Biochimica et Biophysica Acta 1790 (2009) 1161–1169

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

L-cysteine supplementation attenuates local inflammation and restores gut homeostasis in a porcine model of colitis

C.J. Kim [a], J. Kovacs-Nolan [a], C. Yang [b], T. Archbold [b], M.Z. Fan [b], Y. Mine [a,*]

[a] Department of Food Science, University of Guelph, Guelph, ON,Canada N1G 2W1
[b] Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada

ARTICLE INFO

Article history:
Received 12 October 2008
Received in revised form 2 May 2009
Accepted 27 May 2009
Available online 9 June 2009

Keywords:
Inflammatory bowel disease (IBD)
Dextran sodium sulfate (DSS)
L-cysteine
Inflammation
Cytokine
Apoptosis

ABSTRACT

Background: Inflammatory bowel disease (IBD), a chronic inflammation of the gastrointestinal tract, is characterized by a deregulation of the mucosal immune system and resistance of activated T cells to apoptosis. Current therapeutics show limited efficacy and potential toxicity; therefore there is a need for novel approaches for the treatment of IBD. L-cysteine was examined for its ability to reduce colitis symptoms and modulate local gene expression in a DSS-induced porcine model of colitis.
Methods: Colitis was induced via intra-gastric infusion of dextran sodium sulfate (DSS), followed by the administration of L-cysteine or saline. Clinical signs, morphological measurements, histology and gut permeability were assessed for the prognosis of colitis. Local tissue production of cytokines and gene expression in the colon were analyzed by ELISA and real-time RT-PCR.
Results: L-cysteine supplementation attenuated DSS-induced weight loss and intestinal permeability, reduced local chemokine expression and neutrophil influx, and markedly improved colon histology. Furthermore, cysteine significantly reduced the expression of pro-inflammatory cytokines, including TNF-α, IL-6, IL-12p40, IL-1β, and resulted in increased expression of the apoptosis initiator caspase-8 and decreased expression of the pro-survival genes cFLIP and Bcl-xL.
Conclusions and general significance: These results suggest that L-cysteine administration aids in restoring gut immune homeostasis by attenuating inflammatory responses and restoring susceptibility of activated immune cells to apoptosis, and that cysteine supplementation may be a novel therapeutic strategy for the treatment of IBD.

1. Introduction

Inflammatory bowel disease (IBD) is a chronically relapsing inflammation of the gastrointestinal tract that manifests itself in one of two main forms, Crohn's disease (CD) and ulcerative colitis (UC). While the etiology of IBD is still not fully understood, it has been suggested that genetic predisposition, environmental factors, intestinal microflora, and impairment of local tolerance leading to an ongoing activation of the intestinal immune system all play a role [1,2]. Major contributors to IBD pathogenesis include an abundance of pro-inflammatory cytokines, activated lymphocytes, and reactive oxygen species (ROS) in the local inflamed tissue [3,4]. T lymphocytes in particular may play a key role in the pathogenesis of IBD, and the down-regulation of activated T cells via apoptosis is critical in maintaining immune homeostasis. Recent evidence has suggested that mucosal T cell apoptosis may be inhibited in IBD patients, leading to the accumulation of activated T cells and chronic inflammation [5–7].

At present, the pharmacological treatments available include 5-aminosalicylate-based compounds, corticosteroids and immunosuppressive and immuno-regulatory agents, however they have limited therapeutic efficacy and are not suitable for long-term use, and have demonstrated toxic side effects [3,8,9]. As a result, alternative treatment methods have gained increasing attention, especially nutrition and functional foods. Cysteine, a non-essential amino acid, plays a number of roles in protein metabolism. Similar to other amino acids, cysteine serves as a substrate for protein synthesis; however it encompasses diverse non-nutritional functions. Cysteine is the rate-limiting substrate for the synthesis of glutathione (GSH), which is the most important intracellular antioxidant [10]. Furthermore, cysteine is able to spare methionine by 50–80% [11] and is

Abbreviations: CD, Crohn's disease; DSS, dextran sodium sulfate; ELISA, enzyme linked immunosorbent assay; FLIP, Fas-associated-death domain like interleukin-1-converting enzyme-inhibitory protein; GSH, glutathione; HBSS, Hank's balanced salt solution; H and E, hematoxylin and eosin; HTAB, hexadecyl-trimethyl-ammonium bromide; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; MPO, myeloperoxidase; ROS, reactive oxygen species; RT-PCR, reverse transcription polymerase chain reaction; TMB, 3, 3′,5, 5′-tetramethylbenzidine; TNF, tumor necrosis factor; PMSF, phenylmethyl sulfonyl fluoride; BW, body weight; Th, T-helper cell; UC, ulcerative colitis

* Corresponding author. Tel.: +1 519 824 4120x52901; fax: +1 519 824 6631.
E-mail address: ymine@uoguelph.ca (Y. Mine).

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doi:10.1016/j.bbagen.2009.05.018

involved in the synthesis of taurine, both of which are essential for the host defense against oxidative stress [12–14]. Oxidative stress is an important contributor to the disease pathogenesis of IBD; impairment of GSH synthesis, increased oxidative stress, and anti-oxidant supplementation findings support this claim [3,15,16], and DSS-treated animals have been shown to develop acute colitis accompanied by oxidative stress [15]. It has been established that cysteine has potent anti-oxidative roles via GSH synthesis and is able to suppress oxidative stress found in IBD [10]. Moreover, cysteine has been reported to stimulate the synthesis of colonic mucin in rats with colitis, which aids in intestinal epithelial protection [17], and cysteine-containing compounds have been shown to possess anti-inflammatory properties, and to decrease the production of inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-1β in murine models of colitis [10,15]. T lymphocytes have a strong transport activity for cysteine, however circulating cysteine concentrations are typically low (around 10 μM). Plasma concentrations of cystine are much higher, but the membrane transport activity for cystine in human lymphocytes is >10-fold lower than that for cysteine [18]. Therefore, cysteine may be a promising candidate as a novel therapeutic agent for IBD, and its therapeutic mechanism(s) must be further examined.

