Disruption of cyclooxygenase-1 gene results in an impaired response to radiation injury

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Houchen, Courtney W., William F. Stenson, and Steven M. Cohn. Disruption of cyclooxygenase-1 gene results in an impaired response to radiation injury. Am J Physiol Gastrointest Liver Physiol 279: G858–G865, 2000.—Prostaglandins may play an important role in regulating normal renewal of gastrointestinal epithelium, epithelial repair, and initiation or progression of intestinal neoplasia. Synthesis of prostaglandins is catalyzed by either of two cyclooxygenase isoforms, Cox-1 and Cox-2. Cox-1 is the predominant cyclooxygenase isoform found in the normal intestine. In contrast, Cox-2 is present at low levels in normal intestine but is elevated at sites of inflammation and in adenomas and carcinomas. To determine directly whether prostaglandins synthesized by Cox-1 or Cox-2 regulate crypt epithelial cell fate after genotoxic or cytotoxic injury, we examined apoptosis, prostaglandin synthesis, and crypt stem cell survival after γ-irradiation in Cox-1−/− and Cox-2−/− mice. Cox-1−/− mice had increased crypt epithelial cell apoptosis and decreased clonogenic stem cell survival compared with wild-type littermates. PGE2 synthesis was also diminished in Cox-1−/− mice compared with wild-type controls in unstressed intestine and after radiation injury. In contrast, apoptosis, stem cell survival, and intestinal PGE2 synthesis in Cox-2−/− mice after irradiation were the same as in wild-type littermates. Crypt stem cell survival after irradiation was inhibited by a highly specific neutralizing antibody to PGE2, suggesting that this prostaglandin mediates stem cell fate in vivo. These data suggest that prostaglandins synthesized by Cox-1 regulate multiple steps that determine the fate of crypt epithelial cell after genotoxic or cytotoxic injury.

Although prostaglandins are thought to play a key role in protection of the gastrointestinal mucosa from injury and/or injury repair. In contrast, prostaglandins produced through Cox-2 are an important component of the inflammatory response. Cox-2 is expressed at low levels in normal gastric, small intestinal, and colonic epithelium but is induced in macrophages and other cell types at sites of inflammation and injury by proinflammatory cytokines (31–33). Cox-2 is also expressed at high levels in human colon cancers and adenomas and in spontaneously arising adenomas in mice that carry mutations in the APC gene (4, 5, 11, 31, 37). A biological role for Cox-2 in the formation or progression of intestinal adenomas was suggested by the observation that mice with the APC mutation that also lacked functional Cox-2 developed fewer adenomas than mice carrying the APC mutation alone (1, 19).

In the normal intestine and colon, differentiated epithelial cells are continuously and rapidly replaced by replication of undifferentiated cells within the crypt (26). These undifferentiated, replicating crypt epithelial cells are, in turn, derived from a small number of multipotent stem cells located near the base of each crypt. The restoration of normal intestinal epithelial architecture after injury such as that seen with ingested toxins, chemical carcinogens, chemotherapeutic drugs, or γ-irradiation, is a multistep process that must ultimately involve changes in the dynamics of epithelial stem cell replication within the crypt. The response of the intestinal epithelium to γ-irradiation injury has been the most extensively studied model system for investigating the fate of crypt epithelial stem cells and their descendants after injury (3, 21–25, 27, 28). After epithelial injury, crypt epithelial cells undergo apoptosis or cease replicating and are shed as they migrate up the intestinal villi or onto the surface epithelium in the colon. If a crypt contains a surviving stem cell it will proliferate to form a regenerative crypt, and cells from these regenerative crypts will ultimately repopulate the epithelium (21, 27). The fate of epithelial stem cells after injury and their capacity to regenerate the crypt epithelium primarily have been studied using the microcolony formation assay. The number of

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regenerating crypts can be scored by microscopic appearance 3–4 days after injury and used as a surrogate measure of crypt stem cell survival after radiation injury and injury induced by other cytotoxic or genotoxic agents.

