Original Article

Effects of oral curcumin on indomethacin-induced small intestinal damage in the rat

Alessandro Menozzi[1,*], Cristina Pozzoli[2], Enzo Poli[2], Mario Martelli[3], Laura Martelli[3], Chiara Zullian[1], Simone Bertini[1]

[1] *Department of Animal Health, University of Parma, Parma, Italy;*
[2] *Department of Human Anatomy, Pharmacology and Forensic Medicine, University of Parma, Parma, Italy;*
[3] *Department of Chemical Sciences, University of Padova, Padova, Italy.*

ABSTRACT: Nonsteroidal anti-inflammatory drug (NSAID)-induced injury on gastrointestinal tract is well documented, and jejunal inflammation caused by indomethacin in rats is a broadly used experimental model of enteritis. We evaluated the effect of oral curcumin, a compound known to possess anti-inflammatory and anti-oxidant properties, on indomethacin-induced enteritis in the rat. Curcumin (50, 100, and 300 mg/kg) was given to rats by oral gavage 48, 24, and 1 h before enteritis was induced by intragastric administration of 20 mg/kg indomethacin. After 24 h, intestinal macroscopic lesions, myeloperoxidase activity and lipid peroxidation levels were assessed. Curcumin at the dose of 50 mg/kg was uneffective, while at the dose of 100 and 300 mg/kg significantly reduced macroscopic damage caused by indomethacin. By contrast, curcumin at all tested doses was unable to modify indomethacin-induced increases of myeloperoxidase and lipid peroxidation. Curcumin (100 and 300 mg/kg) significantly increased lipid peroxidation level in normal intestinal tissues of rats. Present data show that oral curcumin protects against macroscopic injury induced by indomethacin, leaving unaffected neutrophil infiltration and oxidative cell damage, thus suggesting that this beneficial effect is due to mechanisms not involving anti-inflammatory or anti-oxidant activities.

Keywords: Curcumin, indomethacin, rat, small intestine

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most used classes of drugs, despite their well documented tendency to induce gastroduodenal injury. It is now well established that NSAID use is associated with small intestine and colon damage and worsening of chronic intestinal inflammatory diseases such as ulcerative colitis (UC) or Crohn's disease (CD) (*1*,*2*). While prostaglandin (PG) depletion following cyclooxygenase (COX) inhibition is acknowledged as the crucial pathogenetic event of NSAID-induced gastric damage, the etiology of intestinal injury seems to be more complex, and other mechanisms are likely to be involved, such as mitochondrial dysfunction leading to increased mucosal permeability, luminal bacteria invasion of gut wall, neutrophil-induced oxidative damage and microvascular injury (*3*,*4*). However, a general consensus is gathered around the damaging effects of reactive oxygen species (ROS) released by activated leukocytes in the intestinal mucosa (*5-7*) leading to several studies about the therapeutic potential of antioxidant agents against NSAID-induced enteropathy.

Curcumin (diferuloylmethane) is a bioactive constituent of turmeric (*Curcuma longa*), which has been shown to possess many pharmacological properties, ranging from anti-inflammatory activity to anticancerogenic and antibacterial effects (*8-10*). In particular, curcumin was proven to be effective in protecting against oxidative stress by means of a direct scavenging action of ROS and by the activation of endogenous antioxidant enzymes like catalase, superoxide dismutase and glutathione transferase (*11*,*12*).

The aim of the present study was to evaluate the effects of curcumin on the small intestinal damage induced by acute administration of indomethacin in the rat. Indomethacin-induced enteritis is an experimental model widely used to test new treatments against NSAID-induced enteropathy, and for screening of novel drugs against inflammatory bowel disease (IBD), because of the histo-pathological and functional similarities between human CD and indomethacin jejunal lesions in rats (*13*).

**Address correspondence to:*
Dr. Alessandro Menozzi, Department of Animal Health, University of Parma, Via Del Taglio 10, 43100 Parma, Italy.
e-mail: alessandro.menozzi@unipr.it

2. Materials and Methods

2.1. *Animals*

Male Wistar rats (220-240 g) were purchased from Harlan-Italy (Milan, Italy). They were housed in a restricted access room with controlled temperature (23°C) and a light/dark (12 h:12 h) cycle, and allocated in wire mesh cages with a maximum of 4 subjects per cage. Food and water were provided ad *libitum*. The study was approved by the local Animal Ethic Committee of the Faculty of Medicine, University of Parma, Italy.

