Amelioration of Experimental Colitis by Thalidomide

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Abstract

Background: Rectal administration of iodoacetamide induces colitis by blocking sulphhydryl groups and generating inflammatory mediators. Thalidomide, a non-barbiturate hypnotic, also has an anti-inflammatory effect, presumably by suppressing the production of tumor necrosis factor alpha. In patients with Crohn's disease, neutralization or suppression of TNFα reduces inflammation.

Objectives: To evaluate the effects of thalidomide in a model of experimental colitis.

Methods: Colitis was induced in rats by intracolonic administration of 3% iodoacetamide. In the treatment group, thalidomide 50 mg/kg was given daily by gavage and continued for 7 days until the rats were sacrificed. Their colons were then processed for wet weight, lesion area, weight of mucosal scraping, myeloperoxidase activity and histology. Serum levels of TNF were determined.

Results: Colonic wet weight, lesion area, myeloperoxidase activity and serum levels of TNFα were significantly lower ($P < 0.05$) in the treatment group (iodoacetamide + thalidomide) than the control group (iodoacetamide only). Histologically, colonic inflammation in the treated group was markedly decreased.

Conclusions: Thalidomide effectively decreases colitis induced by iodoacetamide. The mechanism is probably associated with inhibition of TNFα, and should be further studied.

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Thalidomide was used in the past as a non-barbiturate sedative and anti-emetic, but its use was prohibited in most countries due to teratogenicity. Recently, thalidomide has experienced a revival as an immunomodulatory drug for the treatment of certain diseases [1]. Thalidomide is considered the drug of choice for erythema nodosum leprosum and is also beneficial in other conditions such as cachexia due to certain chronic infections and malignancies, oral and esophageal ulcers in AIDS, Behcet's colitis, graft-versus-host disease after bone marrow transplantation, discoid lupus, prurigo nodularis, erythema multiforme and rheumatoid arthritis [2–5]. Recently, the drug was found to be effective in patients with refractory Crohn's disease [6,7], and favorable responses to thalidomide were reported in the past also in a few patients with ulcerative colitis [8].

The mechanism of action of thalidomide has been attributed mainly to the inhibition of tumor necrosis factor alpha by enhancing the degradation of messenger RNA to TNF [9,10]. In addition to selective inhibition of TNF, thalidomide also caused a reduction in the serum levels of interleukin 1 and 6 in a model of lethal bacteremia [11].

Biopsies obtained from patients with ulcerative colitis and Crohn's disease have shown spontaneous production of TNFα by intestinal mucosal cells [12]. In addition, elevated serum concentrations of TNFα were found in children with chronic active inflammatory bowel disease [13]. TNF exerts its activity on endothelial cells, macrophages and T lymphocytes. Anti-inflammatory effects of the drug may also be attributed to modulation of the expression of adhesion molecules on leukocytes, thus inhibiting their migration [4,5,14,15]. Moreover, thalidomide has been found to inhibit angiogenesis [16,17], which might be responsible for its known teratogenic effects [5]. However, its anti-angiogenetic properties probably explain the therapeutic efficacy of thalidomide in some patients with advanced cancer and multiple myeloma [18,19].

In view of the important role of TNFα in inflammatory bowel disease, and the recent reports of favorable clinical responses to thalidomide in a few patients with Crohn's disease, we aimed to study the effects of thalidomide in a model of experimental colitis.

Materials and Methods

Thalidomide was purchased from Andrulis Pharmaceuticals (Maryland, USA), and iodoacetamide from Sigma Chemicals Co. (St. Louis, MO, USA). Male Wistar rats weighing 200–250 g, obtained from the Tel Aviv University Animal Breeding Center, were kept in the animal breeding house of the Wolfson Medical Center and fed Purina rodent chow *ad libitum*. The research was performed on the basis of the agreements of the Helsinki Committee.