Regulation of crypt epithelial apoptosis and stem cell fate after potentially mutagenic or carcinogenic injury may also be an important factor in the initiation of gastrointestinal neoplasia. Gastrointestinal adenomas and carcinomas arise through the acquisition of multiple, independent genetic mutations and subsequent clonal expansion of mutated epithelial stem cells or other long-lived progenitor cells that reside within the crypt (7, 8). Programmed cell death, or apoptosis, is the predominant biological response of crypt epithelial cells to low levels of genotoxic and cytotoxic damage that occur chronically in the small intestine and colon (24, 28). This process results in the removal of individual genetically damaged cells from the crypt epithelium. Thus it has been suggested that apoptosis is an effective cellular strategy for decreasing the probability that any particular crypt epithelial cell will survive injury and acquire the set of multiple mutations necessary for malignancy to occur (25, 28).

Recently, we reported (3) that intestinal prostaglandin synthesis is induced by γ-irradiation and that treatment of mice with indomethacin, a nonselective cyclooxygenase inhibitor, reduced survival of intestinal epithelial stem cells after radiation injury. In the normal intestine, Cox-1 was expressed predominantly in crypt epithelial cells. Radiation injury resulted in a marked increase of Cox-1 within regenerating crypt epithelial cells and a concomitant increase in intestinal PGE \(_2\) levels. Treatment with indomethacin in the period from 24 to 48 h after irradiation decreased PGE \(_2\) levels and decreased the number of surviving crypts. However, recent studies have demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, may affect cell transformation, proliferation, and apoptosis through mechanisms distinct from their effects on prostaglandin synthesis (38). Thus it is difficult to make inferences about the effects of endogenous prostaglandins on crypt epithelial cell fate from experiments using these pharmacological inhibitors.

In this study, we have used mice genetically lacking functional Cox-1 or Cox-2 to define directly the role of prostaglandins synthesized by each of these cyclooxygenases in the response of the intestinal epithelium to radiation injury. We report here that 1) Cox-1 deficient mice had decreased intestinal PGE \(_2\) synthesis, increased crypt epithelial cell apoptosis, and decreased crypt stem cell survival after radiation injury; 2) the response of Cox-2-deficient mice to radiation injury was indistinguishable from the response of wild-type mice; and 3) crypt stem cell survival after irradiation was inhibited by a highly specific neutralizing antibody to PGE \(_2\).

MATERIALS AND METHODS

Animals. The animals used in these studies were cared for in accordance with approved University of Virginia and American Association for Accreditation of Laboratory Animal Care guidelines. Mice were fed standard laboratory mouse chow and lithium and maintained in filter-top microisolator caging in a specific pathogen-free environment, on a 12-h (6:00 AM-6:00 PM) light-dark cycle. Mouse lines with targeted disruption of Cox-1 or Cox-2 were obtained from Dr. R. Langenbach (National Institute of Environmental Health Sciences, Research Triangle Park, NC; Refs. 13, 17). Mice homozygous for the disrupted Cox-1 gene (Cox-1\(^{-/-}\)) or for the disrupted Cox-2 gene (Cox-2\(^{-/-}\)) were obtained by crossing males that were either heterozygous or homozygous for the respective disrupted cyclooxygenase gene with the corresponding heterozygous females. Genotype analysis on the progeny was performed by PCR analysis or Southern blot analysis of tail DNA as previously described (13, 17). Littermates that were homozygous wild type at both the Cox-1 and Cox-2 loci were used as controls. Mice were irradiated at the age of 15–20 wk in a Gamacel 40 cesium irradiator at 0.94 cGy/min. Some mice received dimethyl-PGE2 (10 \(\mu\)g/mouse ip) at the times indicated. FVB/N mice (Taconic, Germantown, NY) were used to determine the effect of neutralizing anti-PGE2 antibody on crypt stem cell survival. There was no statistically significant difference in crypt survival after irradiation in untreated FVB/N mice compared with homozygous wild-type littermates of the Cox-1\(^{-/-}\) or Cox-2\(^{-/-}\) mice (data not shown). For these experiments, mice received a mouse monoclonal neutralizing anti-PGE\(_2\) antibody (2B5, 0.2 mg/mouse ip; gift of Dr. J. Portanova, Monsanto, St. Louis, MO) or an isotype-matched control antibody, MOPC21 (0.2 mg/mouse ip), 24 h before and 24 h after γ-irradiation (16).