2.2. *Induction of intestinal damage*

Enteritis was induced in four groups (n = 6 for each) of unfasted rats, by means of a single intragastric administration of 20 mg/kg indomethacin, suspended in 1% carboxymethylcellulose (CMC), in a total volume of 5 mL/kg b.w. Three groups of indomethacin-treated rats were also pre-treated with curcumin by oral gavage at the dose of 50, 100 or 300 mg/kg. Curcumin was suspended in 5 mL/kg of 1% CMC and administered 48, 24, and 1 h before indomethacin. Control group received an equal volume of 1% CMC p.o. following the same protocol. In order to assess the effect on healthy intestine, separate groups of rats were given curcumin alone, at the same doses and times described above. Rats were sacrificed by cervical dislocation under ether anesthesia 24 h after indomethacin administration and the intestinal lesions were evaluated.

2.3. *Macroscopic evaluation of intestinal damage*

The small intestine was removed from each animal and the first 20 cm of the proximal region, starting from the pylorus, were discarded. The remaining portion of intestine was divided into 8-10 segments of about 5 cm length. Intestinal segments were opened along the antimesenteric border, gently rinsed of fecal contents, fixed on a slide and photographed for macroscopic evaluation of damage. Damaged area of each segment was calculated by means of a digital image analysis software (ImageJ, NIH) and summed per small intestine. The amount of intestinal injury was expressed as a percentage of damaged area over the total examined intestinal mucosa. The examiners were unaware of animal treatment.

2.4. *Myeloperoxidase activity*

Intestinal myeloperoxidase (MPO) activity was assumed as a quantitative index of mucosal inflammation and was measured according to a previously described method with minor modifications (*14*). Briefly, a 5 cm-long segment of jejunum from each rat was homogenized in 1 mL hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% in 50 mM phosphate buffer, pH 6.0) for each 50 mg tissue and centrifuged at 12,000 g for 15 min at 4°C. An aliquot of the supernatant (7 µL) from each sample was then added to 200 µL of a reaction mixture containing 0.167 mg/mL *O*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide in 50 mM phosphate buffer at pH 6.0. Changes in absorbance at 450 nm were measured using a microplate absorbance reader (Tecan Sunrise, Tecan Inc., Mannedorf, Switzerland). One unit of MPO was assumed as that degrading 1 µM hydrogen peroxide per minute at 25°C. Data were expressed as units of MPO per mg of tissue.

2.5. *Lipid peroxidation*

Lipid peroxidation was evaluated as an index of oxidative damage and was assessed by measuring thiobarbituric acid reactive substances in intestinal tissues, according to a previously described technique (*15*), with minor modifications. Samples of jejunum from treated rats were collected, homogenized in 0.15 M KCl (1 mL for 100 mg wet tissue) and centrifuged at 400 g for 10 min. Aliquots (0.5 mL) of supernatants were then mixed to 1 mL 0.6% thiobarbituric acid, 3 mL of 1% phosphoric acid and 83 µL of a 0.2% solution of 2,6-ditert-butyl-4-methylphenol in 95% ethanol. After heating at 85°C for 60 min, samples were then ice-cooled and centrifuged at 2,600 g for 15 min, and the absorbance of the supernatant was measured using a multiplate spectrophotometer (Tecan Sunrise, Tecan Inc., Mannedorf, Switzerland) at a wavelength of 530 nm. Results were expressed as µmoles of malondialdehyde (MDA) per mg tissue.

2.6. *Statistical analyses*

Results were expressed as means ± SEM from 6 rats. Differences among groups were evaluated by one-way analysis of variance, followed by Dunnett's test. A p value less than 0.05 was considered statistically significant. Calculations were performed by a commercial software (GraphPad Prism, ver.3.03, GraphPad Software Inc., San Diego, CA, USA).

2.7. *Drugs*

Drugs and reagents (indomethacin, curcumin and all the analytical chemicals) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All drugs were prepared immediately before use, as suspensions in 1% CMC, and administered by intragastric route in a volume of 5 mL/kg b.w.

