Cyclooxygenase-2-derived prostaglandin D₂ is an early anti-inflammatory signal in experimental colitis

MAUREEN N. AJUEBOR, ANITA SINGH, AND JOHN L. WALLACE
Mucosal Inflammation Research Group, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Received 14 February 2000; accepted in final form 7 April 2000

Ajuebor, Maureen N., Anita Singh, and John L. Wallace. Cyclooxygenase-2-derived prostaglandin D₂ is an early anti-inflammatory signal in experimental colitis. Am J Physiol Gastrointest Liver Physiol 279: G238–G244, 2000.—The ability of nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors to exacerbate inflammatory bowel disease suggests that prostaglandins are important anti-inflammatory mediators in this context. Prostaglandin D₂ has been suggested to exert anti-inflammatory effects. We investigated the possibility that prostaglandin D₂ derived from cyclooxygenase-2 plays an important role in downregulating colonic inflammation in rats. Colitis was induced by intracolonial administration of trinitrobenzene sulfonic acid. At various times thereafter (from 1 h to 7 days), colonic prostaglandin synthesis and myeloperoxidase activity (index of granulocyte infiltration) were measured. Prostaglandin D₂ synthesis was elevated 4-fold above controls within 1–3 h of induction of colitis, preceding significant granulocyte infiltration. Treatment with a selective cyclooxygenase-2 inhibitor abolished the increase in prostaglandin D₂ synthesis and caused a doubling of granulocyte infiltration. Colonic granulocyte infiltration was significantly reduced by administration of prostaglandin D₂ or a DP receptor agonist (BW-245C). These results demonstrate that induction of colitis results in a rapid increase in prostaglandin D₂ synthesis via cyclooxygenase-2. Prostaglandin D₂ downregulates granulocyte infiltration into the colonic mucosa, probably through the DP receptor.

inflammation; inflammatory bowel disease; neutrophil; DP receptor

The confirmation of the existence of at least two isoforms of cyclooxygenase (COX-1 and COX-2) (15, 35) and the availability of selective COX-2 inhibitors made it possible to determine which isoform is responsible for PG synthesis in colitis. Reuter and colleagues (24) demonstrated that most of the PGs produced in inflamed rat colon are derived from COX-2. In addition, an increase in the severity of colitis was observed when rats with colitis were treated with selective COX-2 inhibitors (24). COX-2 has also recently been shown to be the primary source of PG synthesis in colitis in humans (19).

PGD₂ is the major PG produced by mucosal mast cells and has also been shown to be produced by cultured human enterocytes (18). PGD₂ has been shown to suppress the infiltration of leukocytes into the inflamed pleural cavity of the rat (6). In that study, the PGD₂ appeared to be produced primarily by COX-2. It is possible, therefore, that PGD₂ may similarly act in an anti-inflammatory capacity in colitis and that it may be derived predominantly from COX-2. In this study, therefore, we have used a rat model of experimental colitis to examine 1) changes in the production of PGD₂ during colitis, 2) the contribution of COX-2 to

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colonic PGD₂ synthesis, and 3) the possible anti-inflammatory effects of PGD₂ in colitis. We have also attempted to determine whether anti-inflammatory effects of PGD₂ are mediated via the DP prostaglandin receptor or via the peroxisome-proliferator activated receptor-γ (PPAR-γ).

MATERIALS AND METHODS

Animals. Male Wistar rats (175–200 g) were purchased from Charles River Breeding Farms (Montreal, Canada). The animals were fed a standard chow pellet diet, had free access to water, and were maintained on a 12:12-h light-dark cycle. All procedures in this study were approved by the Animal Care Committee of the University of Calgary and were in compliance with the guidelines of the Canadian Council on Animal Care.