Crypt survival. Two hours before death each mouse received 120 mg/kg 5-bromo-2′-deoxyuridine (BrdU; Sigma; St. Louis, MO) and 12 mg/kg 5-fluoro-2′-deoxyuridine (Sigma) to label the S-phase cells. Proximal jejunum was obtained by rapid dissection from mice 84 h after irradiation and fixed in Bouin’s fixative. Paraffin sections (5 \(\mu\)m) were prepared from the proximal jejunum oriented so that the sections were cut perpendicular to the long axis of the intestine. Cells incorporating BrdU were detected by goat anti-BrdU (2), and bound antibody was visualized using horseradish peroxidase-labeled donkey anti-goat IgG followed by staining with 3,3′-diaminobenzidine. A surviving crypt was defined as one containing five or more BrdU-positive cells as previously described (3). The number of surviving crypts per cross section was determined for each mouse by scoring the number of surviving crypts per cross section and dividing the total by the number of cross sections scored. Because differences in the size of regenerating crypts can affect the probability that a regenerating crypt will appear in a cross section (23, 27), we determined the width of 15 representative crypts for each mouse. Crypt survival was expressed as the percentage of control, and Cox-1\(^{-/-}\) or Cox-2\(^{-/-}\) mice compared with homozygous wild-type mice after 14-Gy γ-irradiation (data not shown). Thus differences observed in crypt survival cannot be accounted for by variation in the size of regenerating crypts in the Cox-1\(^{-/-}\) or Cox-2\(^{-/-}\) mice after radiation injury.

Apoptosis. The proximal jejunum was obtained from mice 6 h after irradiation, fixed overnight in 10% neutral-buffered formalin, and embedded in paraffin. Sections were prepared from the proximal jejunum oriented so that the sections were cut perpendicular to the long axis of the intestine. Sections were stained with hematoxylin and eosin, and the number of apoptotic cells per crypt was assessed by morphological criteria as previously described by Potten and Grant (24).
Briefly, only well-oriented crypts in longitudinal sections containing Paneth cells, a crypt lumen, and an uninterrupted column of epithelial cells extending to the crypt-villus junction were scored. An apoptotic cell was defined as a cell containing a single large fragment of condensed chromatin or a group of smaller fragments clustered together within an area that was similar to the size of an adjacent epithelial cell (24). Twenty well-oriented crypts were analyzed in each of eight jejunal cross sections for each mouse. Apoptotic cells were also detected in situ using the terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein nick end labeling (TUNEL) assay (Boehringer Mannheim; Indianapolis, IN) following the manufacturer’s instructions. Before the labeling reaction was initiated, the deparaffinized tissue sections were incubated for 10 min with proteinase K at 20 μg/ml. Incorporated fluorescein-dUTP was detected using horseradish peroxidase-conjugated anti-fluorescein antibody, and bound antibody was visualized with diaminobenzidine. The number of apoptotic cells per crypt as assessed with the TUNEL assay was ~40% higher than those assessed by morphological criteria, but the pattern comparing test groups was identical.

**Measurement of PGE\(_2\) levels.** Lipids were extracted by homogenizing flash-frozen tissue in cold ethanol-0.1 M sodium phosphate, pH 4.0 (70%/30%, vol/vol) followed by shaking incubation at room temperature. An aliquot of the extract was dried down under a stream of nitrogen, and the PGE\(_2\) concentration was determined by a PGE\(_2\)-specific ELISA (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s directions.