Here, the therapeutic efficacy of dietary cysteine supplementation in a dextran sodium sulfate (DSS)-induced porcine model of colitis was evaluated. DSS has been shown in a number of rodent models to induce symptoms of acute colitis, including inflammation in the colon [19–21]. More recently, porcine models of DSS-induced colitis have been described. Pigs share a similar gastrointestinal morphology and physiology with humans [22] and therefore may represent a more appropriate animal model for the study of IBD therapeutics. Free L-cysteine was used since its metabolism and absorption presents a minimal amount of cellular stress, and does not require digestion or ATP expenditure prior to being absorbed. Thus, cysteine would be rapidly absorbed and not exert additional strain on the already compromised cells [23]. In the present study, we examined the ability of L-cysteine to reduce DSS-induced colitis symptoms and pathology, and evaluated the effect of cysteine on local gene expression, in order to further elucidate the effects of cysteine on intestinal inflammation.

2. Materials and methods

2.1. Animals and experimental design

Five-day-old Yorkshire piglets were obtained from the Arkell Swine Research Station (University of Guelph, Guelph, ON). Animals were housed individually in metal floor pens with rubberized floors in a 26 °C temperature controlled 12-hour light/dark cycle room. Piglets were fed three times a day with a commercial milk replacement formula (Soweena® Litter Life; Merrick's Inc. WI) at amounts similar to their *ad libitum* intake level. All animal work was carried out in accordance with the Canadian Council of Animal Care Guide to the Care and Use of Experimental Animals.

Piglets were randomly assigned into one of three groups: a negative control group (Neg, $n=6$) that received only saline throughout the trial; a cysteine group (Cys, $n=7$) that received DSS for the induction of colitis followed by L-cysteine administration; and a placebo group (Pos, $n=8$) that received DSS, but did not receive L-cysteine.

Following a two-day acclimatization period, piglets were fitted with an intra-gastric catheter (Micro-Renathane®, O.D. 0.8 mm; Braintree Scientific, Inc., Braintree, MA). The catheter was anchored to a trimmed inert silicone patch (about 8×12 mm; Access Technologies, Skokie, IL), which was further sutured onto the gastric wall with the catheter end of about 30 mm inserted inside the gastric lumen. A custom-made vest was fitted dorsally on each animal for temporary storage of the exterior segment of the catheter. Following a 3-day recovery period, animals were infused with DSS (MW, 36000–50000; MP Biomedicals, Solon, OH) for five days followed by a five day infusion period of L-cysteine or saline. On day ten, all piglets were sedated through an inhaled anesthetic, isoflurane (Aerrane, Anaquest, WI), and sacrificed via an intra-cardiac injection of Euthanol (pentobarbital) at 0.3 mL/kg·body weight (BW). Colon tissues were collected for cytokine analysis, gene expression analysis, and histology.

2.2. DSS-induced colitis and treatment with L-cysteine

To induce colitis, piglets were given 1.25 g/kg·BW DSS in saline via an intra-gastric catheter twice a day for five days (Pos and Cys groups). Neg animals received only saline. The doses of DSS given to the piglets were determined from previous reported studies of DSS-induced colitis in porcine models [24,25] and were calculated using the animal's weight on the first day of the DSS infusion period. Following the induction of colitis, piglets in the Cys group received L-cysteine (Degussa-Hüls AG, Frankfurt am Main, Germany) suspended in saline, at 60% of the daily recommended feed intake [26], via intra-gastric catheter twice a day for five days. Animals in the Neg and Pos groups were given L-alanine in saline for isonitrogen and isoenergetic balance [27]. The daily dose infusion of true digestible amino acids 0.144 g/kg·BW·day for cysteine and 0.110 g/kg·BW·day for alanine. All solutions were pre-warmed to 37 °C before administration.

2.3. Growth performance and macroscopic observations

Clinical evaluations were made daily of body weight (BW), feed intake, stool consistency and overall animal well-being.

2.4. In vivo intestinal permeability

D-mannitol was used as a biomarker to assess *in vivo* intestinal permeability as previously described [28]. Prior to sacrifice, all animals were infused with 0.6 g/kg·BW of D-mannitol (Sigma-Aldrich, St. Louis, MO), in a total volume of around 13 mL/kg·BW, and blood was collected 0, 35 and 70 min after mannitol administration, into heparin-containing tubes. Plasma was collected by centrifugation at 800 *g* for 5 min, and background interference by polymer organic compounds was removed by boiling in a water bath for 3–5 min followed by centrifugation at 21000 *g* for 60 min. Plasma samples and

Table 1
Quantitative histological grading of colitis.

Feature graded	Grade	Description
Inflammation	0	None
	1	Slight
	2	Moderate
	3	Severe
Extent	0	None
	1	Mucosa
	2	Mucosa and submucosa
	3	Transmural
Regeneration	0	Complete regeneration or normal tissue
	1	Almost complete regeneration
	2	Regeneration with crypt depletion
	3	Surface epithelium not intact
	4	No tissue repair
Crypt damage	0	None
	1	Basal 1/3 damaged
	2	Basal 2/3 damaged
	3	Only surface epithelium intact
	4	Entire crypt and epithelium lost
Percent involvement	1	1–25%
	2	26–50%
	3	51–75%
	4	76–100%