Iodoacetamide-induced colitis

The rats were kept on a liquid diet from the night before the induction of colitis in order to decrease intestinal secretions

TNFα = tumor necrosis factor alpha

that might disturb absorption of iodoacetamide. The animals were first anesthesized by inhalation of ether. Colitis was then induced in two phases: first, the colon was cleaned by enemas of NaCl 0.9%; then 0.1 ml of 3% iodoacetamide dissolved in 1% methylcellulose was administered via a catheter placed 7 cm from the anus, and the intestine was inflated with air to increase surface area and absorption. Thalidomide was dissolved in a solution containing NaCl 0.9% and 20% ethanol.

Experimental design

The rats were divided into three groups of six rats each: colitis, colitis + thalidomide (50 mg/kg), and thalidomide only. In the second group (colitis + thalidomide), 2 days before the induction of colitis, thalidomide administration was initiated at a dose of 50 mg/kg by oropharyngeal gavage, and continued once daily for another 7 days. The control group received only thalidomide for the same time period. All the rats were sacrificed on the tenth day and the large intestines were resected and examined for the following parameters of inflammation: colonic weight (g/cm), surface area of the inflammation (cm^2), weight of colonic scrapings (including inflamed and unaffected tissue), myeloperoxidase activity, and morphological studies. Also, blood was drawn for the determination of serum TNFα levels.

Determination of MPO activity

We added 3 ml of 0.5 % hexadecyltrimethyl-ammonium bromide in 50 mmol/L phosphate buffer (pH 6.0) to 0.3 g of colonic tissue and the tissue was homogenized for 30 seconds three times. The homogenate was then sonicated for 10 sec, freeze-thawed three times and centrifuged for 15 minutes at 40,000 *g*. An aliquot of the supernatant was taken for determination of enzyme activity as described [20].

Determination of serum levels of TNFα

For determination of serum TNFα, MaxiSorp F96 microtiter plates (Nunc Inter Med, Denmark) were coated (overnight at 4°C) with anti-TNFα (2 μg/ml) in 0.1 $NaCO_3$ at pH 8.2. The plates were then washed twice with phosphate-buffered saline containing 0.05% (v/v) Tween 20 (Bio-Rad Laboratories, Hercules, CA, USA). Next, coated plates were blocked (1 hour at room temperature) with PBS-10% fetal calf sera (Biolab, Beverly, MA, USA). Aliquots (100 μl) of diluted (1:4) sera derived from the two groups of rats were incubated overnight (at 4°C) on the antibody-coated plates. The plates were washed four times with PBS and incubated (2 hours at room temperature) with biotinylated anti-cytokine antibody (2 μg/ml of PBS–10% fetal calf serum). After thoroughly washing (6 times), the plates were incubated (30 min at room temperature) with alkaline phosphatase-conjugated streptavidin (1.5 μg/ml in PBS 10%–FCS Jackson ImmunoResearch Laboratories, West Grovw, PA, USA), which binds to the biotinylated antibodies. P-Nitrophenyl phosphate (0.5 mg/ml) with 9.7% diethanol amine in water, pH 9.8, was used as a chromagen. The color that developed in the microtiter wells was read at 405 nm in an enzyme-lined immunosorbent assay reader.

Statistical analysis

The data are presented as means ± SD. Statistical analysis for significant differences was performed by Student's *t*-test for unpaired data.

Results

Intrarectal administration of iodoacetamide caused erosions and ulcerations in the colon after 7 days. In the control rats, which received thalidomide only, the colonic mucosa appeared normal. The lesion area was 4.07 ± 2.04 cm^2 in the rats that received iodoacetamide without thalidomide [Figure 1A].

The control group that was treated with iodoacetamide only had a higher degree of inflammation than the rats that received iodoacetamide and thalidomide. The colonic wet weight, a sensitive parameter of colitis, was 0.37 ± 0.12 g/cm in the rats with iodoacetamide only compared to 0.14 ± 0.03 g/cm in the treated group [Figure 1B]; $P = 0.004$. Moreover, the weight of colonic mucosal scrapings was twofold higher in the iodoacetamide group compared to the group that was also treated with thalidomide. As expected, the wet weight and the weight of the mucosal scrapings were lower in the control group treated with thalidomide only compared to the two other groups [Figure 1B and C]. The activity of MPO in the colonic tissue was 1.7 1.08 U/g in the iodoacetamide group versus 0.14 ± 0.07 U/g in rats that were treated also with thalidomide [Figure 2]; P=0.015. MPO in the iodoacetamide + thalidomide group was similar to that in the control group treated only with thalidomide [Figure 2].