Induction of colitis. Colitis was induced as described previously (21). Briefly, rats were lightly anesthetized with halothane, and the hapten trinitrobenzene sulfonic acid (TNBS; 30 mg in 0.5 ml of 50% ethanol) was administered into the distal colon via a cannula. Untreated rats served as controls. At several time points thereafter (from 1 h to 7 days), groups of rats were euthanized by cervical dislocation. The colon was excised and pinned out on a wax platform. Tissue samples were taken for determination of PG synthesis, for measurement of myeloperoxidase (MPO) activity, and for histological assessment. MPO is an enzyme found primarily in the azurophilic granules of neutrophils and in other cells of myeloid origin. We have previously used MPO activity as a quantitative measure of the number of granulocytes in intestinal tissue and have shown that it correlates well with histological quantification of granulocyte numbers. Thus tissue MPO activity served as an index of granulocyte infiltration into the colon.

PG synthesis and COX-2 mRNA expression. Samples of distal colonic tissue were weighed and placed in an Eppendorf tube containing 1 ml of 10 mM sodium phosphate buffer (pH 7.4). The samples were minced with a scissors for 15 s, then incubated for 20 min in a shaking water bath (37°C). The samples were centrifuged (9,000 g for 1 min), and the supernatants were stored at −20°C. PGD₂ and PGE₂ concentrations in the supernatants were determined using ELISA. Samples of colonic tissue were taken from control rats and from rats 1 and 3 h after TNBS administration. These samples were processed for determination of COX-2 mRNA expression by RT-PCR, as described in detail previously (5).

Effects of selective COX-2 inhibition. To determine which isoform of COX was responsible for PGD₂ synthesis in the early phase of colitis, rats were treated orally with celecoxib (a selective COX-2 inhibitor; 10 mg/kg) or indomethacin (a nonselective COX inhibitor; 10 mg/kg) immediately after instillation of TNBS and were killed 3 h later. Control rats received the vehicle of 0.5% carboxymethylcellulose. The dose of celecoxib used in this study has been shown to selectively inhibit COX-2 activity, and the dose of indomethacin used has been shown to inhibit COX-1 and COX-2 activity (22, 30).

Effects of DP and PPAR-γ receptor agonists. To further examine the potential role for PGD₂ in TNBS-induced colitis, rats were treated intracolonically with PGD₂ (10–100 μg/kg) 1 and 4 h after TNBS administration. Control rats received
an equal volume of saline in place of PGD2. The doses of PGD2 used were recently shown to significantly reduce inflammation in a rat model of pleurisy (6). Other rats were treated with either a DP receptor agonist (BW-245C; 10–50 μg/kg) or a PPAR-γ agonist that is also a metabolite of PGD2 (15-deoxy-Δ12–14PGJ2, subsequently abbreviated as ΔPGJ2; 10–100 μg/kg) 1 and 4 h after TNBS administration. All rats were killed 8 h after TNBS administration for assessment of tissue MPO activity and for histology.

**MPO activity.** Tissue samples were homogenized in hexadecyltrimethylammonium bromide buffer (50 mg/ml). The homogenates were centrifuged (9,000 g for 1 min), and the supernatants were assayed for MPO activity, as described previously (31).

**Histology.** Colonic tissues were fixed in 10% neutral-buffered formalin, dehydrated through graded concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μm thick) were stained with hematoxylin and eosin according to standard protocols, and the slides were coded to prevent observer bias during evaluation. In some experiments, the degree of infiltration of granulocytes into the colonic tissue was semiquantitatively scored with the use of the following criteria: 0 = normal; 1 = mild infiltration; 2 = modest infiltration; 3 = dense infiltration. It should be noted that in the early stages of colitis, the degree of infiltration by granulocytes is so dense that it is virtually impossible to accurately quantify. This is one of the reasons that we utilized a biochemical marker of granulocyte numbers (MPO activity) in most studies.

