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Electrocautery-induced localized colonic injury elicits increased levels of pro-inflammatory cytokines in small bowel and decreases jejunal alanine absorption

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ABSTRACT

Background: Colitis is associated with functional abnormalities in proximal non-inflamed gut areas, but animal models to study small bowel dysfunction in colitis have limitations. This study aims to determine small intestinal alanine absorption and cytokine expression in a novel model of colonic ulceration induced by electro-cautery.
Methods: A descending colon ulcer was induced in rats by a bipolar electro-cautery probe. Ulcer score was determined using Satoh's criteria. Jejunal alanine absorption was measured immediately and at different time intervals post ulcer induction. Levels of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) protein and m-RNA were determined in mucosal scrapings obtained from the colon, duodenum, jejunum and ileum at various time intervals after colonic ulcer induction.
Results: The mean ulcer score was 3 up to 48 h, followed by healing by 96 h post ulcer induction. Small bowel histology was normal throughout. Jejunal alanine absorption was reduced by 12–34% immediately and up to 72 h after cautery and returned to normal at 96 h. IL-1 and TNF-α mRNA increased significantly in the colon, duodenum, jejunum and ileum 3 h post electro-cautery and returned to normal at 48 h, while that of IL-6 increased significantly at 48 h post ulcer induction. Similarly, IL-1, IL-6 and TNF-α protein levels increased in the duodenum, jejunum, ileum and colon up to 48 h post ulcer induction.
Conclusions: Electrically induced localized colonic injury increased production of pro-inflammatory cytokines in non-inflamed segments of the small intestine and was associated with derangements of jejunal absorptive function.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory condition of the colon that commonly manifests with diarrhea, rectal bleeding, hypoproteinemia and sometimes weight loss. These symptoms are thought to arise directly from colonic inflammation. However, there is evidence that colitis may be associated with structural and functional abnormalities in the small intestine. Thus, a decrease in intestinal water, electrolytes, D-xylose, amino acid, glucose, fat and vitamin absorption has been reported [1–7]. These abnormalities may contribute to the clinical manifestations seen in severe colitis. The pathophysiology of this decrease in water and nutrient absorption remains unknown. Although pathological changes in the jejunum of patients with UC have been reported [3,4], these findings have not been reproducible by others [8,9].

Human and experimental colitis has been reported to be associated with increased production of pro-inflammatory and anti-inflammatory cytokines in the inflamed colon as well as in non-inflamed segments of the upper small bowel [10–12]. In addition, localized colitis results in significant structural and functional abnormalities of enteric neurons and their reflexes in non-inflamed areas of the proximal gut [13–16]. These are thought to contribute to the altered small bowel motility, secretory dysfunction and possibly to nutrient mal-absorption [6,17,18]. Although the mechanisms are still largely unknown, others and we have provided

Abbreviations: IL-1, Interleukin-1; (IL-6), interleukin-6; (TNF-α), tumor necrosis factor-α; UC, ulcerative colitis; RT, reverse transcriptase; ELISA, enzyme-linked immunosorbent assay; ENS, enteric nervous system; CNS, central nervous system.

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evidence for a role of capsaicin sensitive primary afferents, the myenteric plexus, cholinergic secretomotor neurons and possible mediation by VIP and nitric oxide [6,17].

While animal models of chemical colitis have provided valuable information on the nature and pathogenesis of small bowel dysfunction in colitis, those models have some limitations in that regard. These include the possibility of leakage of the chemicals used to induce colitis to more proximal part of the intestine causing spreading of inflammation. Moreover, the systemic inflammation that ensues may alter small bowel function. Thus, inducing localized colonic ulceration using methods other than chemicals, such as controlled electrolytic lesions, may provide an alternative procedure for the confirmation of the effects observed with chemical agents. Bipolar electro-cautery is used daily on thousands of patients to control bleeding lesions, and is known to produce localized tissue injury and hemostasis without systemic effect [26]. A detailed description of the technique of electrolytic lesion and its preliminary effects on intestinal absorption and cytokine secretion has been reported previously [19].

Our aim in this study was to determine the effect of inducing an ulcer in the colon by electro-cautery on: (1) Interleukin 1β, Interleukin 6 and TNF-α mRNA expression as well as production in the colon and in distant small intestinal sites, (2) jejunal alanine absorption; and (3) to describe the time course of the change in cytokine expression, production and alanine absorption in relation to ulcer induction and healing.

2. Methods

All animal experiments were approved by the Institutional Review Board animal Care Committee and the University Research Board of the American University of Beirut. Adult Sprague–Dawley rats weighing 180–220 g were used in all experiments. They were kept under standard colony conditions (12 h light/dark cycle, 22 ± 2 °C) and fed lab chow and water ad libitum. For experimental purposes, animals were fasted for 12 h with free access to water. Rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) injection prior to all surgical and invasive procedures.