3. Results

3.1. *Macroscopic damage*

Intragastric treatment with 20 mg/kg indomethacin resulted in a severe intestinal damage, characterized by hyperemia, segmental mucosal ulcerations extending along the mesenteric border of the jejunum and bowel thickening (Figure 1). The area of macroscopically visible damage induced by indomethacin extended for 8.96 ± 0.97% of the total examined intestinal area (Figure 2). Treatment with curcumin at the lowest dose (50 mg/kg) did not modify indomethacin-induced injury (Figure 2). By contrast, intestinal damaged area induced by indomethacin was significantly reduced by curcumin at 100 mg/kg (4.97 ± 0.84%; $p < 0.01$). The highest dose of curcumin employed (300 mg/kg) was able to ameliorate jejunal lesions (5.52 ± 0.78%; $p < 0.05$) even though the effect was not significantly different than that of the lower dose of 100 mg/kg (Figures 1 and 2). Curcumin alone left intestinal mucosa completely unaffected (data not shown).

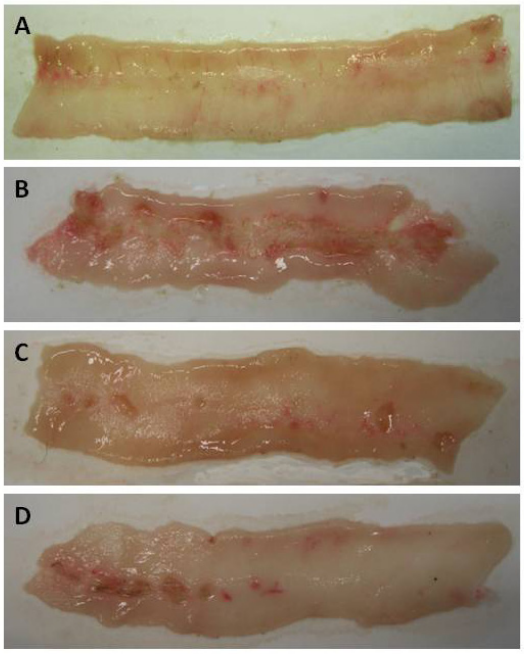

Figure 1. Macroscopic lesions of rat jejunum. A, normal intestine; **B**, indomethacin plus vehicle; **C**, indomethacin plus curcumin 100 mg/kg; **D**, indomethacin plus curcumin 300 mg/kg.

3.2. *Myeloperoxidase assay*

Indomethacin administration caused a more than three-fold increase in mucosal MPO activity (0.067 ± 0.01 U/mg; $p < 0.01$) compared to normal intestine (0.021 ± 0.002 U/mg) (Figure 3). Pre-treatment with curcumin at the dose of 50, 100 or 300 mg/kg did not affect MPO levels in a significant fashion with respect to indomethacin-treated rats (Figure 3). Curcumin at all tested doses did not modify MPO levels of normal intestinal tissues (data not shown).

3.3. *Lipid peroxidation*

Indomethacin administration enhanced lipid peroxidation in intestinal tissue, as demonstrated by the significant increase of MDA levels with respect to vehicle-treated rats (0.065 ± 0.02 *vs*. 0.029 ± 0.003 µmol/mg; $p < 0.01$). Curcumin administered at the dose of 100 mg/kg

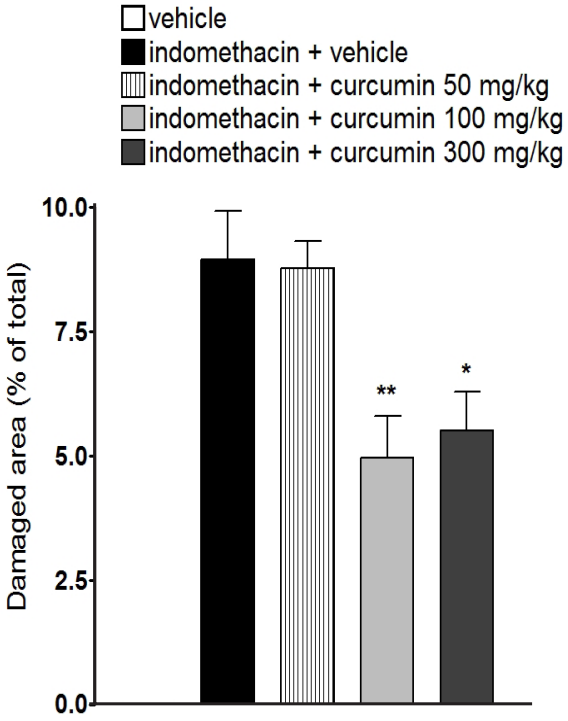

Figure 2. Effect of curcumin (50, 100, and 300 mg/kg) on macroscopic damage induced by indomethacin (20 mg/kg) in the small intestine of rats. Bars represent means ± SEM of 6 experiments. * $p < 0.05$ and ** $p < 0.01$ *vs*. indomethacin + vehicle.