**Expression of PGD synthase and PPAR-γ.** Colonic tissue was homogenized in 1.5 ml of lysis buffer consisting of 0.1% Triton X-100, 500 mM NaCl, 50 mM HEPES, 0.1 mg/ml leupeptin, and 10 mg/ml phenylmethylsulfonyl fluoride. The homogenates were incubated on ice for 30 min, then centrifuged at 400 g for 10 min. The supernatants were collected, and protein concentrations were determined with a Bio-Rad protein colorimetric assay (Bio-Rad, Hercules, CA). An aliquot (50 μg) of total protein lysate was separated on a 10% (PPAR-γ) or 12.5% (PGD synthase) polyacrylamide gel and transferred onto a nitrocellulose membrane (Pall, Ann Arbor, MI). The membrane was incubated overnight at 4°C in blocking buffer (20 mM Tris, 100 mM NaCl, 0.5% Tween 20, and 5% nonfat dried milk), then incubated with either rabbit anti-mouse polyclonal PPAR-γ antibody (1:1,000 dilution; Biomol Research Laboratories, Plymouth Meeting, PA) or rabbit anti-rat PGD synthase antibody (1:800 dilution; Cedarlane Laboratories, Hornby, ON, Canada) for 2 h at room temperature. The membrane was next incubated with horse-radish peroxidase-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h at room temperature. Antibody labeling was then visualized by addition of enhanced chemiluminescence reagent according to the manufacturer’s instructions (Amersham, Little Chalfont, UK).

**Fig. 3.** Western blot analysis of PGD synthase expression at different time points after induction of colitis. A representative blot is shown at top, and the results of densitometric analysis (expressed in arbitrary units) are shown at bottom. Data are shown as means ± SE, with 4 rats per group. **P < 0.01 vs. controls.”

**Fig. 4.** Effect of PGD2 (A), BW-245C (B), and 15 deoxy-Δ12–14PGJ2 (ΔPGJ2; C) on colonic MPO activity 8 h after induction of colitis through intracolonic administration of TNBS. Rats received the test compounds or vehicle intracolonically 1 and 4 h after induction of colitis. Data are shown as means ± SE, with 5–13 rats per group. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the vehicle-treated group.”
Materials. TNBS was obtained from Fluka Chimica (Buchs, Switzerland). PGD$_2$ ELISA kits, ΔPGJ$_2$, and PGD$_2$ were obtained from Cayman Chemical (Ann Arbor, MI). BW-245C [5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropylhydantoin)] was a generous gift from Glaxo-Wellcome (Stevenage, UK). Celecoxib was obtained from Monsanto (St. Louis, MO). Indomethacin and other chemicals were obtained from Sigma Chemical (St. Louis, MO) or VWR Scientific (Edmonton, AB, Canada).

Statistical analysis. All data are shown as means ± SE. Comparisons between two groups of data were performed using a Student’s unpaired *t*-test. Comparisons among three or more groups were performed using a one-way analysis of variance followed by a Dunnett’s multiple-comparison test or a Bonferroni post hoc test. Values of probability <5% (*P* < 0.05) were considered significant.

RESULTS

MPO activity and PG synthesis. As reported previously (32), intracolonic administration of TNBS caused severe ulceration and damage to the distal colon of the
rats. Figure 1 shows the time course of changes in colonic MPO following TNBS administration. A significant increase in MPO activity was first observed at the 8-h time point, and the greatest increase in MPO activity was seen at day 3. Administration of TNBS resulted in significant increases in PGD₂ synthesis 1 h (4.3-fold) and 3 h (3.6-fold) after TNBS but not thereafter (Fig. 1). Thus increases in PGD₂ synthesis occurred before a significant increase in granulocyte infiltration. In contrast, a significant increase in PGE₂ synthesis was not observed until three days after the administration of TNBS. Thus increased PGE₂ synthesis (32-fold) occurred at the same time as the greatest increase in MPO activity (Fig. 1).

Effect of COX inhibitors. Treatment with celecoxib (10 mg/kg) reduced colonic PGD₂ synthesis at 3 h to the levels seen in rats without colitis. Indomethacin caused an even greater suppression of PGD₂ synthesis. Both celecoxib and indomethacin caused a more than doubling of colonic MPO activity (Fig. 2). In contrast, celecoxib and indomethacin did not alter PGE₂ synthesis in colitic rats (data not shown). The increase in PGD₂ synthesis observed at 1 and 3 h after TNBS administration occurred in parallel with significant (P < 0.01) increases in COX-2 mRNA expression (control: 0.4 ± 0.1 densitometry units; 1 h post-TNBS: 1.7 ± 0.3 densitometry units; 3 h post-TNBS: 1.3 ± 0.2 densitometry units; n = 4 per group).

