Phytomedicine 20 (2013) 585–591

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Novel effects of ectoine, a bacteria-derived natural tetrahydropyrimidine, in experimental colitis

Heba Abdel-Aziz[a,*], Walaa Wadie[b], Dalaal M. Abdallah[b], Georg Lentzen[c], Mohamed T. Khayyal[b]

[a] Heliopolis University, 3 Cairo-Belbeis Desert Road, Cairo, Egypt
[b] Department of Pharmacology, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo, Egypt
[c] Research and Development, bitop AG, Stockumer Straße 28, Witten, Germany

ARTICLE INFO

Keywords:
Inflammatory bowel disease
Trinitrobenzenesulfonic acid
Compatible solutes
Ectoines
Intestinal barrier

ABSTRACT

Evidence suggests an important role of intestinal barrier dysfunction in the etiology of inflammatory bowel disease (IBD). Therefore stabilizing mucosal barrier function constitutes a new therapeutic approach in its management.

Ectoine is a compatible solute produced by aerobic chemoheterotrophic and halophilic/halotolerant bacteria, where it acts as osmoprotectant and effective biomembrane stabilizer, protecting the producing cells from extreme environmental stress. Since this natural compound was also shown to prevent inflammatory responses associated with IBD, its potential usefulness was studied in a model of colitis.

Groups of rats were treated orally with different doses of ectoine (30–300 mg/kg) or sulfasalazine (reference drug) daily for 11 days. On day 8 colitis was induced by intracolonic instillation of 2,4,6-trinitrobenzenesulfonic acid, when overt signs of lesions develop within the next 3 days. On day 12, blood was withdrawn from the retro-orbital plexus of the rats and the animals were sacrificed. The colon was excised and examined macroscopically and microscopically. Relevant parameters of oxidative stress and inflammation were measured in serum and colon homogenates.

Induction of colitis led to marked weight loss, significant histopathological changes of the colon, and variable changes in levels of myeloperoxidase, reduced glutathione, malondialdehyde, and all inflammatory markers tested. Treatment with ectoine ameliorated the inflammatory changes in TNBS-induced colitis. This effect was associated with reduction in the levels of TNF-α, IL-1β, ICAM-1, PGE_2 and LTB_4.

The findings suggest that intestinal barrier stabilizers from natural sources could offer new therapeutic measures for the management of IBD.

Introduction

Inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis, are severe chronic inflammatory disorders of the gastrointestinal tract. Although their etiology and pathophysiology are still unclear, an impaired intestinal barrier function, dysbiosis (an imbalance in the intestinal bacterial ecosystem) and immunologic mechanisms have been advocated an important role (Dotan and Mayer, 2002; Melmed and Abreu, 2004; Hanauer, 2006; Shaw et al., 2011; Kiesslich et al., 2012).

IBD affects adversely the quality of life and necessitates long-term dependence on effective drugs (Carty and Rampton, 2003). Mesalazine, sulfasalazine and other 5-aminosalicylic acid (ASA) derivatives are considered currently drugs of choice for the management of most cases, while corticosteroids and immunosuppressants are retained for more severe forms of the disease. Although these drugs are effective, the risk of adverse effects is high, especially considering the chronic and relapsing nature of this condition. Therefore, the search for new safer therapies continues. Due to recent evidence suggesting an important role of intestinal barrier dysfunction in the etiology of IBD, the use of compounds able to stabilize mucosal barrier function constitutes a new therapeutic approach to the disease, especially for the prevention of flares.

Ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid, Fig. 1) is a natural zwitterionic, low molecular weight, strong water binding organic molecule, which was first isolated from *Ectothiorhodospira halochloris.* Later it was also found in several aerobic chemoheterotrophic and halophilic/halotolerant bacteria. Nowadays, commercially available ectoine is produced biotechnologically in high purity (≥95%) using the so-called "bacterial milking" fermentation technology followed by downstream purification (Pastor et al., 2010).

Like other compatible solutes (CS), ectoine confers resistance towards environmental stress conditions such as high temperature,

* Corresponding author at: Scientific Department, Steigerwald Arzneimittelwerk, Havelstr. 5, D-64295 Darmstadt, Germany. Tel.: +49 6151 3305 202; fax: +49 6151 3305 471.
E-mail address: heba.abdelaziz@gmail.com (H. Abdel-Aziz).

0944-7113/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.phymed.2013.01.009

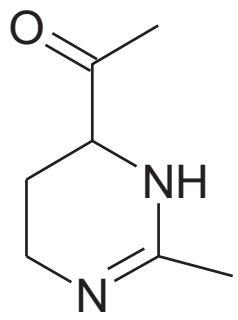

Fig. 1. The chemical structure of ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid).

freezing, extreme dryness and high salinity (Lippert and Galinski, 1992; Galinski, 1993). In addition to their function as osmoprotectants, CSs are characterized by being effective stabilizers of biomolecules, including proteins, nucleic acids and biomembranes. These properties, in addition to the fact that CSs are biologically inert and do not interfere with overall cellular functions, even though they accumulate in high concentration in the cytoplasm, make them potential candidates as safe treatment options for IBD. Since ectoine was also shown to prevent inflammatory responses relevant to IBD (Nielsen et al., 1994; Goke et al., 1997; Danese et al., 2003; Bünger and Driller, 2004; Buommino et al., 2005), the aim of the present study was to investigate its potential usefulness in an animal model of colitis.