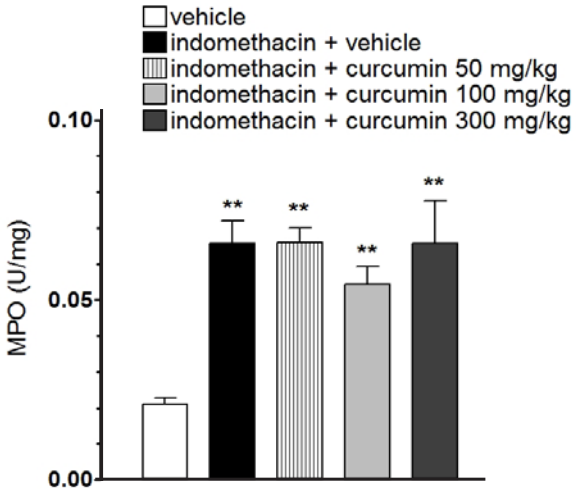

Figure 3. Effect of curcumin (50, 100, and 300 mg/kg) on myeloperoxidase (MPO) activity, as an index of neutrophil infiltration. Results are expressed as means ± SEM of 6 experiments. ** $p < 0.01$ *vs*. vehicle.

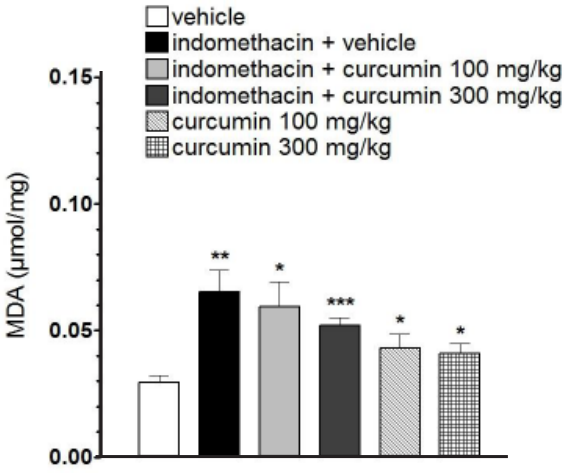

Figure 4. Effect of curcumin (100 and 300 mg/kg) on malondialdehyde (MDA) levels, as an assay of lipid peroxidation. Results are expressed as means ± SEM of 6 experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ *vs*. vehicle.

and 300 mg/kg was devoid of significant effects on indomethacin-treated rats (Figure 4). Curcumin alone was able to significantly increase lipid peroxidation in healthy intestinal mucosa at both 100 and 300 mg/kg (0.043 ± 0.005; $p < 0.05$ and 0.041 ± 0.004; $p < 0.05$, respectively) (Figure 4).

4. Discussion

The purpose of this study was to evaluate the effects of oral curcumin on an experimental model of intestinal inflammation, the enteritis induced in rats by indomethacin administration. As in previous works (*3*,*5*), indomethacin induced a severe inflammation in the rat small intestine with hyperemia, erosions and linear ulcerations of the mucosa. Besides macroscopically visible damage, indomethacin caused a marked mucosal neutrophil infiltration and increased lipid peroxidation as evidenced by the enhanced MPO and MDA levels, respectively. Curcumin gavage at the dose of 50 mg/kg was unable to modify the area of damage, while at 100 or 300 mg/kg resulted effective in reducing the extension of macroscopic lesions, showing a dose-independent protecting effect. By contrast, all tested doses of curcumin were uneffective on indomethacin-induced increases of MPO activity or MDA levels. Interestingly, curcumin given alone, caused a significant increase of intestinal lipid peroxidation in normal rats both at 100 and 300 mg/kg, while leaving MPO activity unaffected.