Expression of PGD synthase. Since a significant increase in PGD₂ synthesis was observed at the 3 h time point, we investigated whether the expression of PGD synthase (the enzyme that catalyzes the conversion of PGH₂ to PGD₂) was altered following induction of colitis. As depicted in Fig. 3, PGD synthase expression in colonic tissue at 3 h after TNBS was similar to that in rats without colitis. Moreover, PGD synthase expression was significantly reduced from 24 h to 7 days after induction of colitis.

Effect of exogenous PGD₂ on colonic granulocyte infiltration. Because inhibition of PGD₂ synthesis with COX inhibitors resulted in a significant increase in granulocyte infiltration, we next examined whether administration of PGD₂ would reduce TNBS-induced granulocyte infiltration. As shown in Fig. 4, the highest dose of PGD₂ (100 μg/kg) significantly reduced colonic MPO activity, but the lower doses had no effect. Histologically, the colonic tissue from rats that received intracolonic TNBS but were then treated with vehicle exhibited widespread epithelial injury and marked submucosal edema (Fig. 5). Granulocyte infiltration (mainly neutrophils) was evident in the submucosa and mucosa. The density of the granulocyte infiltrate was blindly scored on a 0 (normal) to 3 (dense infiltrate) scale. In rats that received PGD₂ after TNBS administration, the colonic tissue exhibited greater preservation of the epithelium and reduced levels of submucosal edema. Again, granulocyte infiltration was evident (mean histological score of 1.0 ± 0.3 with the highest dose) but was not as extensive as in the vehicle-treated group (mean histological score of 2.5 ± 0.3; P < 0.05).

To assess the potential mechanism through which PGD₂ mediated its anti-inflammatory effects, two additional studies were performed. It is well known that PGD₂ can activate the DP receptor, so we investigated the effect of a DP receptor agonist (BW-245C) on TNBS-induced granulocyte infiltration. The highest dose of BW-245C (50 μg/kg) significantly reduced MPO activity in the colon (Fig. 4). Histologically, the colonic tissue from rats treated with the highest dose of BW-245C were very similar to those from rats treated with PGD₂; that is, there was clear preservation of the epithelium and reduced submucosal edema (Fig. 5). In rats treated with the highest dose of PGD₂, the mean histological score was 1.0 ± 0.3, significantly less (P < 0.05) than that in the vehicle-treated controls. In contrast, intracolonic administration of the PPAR-γ agonist, ΔPGJ₂, significantly enhanced MPO activity at the highest dose tested, whereas the lower doses had no effect (Fig. 4). The histological score for granulocyte infiltration in the group treated with the highest dose of ΔPGJ₂ did not differ significantly from that of the vehicle-treated group (mean score of 2.7 ± 0.3).

Expression of PPAR-γ. Western blot analysis showed the presence of a 50-kDa band corresponding to PPAR-γ in the colon of naïve rats. Expression of this protein was significantly higher in rats with colitis at 3 and 24 h (Fig. 6).

