Divergent Effects of Nicotine Administration on Cytokine Levels in Rat Small Bowel Mucosa, Colonic Mucosa, and Blood

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Abstract

Background: Chronic nicotine administration has a dual effect on inflammatory bowel disease: augmentation of jejunitis and amelioration of colitis. We previously showed that chronic nicotine administration has divergent regional effects on small bowel and colonic mucosal mediators and blood flow.

Objective: To examine the effects of nicotine administration on cytokine levels in normal rat small bowel mucosa, colonic mucosa, and blood.

Methods: Male Sprague-Dawley rats weighing 200–250 g were given nicotine (12.5 μg/ml) that was dissolved in tap water. Rats were sacrificed on days 1, 2, 7 and 14 after nicotine initiation; blood was withdrawn, and small bowel and colon were resected, washed and weighed. Mucosal scrapings were extracted in 2 ml Krebs-Hemselest buffer for determination of interleukins-2, 6 and 10 using the Biosource International Immunoassay Kit.

Results: Nicotine decreased IL-10 and increased IL-6 levels in small bowel mucosa (from 3.5 ± 0.5 to 0.4 ± 0.1 pg/ml and from 1.9 ± 0.4 to 13.6 ± 0.4 pg/ml respectively; $P < 0.05$). Nicotine decreased IL-2 levels in the colon (from 15.8 ± 3.0 to 7.9 ± 1.0 pg/ml; $P < 0.05$), having no effect on IL-10 or IL-6 levels. Rats treated with nicotine had lower IL-6 and IL-2 blood levels compared to control rats.

Conclusions: Nicotine has different regional effects on small bowel and colonic cytokine mucosal levels, which might explain some of its opposite effects on small bowel and colonic inflammation.

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Cigarette smoking has long been known to alter the course of inflammatory bowel disease [1–6]. Although the two forms of inflammatory bowel disease – ulcerative colitis and Crohn's disease – share many clinical features, the effects of cigarette smoking on the course of UC and CD are quite distinct. In ulcerative colitis, cigarette smoking appears to be uniquely beneficial. Most patients with UC are non-smokers, and the onset of both the first bout of UC and disease recurrence correlate statistically with the cessation of cigarette smoking [1–3]. Nicotine is one of the main active components of cigarette smoke and therefore has been suggested as an active agent in this protective effect. Nicotine patches were shown in a controlled double-blind placebo study to ameliorate active UC [4]. In contrast, smoking potentates the course of Crohn's disease and smokers have a high risk of both clinical and surgical recurrences of CD, although some studies show a lack of correlation between smoking and CD [2,3,5,6]. The mechanisms underlying this major discrepancy in the effects of cigarette smoking in the two forms of inflammatory bowel disease remain unclear. One obvious difference is the site of involvement. UC involves the various regions of the colon in a contiguous manner commencing in the rectum; whereas CD often spares the rectum, but most frequently involves any portion of the small intestine, usually with predominant features in the terminal ileum.

The effect of nicotine administration in animal models of gastrointestinal injury was first examined in the colon. We have shown a biphasic effect of chronic nicotine treatment in a trinitrobenzene sulfonic acid-induced colitis model with features mimicking CD. While lower doses of nicotine (12.5–25 μg/ml) in the drinking water started 10 days prior to TNBS administration and until sacrifice were protective, higher doses (250 μg/ml) were deleterious [7]. The effects of cigarette smoking or nicotine on small intestinal inflammation have been less well characterized. Chronic nicotine administration at a dose protective to the colon in the TNBS and the iodoacetamide models (12.5 μg/ml) aggravated jejunitis in a model of small intestine inflammation induced by iodoacetamide delivery [8]. We later demonstrated the same effects in the same animal in a model of jejunitis and colitis in IL-10 knockout mice [9].

In order to further assess the impact of regional effects of nicotine on intestinal inflammation, we investigated the effects of nicotine administration on cytokine profile in small bowel, colonic mucosa and blood of normal rats.

Methods

Animals

Male, Sprague-Dawley rats weighing 200–250 g were housed in our standard laboratory facility, given standard rat laboratory chow and drinking water *ad libitum*, and exposed to an alternating 12 hour light-dark cycle.

Nicotine (12.5 μg/ml) was dissolved in tap water with rats drinking *ad libitum*; control rats received tap water alone *ad libitum*. The average daily nicotine intake was 106 μg/rat/24 hours. Nicotine administration was given for up to 14 days. Nicotine alone had no effect on daily food or water intake of rats compared with controls.

IL = interleukin
UC = ulcerative colitis
CD = Crohn's disease

TNBS = trinitrobenzene sulfonic acid

The dose of nicotine chosen was previously reported to produce the most effective colonic protection in rats, producing plasma cotinine levels similar to those reached in typical cigarette smokers [7]. Rats were sacrificed 1, 2, 7 and 14 days after nicotine was initiated.