Methods

Drugs

2,4,6-Trinitrobenzene sulfonic acid (TNBS) was purchased from Sigma-Aldrich (Schnelldorf, Germany). Ectoine was provided by bitop AG (Witten, Germany) and administered as an aqueous solution. Sulfasalazine was a gift from El-Kahira Pharmaceutical Company (Cairo, Egypt) and was used as a suspension in 1% methylcellulose.

Animals

Adult male Wistar rats, weighing 150–200 g each, were obtained from 'The Modern Veterinary Office for Laboratory Animals', Cairo, Egypt and left to acclimatize at the animal facility of the Faculty of Pharmacy, Cairo University, for one week, before subjecting them to experimentation. They were provided with a standard pellet diet and given water *ad libitum*. The animals were kept at a temperature of $22 \pm 3\,^{\circ}C$ and a 12-h light/dark cycle as well as a constant relative humidity throughout the experimental period. The study was carried out according to The European Communities Council Directive of 1986 (86/609/EEC) and approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University.

Induction of colitis

Colitis was induced using 2,4,6-trinitrobenzene sulfonic acid (TNBS) as described by Morris et al. (1989). In brief, rats that had been deprived of food but not water for 36 h were lightly anesthetized with diethyl ether. A polyethylene catheter (3 mm diameter) fitted onto a 1-ml syringe was inserted rectally into the colon so that the tip was 8 cm proximal to the anus. In a head-down position, 0.25 ml of 50% (v/v) ethanol containing 50 mg/kg TNBS was then slowly instilled into the lumen of the colon. After delivering the required dose of TNBS/ethanol solution, the catheter was left in place for 30 s and then removed gently. Rats were kept for another 30 s in this position to avoid leakage of the instillate.

Experimental design

Determination of the potential protective effect of ectoine

Rats were randomly allocated to 8 groups of 8 animals each. Two groups were given distilled water and were designated as normal control and TNBS control groups. The other groups were treated either with ectoine (30, 50, 100, 200 and 300 mg/kg) or sulfasalazine (300 mg/kg) as a reference drug. The drugs were administered by oral gavage once daily throughout the experimental period (11 days). On day 8, colitis was induced by intra-colonic injection of TNBS in all groups except the normal control group which received vehicle instead. Animals were weighed immediately before colitis induction and just before autopsy to determine the effect of colitis on body weight.

The rats were sacrificed on day 12 by cervical dislocation and the distal 8 cm portion of the colon was excised, opened longitudinally and thoroughly rinsed in ice-cold normal saline. The colon segments were placed on an ice-cold dissecting surface, cleaned of fat and mesentery, blotted on filter paper and weighed. Mucosal damage was assessed by measuring the ulcerative area (cm^2) in the mucosa. The colon mass index (ratio of colon weight to total body weight) was calculated in terms of mg/g. The index was taken as a measure of the degree of colonic edema and the severity of inflammation.

The excised portion of the colon was then homogenized in ice-cold bi-distilled water to obtain a 10% (w/v) homogenate and divided into two aliquots which were used for spectrophotometeric estimation of reduced glutathione (GSH) (Beutler et al., 1963) and myeloperoxidase (MPO) activity (Krawisz et al., 1984).

Mechanisms underlying the protective action of ectoine

A further four groups of animals, each consisting of 8 rats were designated as normal control, TNBS control, ectoine treated (100 mg/kg) and sulfasalazine treated (300 mg/kg). The groups were subjected to the same protocol of treatment mentioned above.

On day 12 (day of sacrifice), blood samples were withdrawn from the retro-orbital plexus of each rat under light ether anesthesia. The separated sera were divided into several aliquots. One aliquot was used for the spectrophotometric estimation of total nitrate/nitrite (NO_x) (Miranda et al., 2001). The remaining aliquots were used to determine tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-10 (IL-10), intercellular adhesion molecule-1 (ICAM-1), leukotriene B4 (LTB_4) and prostaglandin E_2 (PGE_2) using specific ELISA kits (R&D Systems GmbH, Wiesbaden, Germany).

After withdrawal of blood, the rats were sacrificed and the distal 8 cm section of the colon was excised and homogenized. The homogenate was divided into two aliquots:one was used for the estimation of malondialdehyde (MDA) (Mihara and Uchiyama, 1978), while the other was centrifuged at 13,000 rpm for 30 min at $4\,^{\circ}C$ and the resultant supernatant was frozen at $-20\,^{\circ}C$ for assaying TNF-α, IL-1β, IL-10, ICAM-1, PGE_2, LTB_4 and NO_x.

In addition, the spleen was excised, blotted on filter paper and weighed. The spleen mass index (ratio of spleen weight in mg to animal weight in g) was taken as a further marker of inflammation (Cuzzocrea et al., 2003).

The colon segments from 2 randomly chosen animals of each group were fixed in 10% (v/v) formalin and preserved for histological examination.