**Statistical methods.** Pairwise \(t\)-tests using the pooled estimate of variance and Bonferroni’s correction of the \(P\) values for multiple comparisons were used for analysis of the effects of cyclooxygenase genotypes on crypt survival and radiation-induced apoptosis. Pairwise Wilcoxon Mann-Whitney tests with Bonferroni’s adjustment of \(P\) values for multiple comparisons were used for analysis of the effects of radiation and cyclooxygenase genotype on intestinal PGE\(_2\) levels.

**RESULTS**

**Radiation-induced apoptosis was increased in mice lacking functional Cox-1.** In view of the apparent roles of both Cox-1 and Cox-2 in epithelial injury repair and in the development of intestinal neoplasia as well as the potential importance of apoptosis as a mechanism for removing genetically damaged epithelial cells, we examined crypt epithelial cell apoptosis in wild-type mice and in mice lacking functional Cox-1 or Cox-2. Apoptosis induced by radiation injury was significantly increased in intestinal crypt epithelial cells of Cox-1\(^{-/-}\) mice compared with wild-type littermates (Fig. 1). Spontaneous apoptosis occurred infrequently in the crypts of uninjured wild-type mice (0.21 apoptotic cells/crypt), Cox-1\(^{-/-}\) mice (0.28 apoptotic cells/crypt), or Cox-2\(^{-/-}\) mice (0.20 apoptotic cells/crypt). Radiation injury resulted in a large induction of apoptosis within the crypt epithelium of all groups. The number of apoptotic cells per crypt 6 h after 8-Gy \(\gamma\)-irradiation was 1.9-fold higher in the Cox-1\(^{-/-}\) mice (5.0 apoptotic cells/crypt) than in wild-type mice (2.6 apoptotic cells/crypt) (Fig. 1). The lack of functional Cox-2 did not affect radiation-induced apoptosis; the number of apoptotic cells in the crypts of Cox-2\(^{-/-}\) mice after irradiation (2.5 apoptotic cells/crypt) was not significantly different from that observed in wild-type mice (2.6 apoptotic cells/crypt). Although apoptotic cells were found throughout the crypts, the majority of apoptotic cells were located in the lower half of each crypt both in Cox-1\(^{-/-}\) mice and wild-type littermates (Fig. 2). Apoptotic cells were scarce in the villus epithelium of either Cox-1\(^{-/-}\) mice or wild-type littermates (data not shown). These data suggest that the increase in apoptosis was not caused by a change in gradient of susceptibility to apoptosis along the crypt-to-villus axis of the gut. Clearly, it was not simply the presence or absence of Cox-1 that determined the ability of epithelial cells to undergo radiation-induced apoptosis because prior studies have shown that in wild-type mice, differentiation of the epithelial cells as they emerge onto the villus is associated with both loss of Cox-1 expression (3) and loss of the ability to undergo apoptosis in response to radiation (21, 22, 24), whereas we observed that disruption of the Cox-1 gene resulted in increased radiation-induced apoptosis in crypt epithelial cells.

