5% acetic acid induced acute colitis in the distal part of the colon in rats, which was characterised by epithelial necrosis and oedema (22). Epithelial destruction and influx of inflammatory cells was found after the administration of acetic acid into the colon of mice (5).

In this study, histidine reduced the severity of congestion, haemorrhages, oedema, and leukocyte infiltration, as well as the degenerative changes induced by the intracolonic instillation of acetic acid. It has been reported that L-histidine, but not D-histidine, attenuates brain oedema induced by cryogenic surgery in rats (16). Intraperitoneal injection of histidine suppressed neutrophil recruitment and decreased the number of CD4+ lymphocytes in ischaemic rat brains (14). In the chronic alcoholic liver injury model in rats, mRNA expression of interleukine-6 and tumour necrosis factor-α was down, regulated by histidine (21). In the interleukin-10-deficient cell transfer model of colitis, dietary histidine suppressed inflammatory responses and cytokine production in the colon in mice (1). It was reported that histidine protected the mouse’s small intestine from Salmonella Typhimurium-induced damage. They demonstrated that the efficacy of L-histidine in protecting the infected intestinal tissue was attributed to the anti-oxidative capacity of L-histidine on reactive oxygen species formed in response to lipopolysaccharide (25). Moreover, it was reported that histidine eliminated the nuclear factor κ B-dependent activation of the interleukin-8 promotor induced by tumour necrosis factor α and inhibited the nitrogen dioxide- and tumour necrosis factor-α-induced interleukin-8 secretion (28). On the other hand, the reduced number of interleukine-10-positive lymphocytes in the intestinal mucosa of histamine-deficient, histidine decarboxylase (HDC) knockout mice and the altered faecal bacterial flora in these animals suggest that histamine and its precursor histidine may be involved in the pathophysiology of inflammation in the colon of normal animals (2). Intraperitoneal injection of histidine produced an analgesic effect in an inflammatory model of pain in mice (29). It seems that the anti-inflammatory effect of histidine observed in the present study may be related to the anti-oxidative and anti-cytokine effects of histidine because the involvement of reactive metabolites of oxygen and nitrogen, tumour necrosis factor α, interleukins 12 and 18, and nuclear factor κ B in the pathogenesis of colitis, have been well described (13, 19, 24).

The results also showed that ketotifen suppressed the pathological changes such as congestion, haemorrhages, oedema, and leukocyte infiltration, as well as the degenerative lesions induced by acetic acid in the colon of rats. It has been reported that ketotifen produced a protective effect in the acute ulcerative colitis in children (18). Ketotifen prevented mucosal damage in the colitis induced by trinitrobenzene sulfonic acid and acetic acid (3), and produced a protective effect in capsaicin-augmented acetic-acid-induced colitis (4). In another rat model of ulcerative colitis, it was observed that ketotifen decreased mucosal injury and myeloperoxidase activity (8). Ketotifen inhibited lipopolysaccharide-induced oedema by reducing the plasma leakage in the skin in rats (17). Besides its inhibitory effect on mast cell degranulation (11), ketotifen has anti-oxidant activity (33), and anti-cytokine (9) and anti-chemokine (15) production properties. The roles of reactive oxygen species, cytokines, and chemokines in the pathogenesis of colitis have been reported (13, 19, 23, 24).

In the present study, ketotifen enhanced the inhibitory effects of histidine on the inflammatory responses and on the degenerative changes. Histidine and ketotifen produced suppressive effects on oxidative stress (27, 28) and exerted inhibitory effects on the production of cytokines (1, 9) and chemokines (14, 15). Histidine attenuated brain oedema (16) and ketotifen suppressed vascular permeability (17). Carnosine (β-alanyl-histidine), as a precursor of histidine (32), was reported to attenuate mast-cell degranulation and histamine release (26). Ketotifen is well known as a mast-cell stabilizer, as well as a histamine H1 receptor antagonist (11). It seems that both histidine and ketotifen may use the same mechanisms in producing suppressive
effects on the pathological changes induced by acetic acid in the colon in rats observed in the present study.

In conclusion, the results of this study showed that intracolonic instillation of acetic acid produced colitis. Both ketotifen and histidine induced anti-inflammatory effects in acetic-acid-induced colitis. In addition, ketotifen enhanced the suppressive effects of histidine.

References