Laparotomy was performed under light anesthesia, Avertin 2,2,2-tribromoethanol in 2-methyl-2-butanol at 20 µl/g mice (Aldrich, Milwaukee, USA), and 1 ml of blood was aspirated by cardiac puncture. Following laparotomy, the colon and entire small bowel were isolated, washed, weighed and inspected in entirety; mucosa was scraped and extracted for cytokine determination.

Determination of mucosal cytokine levels

Mucosal scrapings were placed in 2 ml Krebs-Hemselest buffer, homogenized with polytron, centrifuged for 15 minutes at 4°C and supernatant was used for the determination of rat IL-2, 6, and 10 activity, using the Biosource International Immunoassay Kit (Biosource International Inc., Camarillo, CA, USA) (pg/ml).

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis for significant differences was performed according to the Student's *t*-test for unpaired data and the non-parametric Mann-Whitney U test.

Results

Effect of nicotine on cytokine profile in small bowel mucosa

The group of rats treated with 12.5 µg/ml nicotine all showed normal appearing mucosa. Twenty-four hours after the beginning of nicotine administration (12.5 µg/ml in the drinking water), IL-2 levels in small bowel mucosa decreased significantly compared to control rats: 5.5 ± 1.0 versus 19.2 ± 4.9 pg/ml respectively; $P < 0.05$; n=5–10). The levels returned to baseline after 2 and 7 days of nicotine administration and decreased significantly again when measured after 14 days of nicotine administration (9.2 ± 1.4 pg/ml; $P < 0.05$) [Figure 1].

Mucosal IL-6 levels increased by 8–12 fold over time in nicotine-treated rats, from 1.9 ± 0.4 pg/ml at baseline to 13.6 ± 0.4 pg/ml after 7 days of nicotine administration ($P < 0.05$), and returned to baseline after 14 days of treatment [Figure 1]. Small bowel mucosal IL-10 levels decreased significantly after 1, 2 and 7 days of nicotine treatment compared to control rats: 0.42 ± 0.1 vs. 3.5 ± 0.5 pg/ml respectively ($P < 0.05$; n=5–10), and returned to baseline after 14 days [Figure 1]. Thus, acute nicotine administration (1 or 2 days) significantly decreased IL-2 and IL-10 jejunal levels and increased IL-6 levels, while chronic administration (7 days) caused an increase in IL-6 and a decrease in IL-10 levels.

Effect of nicotine on cytokine profile in colonic mucosa

The addition of nicotine to the drinking water had no effect on colonic appearance, weight, myeloperoxidase and nitric oxide synthase activities and prostaglandin E_2 generation in normal rats (data not shown). At 1, 2 and 14 days after nicotine administration (12.5 µg/ml in drinking water), IL-2 levels in colonic mucosa decreased significantly compared to control rats (7.9 ± 1.0 vs.15.8 ± 3.0 respectively; $P < 0.05$; n=5–9 rats). There was no change from baseline of IL-6 and IL-10 mucosal levels at any given time point (P = not significant). Thus, the colonic cytokine profile behaved totally different to both acute and chronic nicotine exposure compared to small bowel mucosa [Figure 1].

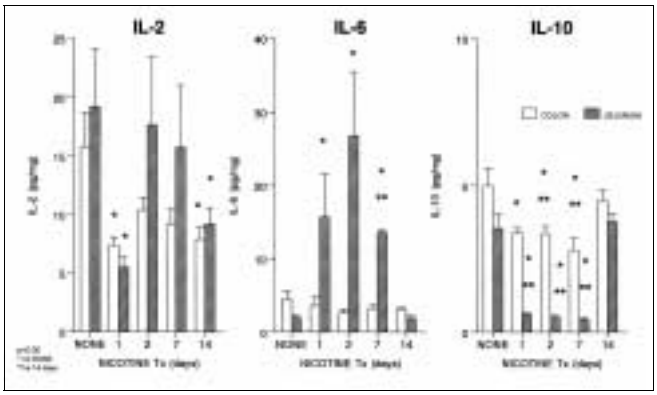

Figure 1. Rats were given nicotine, 12.5 µg/ml, in their drinking water. They were sacrificed after 1, 2, 7 and 14 days; their colon and small bowel were isolated, washed and weighed and their mucosa scraped and extracted for cytokine determination as described in the Methods section.
Results are mean ± SE. * denotes $P < 0.05$ versus control rats.