The serum levels of TNF in iodoacetamide-induced colitis were 2.4-fold higher in rats that were not treated with thalidomide [Figure 3]; $P = 0.0006$.

Histological examination of these intestines showed deep mucosal and submucosal ulcerations associated with an inflammatory infiltrate consisting mainly of lymphocytes and macrophages [Figure 4A]. In the rats that received thalidomide (in addition to iodoacetamide) the lesion area was significantly smaller (0.78 ± 0.84 cm^2, $P = 0.008$) and histological analysis demonstrated only mild focal inflammation with small erosions limited to the mucosa [Figure 4B].

Discussion

In the present study, thalidomide was found to be effective in ameliorating iodoacetamide-induced colitis in rats. Improvement was observed in all parameters of inflammation: surface area, wet weight of the intestinal segment, weight of the intestinal scraping, MPO activity, and histology. Thalidomide decreased serum levels of TNFα, which may represent the mechanism of action. Recently, a beneficial therapeutic response to thalidomide was demonstrated in some patients

MPO = myeloperoxidase
PBS = phosphate-buffered saline
FCS = fetal calf serum

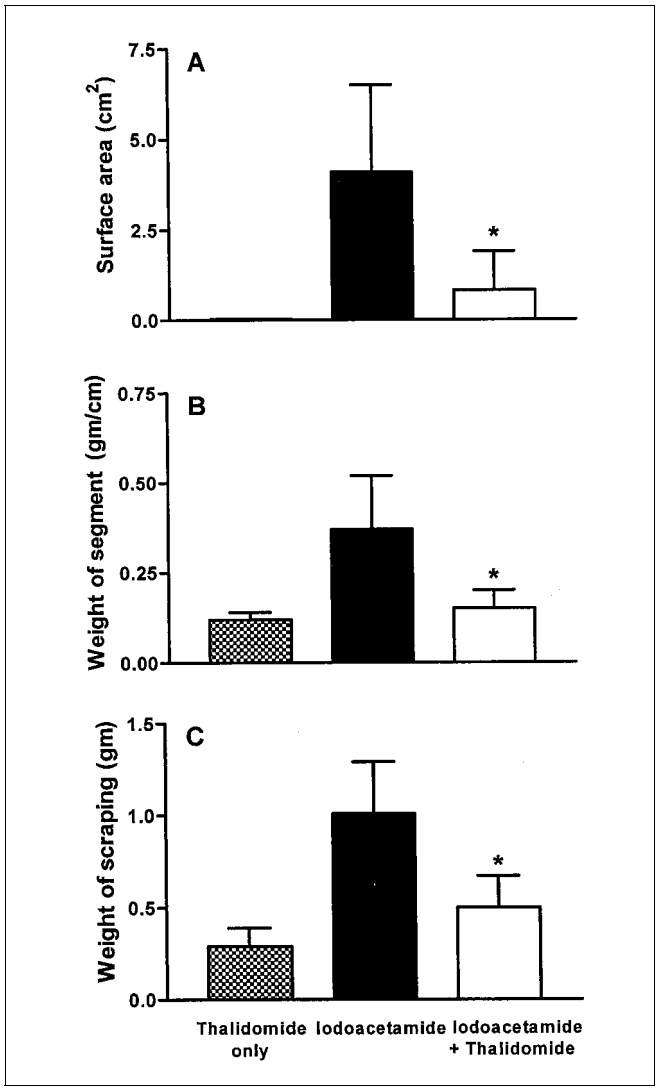

Figure 1. Effect of thalidomide on colonic lesion area **[A]**, colonic weight **[B]**, and weight of scrapings **[C]** in iodoacetamide-treated rats. Colitis was induced by administration of 0.1 ml 3% iodoacetamide dissolved in 1% methylcellulose via a catheter into the rectum. Thalidomide (50 mg/kg, given orally by gavage) was administered once daily, and all rats were sacrificed 10 days after the induction of colitis. The colon was isolated and weighed and the lesion area measured. Results are expressed as mean SD for six rats in each group.