Several authors have already demonstrated the efficacy of this compound against damage induced by various stimuli both in the stomach (*16*,*17*) and in the intestine (*18-20*) of experimental animals and there seems to be general consensus that the antioxidant properties of curcumin play an important role in its protective effects (*9-12*). Indomethacin-induced gastric damage was reduced by curcumin in rats and this beneficial effect was linked to the scavenging of ROS exerted by the drug and to a protective activity on endogenous antioxidant enzymes (*21*). Moreover, in previous studies, intestinal damage caused by indomethacin in rat small intestine was ameliorated by intraperitoneal administration of curcumin, which reduced MDA levels and restored catalase and glutathione peroxidase activity (*22*) or decreased oxidative stress in mithocondria (*23*). In our experiments, curcumin, administered by a different route, *i.e.* by oral gavage, was effective in reducing the extent of indomethacin-induced lesions, in accordance to data shown in the study by Sivalingam *et al*. (2007), even if the protective dose in our study was 5 times higher. This is not surprising, as it could be due to lower bioavailability by the intragastric route. Remarkably instead, oral curcumin failed to decrease neutrophil infiltration or lipid peroxidation in inflamed intestinal tissues of rats. Moreover, curcumin was able to increase MDA level in normal mucosa at both doses which were protective against indomethacin-induced macroscopic injury. Actually, curcumin has been shown to possess a dual behavior of anti-oxidant/pro-oxidant, being able to reduce the formation of ROS as well as to increase it, and to promote cell apoptosis by stimulating the production of oxidative radicals (*24-26*). The enhancement of lipid peroxidation in healthy jejunum of rats induced by curcumin could therefore explain the lack of efficacy of this drug on MDA levels in inflamed intestine, and suggest that ROS concentrations are not crucial in the protective effect exerted by curcumin on indomethacin-induced enteritis.

However, pathogenesis of NSAID-induced intestinal damage is unanimously considered multifactorial and several mechanisms other than peroxidation of membrane lipids by means of neutrophil-derived oxidative radicals are involved. Microvascular injury, due to the lack of vasodilation by PGs and to occlusions following the accumulation of inflammatory cells in the lumen of microvessels, which is thought to be fundamental for the developing of NSAID-induced lesions in the stomach (*27*,*28*), plays a role in the etiology of intestinal damage as well (*5*). The importance of vasodilation in the protection of gastrointestinal mucosa against the noxious effects of NSAIDs is demonstrated by the lack of lesivity of nitric oxide (NO)-donor NSAIDs (*29*) or by the protective effects of vasodilators on indomethacin-induced gastric and intestinal damage (*30*,*31*). We could therefore hypothesize that the beneficial effect of curcumin shown by our study was possibly due to a vasodilator activity and, by literature data, this compound was indeed shown to possess relaxant properties on isolated rat aorta and on porcine coronary arteria (*32*,*33*). Moreover, a previous study has shown that curcumin is effective in ameliorating dinitrobenzene sulfonic acid

(DNBS)-induced colitis in mice by acting as an agonist on transient potential vanilloid receptor 1 (TRPV1) (*34*) and could therefore induce the release of vasorelaxant mediators such as substance P, calcitonine gene – related peptide (CGRP) or NO (*35*) from sensory nerve terminals. There is also evidence that NSAIDs are able to block TRPV1 receptors (*36*) and this might contribute to the antiinflammatory and analgesic effects, as well as to the damaging activity, since it was demonstrated that TRPV1 mediates protection against experimental colitis in mice and rats (*37*,*38*). We cannot therefore exclude that these mechanisms may also be involved in the protective effect exerted by curcumin against indomethacin-induced enteritis. On the other hand, the TRPV1-mediated release of vasoactive substances could cause an increase of vascular permeability and subsequent neutrophil infiltration of intestinal mucosa, thus explaining the lack of efficacy of curcumin on MPO levels in our experimental conditions.

In conclusion, this study demonstrated that curcumin, given orally, is able to protect against acute macroscopic injury caused by indomethacin in rat small intestine and that this effect is not likely involving antioxidant properties. Further experiments, however, will be necessary to elucidate the mechanisms underlying the beneficial activity exerted by this drug on NSAID-induced enteritis.

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