Crypt epithelial apoptosis was not significantly different in wild-type or Cox-1\(^{-/-}\) mice treated with a single dose of dimethyl-PGE\(_2\) 12 h before irradiation than in untreated controls (Fig. 3). However, dimethyl-PGE\(_2\) inhibited radiation-induced apoptosis in wild-type mice by 44% when given 1 h before irradiation and by 77% when given 1 h after irradiation. Treatment of Cox-1\(^{-/-}\) mice with dimethyl-PGE\(_2\) either 1 h before irradiation or 1 h after irradiation reduced the number of apoptotic cells per crypt to the levels observed in similarly treated wild-type mice. These data suggest that the critical time period for the effects of prostaglandins on apoptosis was short and occurred near or after the time of injury. The observation that dimethyl-PGE\(_2\) was able to suppress apoptosis when given after radiation injury had occurred also suggested that prostaglandin-induced downregulation of apoptosis was not a consequence of alteration in the amount or spectrum of radiation-induced damage.
Mice lacking functional Cox-1 had impaired crypt stem cell survival after irradiation. Our previous study (3) showed that indomethacin, a nonspecific cyclooxygenase inhibitor, decreased crypt stem cell survival when given from 24 to 48 h after irradiation. Crypt survival was also markedly diminished in Cox-1−/− mice (4.9 surviving crypts/cross section) compared with wild-type littermates (10.1 surviving crypts/cross section) (Fig. 4). However, crypt survival in Cox-2−/− mice was the same as in wild-type mice. The 52% reduction in crypt survival seen in the Cox-1−/− mice is similar to when given from 24 to 48 h after irradiation. Crypt survival was also markedly diminished in Cox-1−/− mice (4.9 surviving crypts/cross section) compared with wild-type littermates (10.1 surviving crypts/cross section) (Fig. 4). However, crypt survival in Cox-2−/− mice was the same as in wild-type mice. The 52% reduction in crypt survival seen in the Cox-1−/− mice is similar to
the ~60% reduction in crypt survival previously reported in wild-type FVB/N mice receiving indomethacin in the period after radiation compared with mice not receiving indomethacin (3). Thus the lack of functional Cox-1, but not Cox-2, recapitulated the effects of indomethacin on intestinal crypt survival, and this suggests that prostaglandins produced by Cox-1 mediate the survival of intestinal epithelial stem cells after irradiation.

**Intestinal PGE2 synthesis was impaired in Cox-1−/− mice after irradiation.** We previously found (3) that γ-irradiation induced a progressive rise in intestinal PGE$_2$ levels in wild-type FVB/N mice. The induction of PGE$_2$ synthesis by γ-irradiation was associated with enhanced crypt stem cell survival. In the present study, nonirradiated Cox-1−/− mice had diminished intestinal PGE$_2$ levels compared with their wild-type littermates (4.7 vs. 16.3 pg/mg; $P = 0.006$) (Fig. 5). In contrast, intestinal PGE$_2$ levels were not affected by the absence of functional Cox-2; PGE$_2$ levels in nonirradiated Cox-2−/− mice were similar to those in wild-type mice (16.1 vs. 16.3 pg/mg; $P = 0.94$). In mice that were homozygous wild type for both Cox-1 and Cox-2, intestinal PGE$_2$ levels increased 3.8-fold by 3.5 days after 13.8-Gy γ-irradiation (16.3 to 61.2 pg/mg; $P = 0.007$), an induction of PGE$_2$ synthesis similar to that previously seen in FVB/N mice after γ-irradiation (4). A similar increase in PGE$_2$ levels was observed after irradiation of Cox-2−/− mice (Fig. 5). However, intestinal PGE$_2$ levels failed to rise after irradiation in the Cox-1−/− mice. Rather, PGE$_2$ levels in the Cox-1−/− mice fell after irradiation to levels that were significantly lower than present in nonirradiated Cox-1−/− mice (2.7 vs. 4.7 pg/mg; $P = 0.041$). These data suggest that the intestinal mucosa could not fully compensate for the chronic absence of functional Cox-1 by increased Cox-2-mediated prostaglandin synthesis.