* Significantly different from iodoacetamide only ($P < 0.01$).

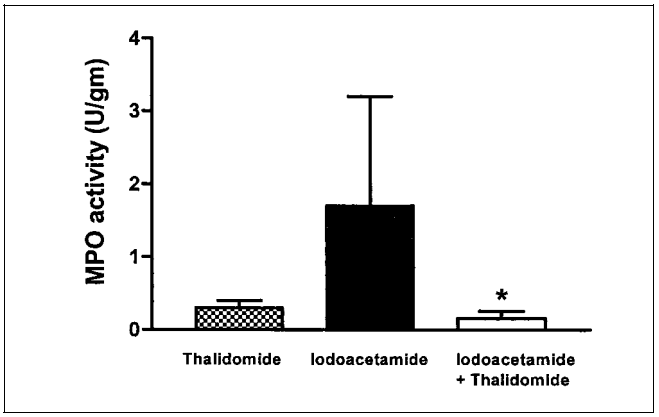

Figure 2. Effect of thalidomide on colonic myeloperoxidase activity in iodoacetamide-treated rats. Colitis was induced with iodoacetamide and thalidomide treatment was given as described in Materials and Methods. The distal 10 cm-long colonic segment was isolated and the mucosa scraped for determination of MPO activity.

* Significantly different from iodoacetamide only ($P = 0.015$).

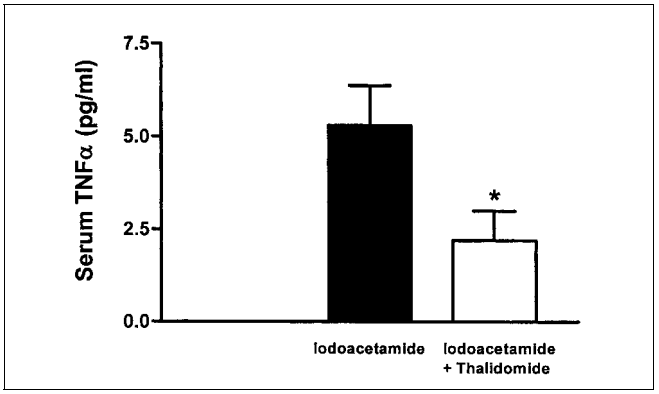

Figure 3. Effect of thalidomide on serum levels of TNFα in iodoacetamide-treated rats. Serum TNF levels were determined 10 days after the induction of colitis as described in Materials and Methods.

* Significantly different from iodoacetamide only ($P = 0.0006$).

with refractory Crohn's disease, assumed to be due to its anti-TNFα effect [6,7]. A few case reports published more than 20 years ago described improvement in inflammatory bowel disease symptoms after treatment with thalidomide. Although animal models of experimental colitis are not identical to human colitis there are still many similarities to IBD.

In our study, we used the model of iodoacetamide-induced colitis. Iodoacetamide blocks sulfhydryl groups that are crucial for the vitality of the intestine. Glutathione serves as the main pool of sulfhydryl groups and in its reduced form (GSH) protects epithelial cells from oxidative stress. Intrarectal administration of iodoacetamide causes maximal injury to the intestine after 3–7 days [21]. Histologically, the resulting inflammation is characterized by deep ulcers affecting the mucosa and the submucosa and by an extensive inflammatory infiltrate involving all layers of the intestine, findings that were also observed in our study. In this model of experimental colitis, increased levels of prostaglandin estradiol, leukotriene B_4 and nitric oxide