2.1. Reagents and chemicals

Chloroform, isopropanol, PEPC (di-ethyl pyrocarbonate) water, agarose, and ethidium bromide were obtained from (Amresco, Solon, Ohio). Trizol (AB gene, UK), tris, primers for the cytokines, and RT-PCR kits were obtained from AB Gene, USA. Alanine was obtained from sigma Chemicals, St. Louis, MO, USA and ^{14}C-labeled alanine from Amersham, USA.

2.2. Electro-cautery

The details of the procedure were published previously [19]. The electro-cautery probe is made of 20 cm plastic tube that covers two cauterizing electrodes wrapped by a catheter (P 100). The electrodes are made of two electrical wires insulated except for both extremities. The ball-shape distal extremities of the wires (distance 1–3 mm) were introduced into the descending colon to be placed in contact with the mucosa; the other extremities were connected to the pole of a direct current generator.

Under light anesthesia, the probe is introduced through the anal verge into the descending colon for a distance of 6.5–8.5 cm. The correct positioning of the electrodes is verified by the finger of the experimenter by following their trajectory in the lower abdominal cavity. A direct bipolar current (3–7 mA) is delivered through the electrodes for 30 s and then for another 30 s after reversing the polarity of the electrodes. Then, the probe is moved for a distance of ±0.5 cm and the current injection is repeated. In sham rats the same procedure was repeated (introduction of the probe) without current delivery.

2.3. Ulcer assessment and histopathological examination

Rats were sacrificed at various time intervals after electro-cautery (3–96 h). The descending colon was opened to determine ulcer severity and tissue biopsies were obtained from the duodenum, jejunum, ileum and areas of the colon proximal or distal to the lesion. Satoh's criteria [20] were used to determine the severity of colonic inflammation which was visually assessed at the end of the experiments using a scale of 0–3; a score of zero was given to normal mucosa, 1 to mucosal erosion, 2 to moderate lesion and 3 to deep ulcer. Segments from small and large intestines were removed, washed with physiologic saline and fixed with 4% paraformaldehyde. Then, they were cut into 5 μm thick sections for light microscopy. Sections were stained with Hematoxylin and Eosin, and Periodic Acid Schiff's reagent. Pathologic examination was done by an expert who was blinded to the experimental protocols. Any change in the structure and shape of the intestinal layers and the presence and severity of any inflammatory cell infiltrates were recorded [19].

2.4. Jejunal alanine absorption

Measurement of alanine absorption in vivo was done using the previously described single pass intraluminal perfusion technique [21]. Briefly, the abdominal cavity of anesthetized rats was opened by a midline incision and the jejunum exposed with its blood supply kept intact.

A segment of the proximal jejunum was cannulated by an inlet catheter placed 5 cm distal to the ligament of Treitz and an outlet catheter placed 15–20 cm distal to it. The segment was repositioned in the abdominal cavity and covered with paraffin oil. The temperature of the animal was kept at 37 °C. The cannulated jejunal segment was perfused by a peristaltic pump at a constant rate of 0.7 ml/min for a period of 200 min. The perfusate consisted of modified Ringer solution [(in mM) 140 NaCl, 10 $KHCO_3$, 1.2 K_2HPO_4, 1.2 $MgCl_2$, and 1.2 $CaCl_2$] containing 1 mM cold alanine, 10 mCi 14 C alanine, and 15 mg/l of phenol red. The non-absorbable indicator was used to correct for any changes in alanine absorption resulting from intestinal water transport. The effluent solution was collected at 20-min intervals for a period of 180 min. One-milliliter aliquots of the initial and effluent solutions were assayed for phenol red concentration and radioactivity content. Absorption was calculated from the rate of disappearance of labeled alanine from the perfusate, taking into account water movement as measured by the change in phenol red concentration. Alanine absorption was measured in sham rats and in experimental rats immediately, and at 3, 12, 24, 48 and 72 h after delivery of current.

2.5. Tissue processing and IL-1, IL-6 and TNF-α protein determination

Full thickness strips of duodenal, jejunal, ileal or colonic segments (next to the induced ulcer) weighing 0.08–0.12 g per strip were homogenized on ice in PBS [0.818 g of NaCl, 0.02 g of KCl, 0.02 g of KH_2PO_4 and 0.115 g of Na_2HPO_4 in 100 ml DDW (pH = 7.2–7.4)] modified by adding 2.3376 g of NaCl, 0.5% of Bovine Serum Albumin, 0.05% of Tween 20 and protease inhibitor cocktail tablets for the complete inhibition of protease during tissue processing. Homogenates were then centrifuged at 4 °C for 1 h at a speed of 11,000 rpm, and samples were taken from the supernatant and used for protein determination and cytokine