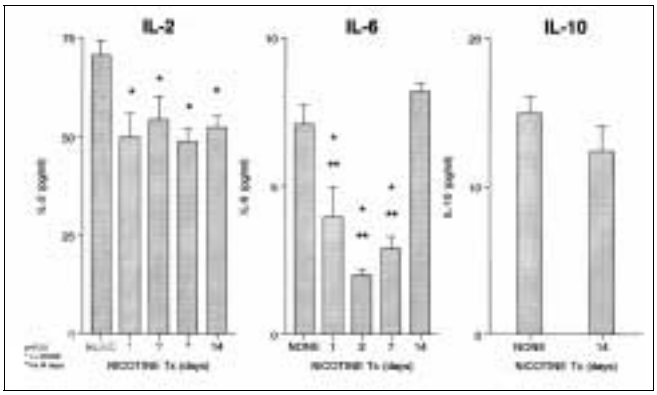

Figure 2. Rats were given nicotine, 12.5 µg/ml, in their drinking water. They were sacrificed after 1, 2, 7 and 14 days. One milliliter of blood was aspirated by cardiac puncture and assessed for cytokine levels. Results are mean ± SE. * denotes $P < 0.05$ versus control rats.

Effect of nicotine on cytokine profile in the blood

The addition of nicotine (12.5 µg/ml in drinking water) to rats significantly changed blood IL-2 and IL-6 levels, but had no effect on IL-10 colonic mucosal levels [Figure 2]. The decrease in IL-2 levels was found in all time periods tested (70.5 ± 3.8 at baseline to 52.4 ± 3.0 pg/ml after 14 days of nicotine administration, $P < 0.05$; n=5–16 rats). IL-6 blood levels showed a similar pattern but returned to baseline after 14 days of nicotine administration (7.1 ± 0.66 at baseline, 2.9 ± 0.4 after 7 days and 8.2 ± 0.3 pg/ml after 14 days; n=5–16).

Discussion

Cigarette smoking has been identified as the only environmental factor that alters the course of inflammatory bowel disease.

However, the precise mechanisms of its actions remain elusive. Further, a discrepancy emerges in its effects in the disorders comprising inflammatory bowel disease: namely, amelioration in ulcerative colitis and, in most studies, aggravation of the course of Crohn's disease. While explanations for these differences remain unclear, our laboratory has demonstrated that nicotine exacerbates jejunal injury induced by iodoacetamide in rats, while protecting against colonic inflammation induced by the same agent and at the same dose [8]. In addition, we have demonstrated that nicotine aggravates jejunitis but protects against colitis in the IL-10 -/- mouse model [9]. The divergent effects of cigarette smoking and/or nicotine in human UC versus CD (characterized by small intestinal involvement), and in small bowel compared to colonic injury induced by iodoacetamide, suggest that regional differences may exist between mechanisms of mucosal defense in the small intestine versus the colon. In our studies, interesting regional differences emerged: in the rat normal jejunum, chronic nicotine administration decreased PGE_2 generation and increased NOS activity but had no effect on the microcirculation; whereas microcirculation in the colon was enhanced, but NOS activity or PGE_2 generation was not affected by nicotine in the normal colon [8].

In the mouse model, we examined the regional effects of nicotine on mRNA level proteins – intestinal trefoil factor and somatostatin – which we found to be protective against gastrointestinal injury. Intestinal trefoil factor is a member of the recently described trefoil family of peptides, a group of proteins expressed primarily by mucus cells throughout the gastrointestinal tract and whose compact structure imparts unique stability against pH changes and enzymatic degradation in the gut [9]. Intestinal trefoil factor has been shown to enhance epithelial restitution of colonic and intestinal epithelial cells, a mechanism important in gastrointestinal mucosal defense, and increase the viscosity of intestinal mucus – two mechanisms that may, at least, in part, explain intestinal trefoil factor's protective effect against injury in the gastrointestinal tract [9]. We demonstrated that nicotine consistently increased colonic intestinal trefoil factor mRNA expression as compared to untreated wild-type mice, having no effect in the jejunum.

The present study provides further evidence for distinct and different effects of nicotine on small and large bowel. Nicotine, at a dose protective to the colon, decreased IL-10 and increased IL-6 production in the small bowel, and had a biphasic effect on IL-2. On the other hand, its only effect in the colon was to significantly decrease IL-2 levels in both the acute and chronic phases of administration. Thus nicotine administration decreased anti-inflammatory mediator (IL-10) levels in the small bowel and increased the levels of pro-inflammatory mediator (IL-6), possibly contributing to mucosal damage in that region. Nicotine decreased the levels of the pro-inflammatory mediator (IL-2) in colonic mucosa. There was no correlation between mucosal and blood levels of the cytokines examined. Other authors have found, in different settings, similar effects of smoking/nicotine on the pro-inflammatory mediators IL-8, IL-1 and tumor necrosis factor in colonic mucosa [10,11].

In summary, chronic nicotine administration at a dose of 12.5 μg/ml in the drinking water had different effects on cytokine expression in the small and large bowel of normal rats. Taken together with other studies and our previous findings, these differential regional effects of nicotine on both sites may explain in part the divergent effects of smoking on either UC or Crohn's disease.

PGE_2 = prostaglandin E_2
NOS = nitric oxide synthase

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Because it's there

George Leigh Mallory (1886-1924), British mountaineer