Chemico-Biological Interactions 205 (2013) 72–80

Contents lists available at SciVerse ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

The role of cholinergic anti-inflammatory pathway in acetic acid-induced colonic inflammation in the rat

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ARTICLE INFO

Article history:
Received 11 April 2013
Received in revised form 23 May 2013
Accepted 11 June 2013
Available online 26 June 2013

Keywords:
Acetic acid
Cholinergic
Colitis
Huperzine A
Nicotine
Rat

ABSTRACT

The "cholinergic anti-inflammatory pathway" provides neurological modulation of cytokine synthesis to limit the magnitude of the immune response. This study aimed to evaluate the impact of the cholinergic anti-inflammatory pathway on the extent of tissue integrity, oxidant-antioxidant status and neutrophil infiltration to the inflamed organ in a rat model of acetic acid-induced colitis. Colitis was induced by intrarectal administration of 5% acetic acid (1 ml) to Sprague–Dawley rats (200–250 g; $n = 7–8$ per group). Control group received an equal volume of saline intrarectally. The rats were treated with either nicotine (1 mg/kg/day) or huperzine A (0.1 mg/kg/day) intraperitoneally for 3 days. After decapitation, the distal colon was scored macroscopically and microscopically. Tissue samples were used for the measurement of malondialdehyde (MDA) and glutathione (GSH) levels, and myeloperoxidase (MPO) activity. Formation of reactive oxygen species was monitored by using chemiluminescence (CL). Nuclear factor (NF)-κB expression was evaluated in colonic samples via immunohistochemical analysis. Trunk blood was collected for the assessment of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-10, resistin and visfatin levels. Both nicotine and huperzine A reduced the extent of colonic lesions, increased colonic MDA level, high MPO activity and NF-κB expression in the colitis group. Elevation of serum IL-1β level due to colitis was also attenuated by both treatments. Additionally, huperzine A was effective to reverse colitis-induced high lucigenin-enhanced CL values and serum TNF-α levels. Colitis group revealed decreased serum visfatin levels compared to control group which was completely reversed by nicotine. In conclusion, modulation of the cholinergic system either by nicotine or ACh esterase inhibition improved acetic acid-induced colonic inflammation as confirmed by macroscopic and microscopic examination and biochemical assays.

1. Introduction

Inflammatory bowel diseases (IBDs), namely ulcerative colitis and Crohn's disease, are two chronic idiopathic diseases characterized by prominent intestinal inflammation [1]. The pathogenesis of IBD involves an interaction between genetic and environmental factors. Whatever the precise mechanism responsible for initiating and perpetuating intestinal inflammation, there is an ample evidence for an intense local immune response, associated with recruitment and activation of lymphocytes and macrophages. The subsequent release of soluble cytokines and other inflammatory mediators causes tissue damage and contributes to many of the clinical features of these diseases and to the amplification and perpetuation of the local immune response [2].

Stimulation of vagus nerve signals was shown to significantly inhibit tumor necrosis factor (TNF) release in animals receiving lethal amounts of endotoxin [3]. Subsequent work established that vagus nerve signaling inhibits cytokine activities and improves disease endpoints in experimental models of sepsis, ischemia/reperfusion, hemorrhagic shock, myocardial ischemia, ileus, experimental arthritis, and pancreatitis [3,4–11]. The mechanism for inhibition of cytokine synthesis is attributable to acetylcholine (ACh), the major vagus nerve neurotransmitter [3,12,13]. Macrophages and other inflammatory cells releasing cytokines express ACh receptors, which transduce an intracellular signal to suppress cytokine synthesis and release [12,13]. The best characterized cholinergic receptor that inhibits cytokines is the α7 subunit of the nicotinic ACh receptor (α7 nAChR).

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http://dx.doi.org/10.1016/j.cbi.2013.06.009

Stimulation of the vagus nerve or administration of α7 nAChR agonists, inhibits both TNF and interleukin (IL)-1, IL-6, IL-8 and high-mobility group box 1 (HMGB1) [13]. The mechanism of signal transduction probably involves ligand-receptor interaction on cytokine-expressing cells to decrease nuclear translocation of nuclear factor (NF)-κB as well as activation of the transcription factor signal transducer and activator of transcription (STAT)-3 via phosphorylation by janus kinase (JAK)2, which is recruited to the α7 nAChR [8,13]. Thus, vagus nerve-mediated cholinergic pathway which was termed the "cholinergic anti-inflammatory pathway" provides neurological modulation of cytokine synthesis to limit the magnitude of the immune response.

The discovery of the critical role for the α7 nAChR in mediating cholinergic anti-inflammatory signaling led to the utilization of nicotine and other α7 nAChR agonists in mechanistic studies. Nicotine has been demonstrated to inhibit resident peritoneal macrophage activation ex vivo, attenuating pro-inflammatory cytokine release through α7 nAChR-mediated activation of the JAK/STAT pathway [8]. The activation of the cholinergic anti-inflammatory pathway by systemic cholinesterase inhibition has also been demonstrated to control inflammation. Cholinesterase inhibition by physostigmine and neostigmine conferred similar protection against septic shock induced by cecal ligation and puncture along with down-regulation of NF-κB and reduction of the circulating levels of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 [14].