DISCUSSION

Although PGE₂ and PGJ₂ can contribute to inflammatory reactions by virtue of their vasodilator properties (promoting edema formation) and through effects on sensory afferent nerves (promoting pain), it is also clear that these PGs and other members of this family of lipid mediators can exert many anti-inflammatory actions. For example, PGs can inhibit leukocyte adher-
ence (2), inhibit the release of reactive oxygen metabolites from neutrophils (34), prevent mast cell degranulation (12), and inhibit the generation of a number of other inflammatory mediators and cytokines from various cells (9, 11, 16). A recent study of experimental pleuritis highlighted the anti-inflammatory properties of PGD2 (6). Production of this prostanoid was markedly elevated during the resolution of acute inflammation in that model, and exogenous PGD2 was found to significantly reduce neutrophil levels in the inflammatory exudate. Moreover, the increase in PGD2 production paralleled changes in COX-2 expression and was suppressed by a selective COX-2 inhibitor. In the present study, we attempted to determine whether PGD2 might similarly be an important anti-inflammatory mediator in experimental colitis, where it has already been shown that NSAIDs and selective COX-2 inhibitors exacerbate the inflammatory response and the tissue injury (24, 31). Interestingly, PGD2 production was elevated very early after induction of colitis (1–3 h) but not thereafter. The increase in PGD2 synthesis preceded significant changes in PGE2 synthesis, as well as preceding significant granulocyte infiltration. This suggested to us that PGD2 may play a role in modulating the initial granulocyte infiltration in this model. Indeed, we observed that exogenous PGD2 could significantly reduce granulocyte infiltration into the colon, supporting this hypothesis. Moreover, selective inhibition of COX-2 (with celecoxib) resulted in a complete blockade of the increase in colonic PGD2 synthesis, and this was accompanied by a more than doubling of granulocyte infiltration, as measured by MPO activity. Together, these results strongly suggest that PGD2 derived from COX-2 is an important early signal in colitis that acts to downregulate granulocyte infiltration.

It is not clear why the increase in PGD2 synthesis occurred in such a narrow window of time. PGE2 synthesis was not significantly increased at the same time as PGD2 synthesis, and we know from previous studies (24) that, at least at 72 h after TNBS administration, PGE2 is produced primarily via COX-2. Moreover, the initial increase in PGD2 synthesis occurred in parallel with an increase in COX-2 expression, but it declined despite the continued elevation of COX-2 expression, as we have previously demonstrated (24). This suggests that secondary enzymes in the synthesis of PGD2 and PGE2 may have been more important in regulating their production during acute colitis than COX-2. Indeed, our observation that the expression of PGD synthase significantly decreased at 24 h after induction of colitis is consistent with the diminution of PGD2 synthesis. The factors responsible for the downregulation of PGD synthase expression remain to be identified.

PGD2 can interact with both cell surface receptors, such as the DP prostaglandin receptor, and nuclear receptors, such as PPAR-γ (20). We attempted to determine whether actions at one of these receptors accounted for the ability of PGD2 to reduce granulocyte infiltration in acute colitis by assessing the effects of a DP receptor agonist (BW-245C) and a PPAR-γ agonist (ΔPGJ2). BW-245C was found to significantly diminish colonic MPO activity, whereas ΔPGJ2 significantly increased colonic MPO activity (each at the highest dose tested). Thus it seems likely that the anti-inflammatory activity of PGD2 was mediated via the DP receptor. It is interesting, however, that expression of the PPAR-γ receptor was markedly upregulated in the inflamed colon. Several anti-inflammatory activities of PPAR-γ agonists (such as ΔPGJ2) have been demonstrated, including inhibition of cytokine expression (13, 29), suppression of expression of inducible nitric oxide synthase (3, 26), and inhibition of cell migration (7). Moreover, Su and colleagues (29) recently demonstrated that agonists of the PPAR-γ receptor diminished the severity of injury in a mouse model of colitis. It is possible, therefore, that treatment with a PPAR-γ agonist over a more prolonged period of time, or at a different time point after induction of colitis, might have a significant anti-inflammatory effect.

In summary, the results of the present study demonstrate that COX-2-derived PGD2, most probably acting via the DP receptor, acts to downregulate granulocyte infiltration into colonic mucosa during the early stages of the inflammatory response induced by TNBS. The ability of selective COX-2 inhibitors to exacerbate colitis may be due, at least in part, to suppression of the synthesis of PGD2.

We are grateful to Webb McKnight and Michael Dicay for their assistance in performing these studies. This work was supported by a grant from the Medical Research Council of Canada (MRC). M. N. Ajibor is supported by an Alberta Heritage Foundation for Medical Research (AHFMR) Fellowship. J. L. Wallace is a MRC Senior Scientist and an AHFMR Senior Scientist.

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