IBD = inflammatory bowel disease

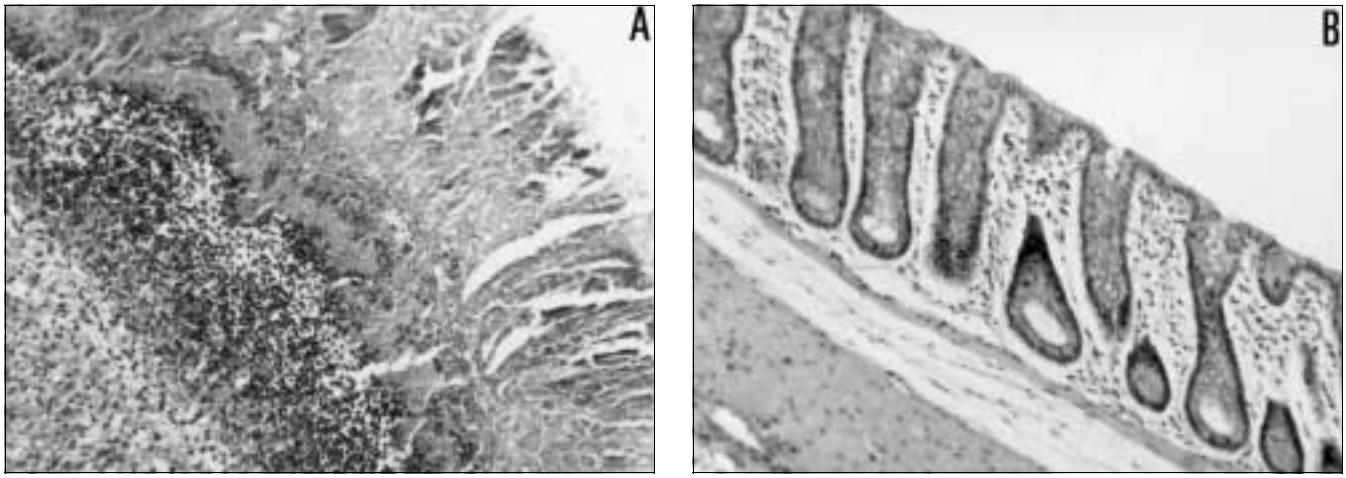

Figure 4. Histological section of the colon isolated from rats with iodoacetamide-induced colitis. **[A]** Wide mucosal ulceration with an extensive inflammatory cell infiltrate involving all layers of the intestinal wall can be seen in iodoacetamide-treated rats. **[B]** Only mild superficial inflammatory cell infiltration with no ulceration can be seen in the colonic section of rats treated with iodoacetamide and thalidomide. (hematoxylin & eosin, original magnification x80).

synthase were found in the colonic mucosa [22]. TNFα, a pro-inflammatory cytokine involved in chronic granulomatous inflammations, was measured in high concentrations in the mucosa of patients with Crohn's disease [12]. We found high levels of TNFα in the serum of rats with experimental colitis, as described also in children suffering from IBD [13]. The elevated serum levels of TNFα may reflect the high intestinal mucosal levels that have been reported in IBD. In our study, in the rats in which the colitis was ameliorated by thalidomide, serum levels of TNFα were markedly decreased [Figure 3]. TNFα is produced mainly by activated macrophages and lymphocytes after they reach the site of inflammation. In the model of iodoacetamide-induced colitis, 7 days after the induction of colitis, the cellular infiltrate consists mostly of lymphocytes and macrophages. It is questionable whether a beneficial effect of thalidomide could have occurred earlier when the inflammatory infiltrate consisted mainly of polymorphonuclear cells, although it has been reported that neutrophils can contribute to TNFα production too [9], especially in the acute phase of the inflammation. Other authors, using the model of experimental colitis induced in mice by dextran sulfate, reported that neutralization of TNFα proved to be beneficial only in the chronic phase of inflammation [23]. The production of TNFα by activated macrophages and lymphocytes, only after they reach the site of inflammation, is not unique to iodoacetamide colitis and has been found in other models of colitis as well [24].

To our knowledge, this is the first study reporting apparent anti-inflammatory and TNF-lowering effects of thalidomide in a model of experimental colitis. An optic isomer of thalidomide, D (+), has been described, which lacks the teratogenic effect without losing its inhibitory action on TNFα [25]. This drug holds promise in the future also for use in women with childbearing potential.

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