In this study, we aimed to evaluate the impact of the cholinergic anti-inflammatory pathway on the extent of tissue integrity, oxidant-antioxidant status and neutrophil infiltration to the inflamed organ in a rat model of acetic acid-induced colitis, using nicotine and huperzine A, a cholinergic nicotinic receptor agonist and a potent and reversible inhibitor of ACh esterase, respectively.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats of either sex (200–250 g; $n = 7$–8 per group) were kept in a room at a constant temperature (22 ± 1 °C) with 12 h light/dark cycles and fed standard pellet chow and water ad libitum. The study protocol was approved by Marmara University, School of Medicine, Animal Care and Use Committee.

2.2. Induction of colitis

After an overnight fasting, colonic inflammation was induced under light ether anesthesia by intrarectal administration of 1 ml of 5% (v/v) acetic acid in 0.9% NaCl with a 8 cm long cannula [15]. The rats in the control group were subjected to the same procedure with the exception that isotonic saline was substituted for acetic acid. In treatment groups, the rats were treated with either nicotine (1 mg/kg/day; intraperitoneally) or huperzine A (0.1 mg/kg/day, intraperitoneally) or vehicle (0.9% NaCl, 1 ml/kg; intraperitoneally) 20 min before induction of colitis and the treatment was continued for 3 consecutive days.

On the fourth day after the induction of colitis, all rats were decapitated. The last 8 cm of the colon was excised, opened longitudinally, and rinsed with saline solution. Then, the distal colon was weighed and the mucosal lesions were scored macroscopically using the criteria outlined in Table 1 [16]. Tissue samples were taken for histologic evaluation of the lesions by light microscopy. Other tissue samples were stored at −70 °C for subsequent measurement of malondialdehyde (MDA) and glutathione (GSH) levels, and myeloperoxidase (MPO) activity. Formation of reactive oxygen species in colonic samples was monitored by using chemiluminescence method. Trunk blood was collected for the assessment of pro-inflammatory cytokines TNF-α and IL-1β, anti-inflammatory cytokine IL-10 and, adipokines resistin and visfatin levels.

Table 1
Criteria for macroscopic scoring of colonic lesions.

Score	Appearance
0	No damage
1	Focal hyperemia, no ulcers
2	Hyperemia or bowel wall thickening without linear ulceration
3	Ulceration with inflammation at one site
4	Two or more sites of ulceration/inflammation
5	Major sites of damage extending more than 1 cm along the length of colon
6–10	If damage extends more than 2 cm along the length of colon, the score is increased by one for each additional 1 cm

2.3. Histological evaluation

For light microscopic investigation, samples from distal colon were placed in 10% formaldehyde, dehydrated in ascending alcohol series (70%, 90%, 96% and 100%), and embedded in paraffin. For each animal, four randomly taken tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) and examined under an Olympus BH 2 photomicroscope. Histologic scoring was performed by the criteria shown in Table 2 [17]. Here, tissue damage refers to both architectural disturbance and ulceration. Vasculitis refers to thickness and inflammation of the nearby vessels. And, the inflammatory cell infiltration refers to the infiltration of the tissue mainly by neutrophils and lymphocytes. All tissue sections were examined by an experienced histologist (F.E.) who was unaware of the treatment groups.

2.4. Measurement of MDA and GSH levels

The colon samples were homogenized in 10 volumes of ice-cold 10% trichloracetic acid and centrifuged at 700*g* for 15 min at 4 °C. Supernatant was removed and recentrifuged at 10,000*g* at 4 °C for 8 min. GSH was determined by a spectrophotometric method which is a modification of Ellman procedure [18]. Lipid peroxide levels are expressed in terms of MDA equivalents as nmol MDA/g tissue [19].

2.5. Measurement of MPO activity

Colonic MPO activity – an indicator of neutrophil accumulation – was assessed by measuring the H_2O_2-dependent oxidation of o-dianizidine 2HCl. One unit of enzyme activity was defined as the amount of MPO present that causes a change in absorbance of 1.0 unit min at 460 nm and 37 °C and expressed in units per g tissue [20].

2.6. Chemiluminescence assay

Chemiluminescence (CL) assay is a direct noninvasive method for measuring reactive oxygen species. Due to limitations, i.e., potential variability and low intensity of native CL, luminol and

Table 2
Criteria for microscopic scoring of colonic lesions.

Score	Appearance
0 None; 1 Mild; 2 Moderate; 3 Severe	Submucosal edema
0 None; 1 Localized; 2 Moderate; 3 Severe	Damage/necrosis
0 None; 1 Mild; 2 Moderate; 3 Severe	Inflammatory cell infiltration
0 None; 1 Mild; 2 Moderate; 3 Severe	Vasculitis
0–; 1+	Perforation