**Crypt stem cell survival after irradiation was mediated by PGE2.** To determine whether the diminished crypt survival in Cox-1−/− mice was specifically related to decreased PGE$_2$ levels as opposed to changes in the levels of other eicosanoids, we treated wild-type mice with a neutralizing anti-PGE$_2$ antibody, 2B5, beginning 24 h before 14-Gy γ-irradiation. This antibody is able to inhibit the biological activity of PGE$_2$ in several experimental models of inflammation and has <1% cross-reactivity with other eicosanoids, with the exception of PGE$_1$ (16, 20). Treatment of mice with anti-PGE$_2$ reduced crypt survival to 51% of the level compared with mice treated with MOPC21, an isotype-matched control antibody (Fig. 6). Thus treatment with anti-PGE$_2$ beginning before irradiation recapitulated the effects of both Cox-1 gene disruption and indomethacin administration. This suggests that the decrease in crypt survival seen in indomethacin-treated mice and in Cox-1−/− mice was specifically caused by decreased levels of PGE$_2$.

**DISCUSSION**

We report here that Cox-1−/− mice had increased crypt epithelial apoptosis, decreased survival of clonogenic crypt stem cells, and impaired induction of prostaglandin synthesis after radiation injury. Whether the lack of functional Cox-1 would also affect the epithelial response to other types of injury (e.g., bacterial infection, chemotherapeutic agents, cytokines, reactive oxygen species) or the response to other agents that can induce apoptosis (e.g., chemical carcinogens, tumor necrosis factor-α, fas ligand) has not yet been determined. Cox-1 is present in far greater abundance than Cox-2 in the intestine after radiation injury (3). The enhanced radiation-induced apoptosis and diminished crypt survival observed in Cox-1−/− mice combined with the absence of effects on these processes in Cox-2−/− mice suggest that the prostaglandins that regulate these early events during the epithelial response to radiation injury are produced through Cox-1. However, the relative importance of prostaglandins synthe-
sized by Cox-1 or Cox-2 in epithelial wound repair may depend on the nature of the injury and whether it is accompanied by an inflammatory response. In circumstances in which epithelial injury is accompanied by significant inflammation or in adenomatous polyps, levels of Cox-2 are much higher than are found in either the normal intestine or in the intestine after radiation injury (4, 5, 11, 29, 33, 37). Recent studies demonstrated that prostaglandins synthesized by Cox-2 can accelerate healing of gastric ulceration (15, 30). Prostaglandins derived from Cox-1 and/or Cox-2 appear to play a role in reducing epithelial injury in dextran sodium sulfate-induced colitis (18, 35). Thus it is possible that prostaglandins produced by Cox-2 can also prevent epithelial damage or improve healing of epithelial injuries by suppressing crypt epithelial apoptosis or enhancing stem cell survival. Nevertheless, an important role for prostaglandins produced through Cox-1 in the regulation of crypt epithelial apoptosis and stem cell survival during epithelial injury repair would be consistent with the hypothesis that combined Cox-1 and Cox-2 inhibitors such as indomethacin increase epithelial cell death after injury or impair epithelial wound healing in addition to the direct toxic effects of these NSAIDs on the gastric and intestinal mucosa (29, 34).

After damage produced by γ-irradiation or other agents that produce DNA damage, crypt epithelial cells can undergo apoptosis or can attempt to repair the DNA damage (28). Mutations may result within the surviving epithelial cells via errors in the DNA repair process. Previous studies have suggested that neoplasia in the intestinal epithelium requires acquisition of multiple, independent somatic mutations within functionally anchored epithelial stem cells and other long-lived progenitor cells in the crypt (7, 8). Apoptosis induced by radiation injury, chemical carcinogens, or other DNA-damaging agents occurs most prominently in the lower half of the intestinal crypt epithelium, a region of the intestinal epithelium where these epithelial progenitor cells reside (10, 14, 25, 28). Studies in mice that have a mutation in the mouse homolog of the gene responsible for familial adenomatous polyposis (APC) suggest that prostaglandins produced by both Cox-1 and Cox-2 are important in regulating the development of adenomas arising from the intestinal epithelium (1, 19). Cox-2 was not present in the normal intestine or colon of mice bearing a mutant APC but was induced at very early stages of adenoma formation (19). Mice that had a mutant APC and were homozygous for a disrupted Cox-2 gene (Cox-2−/−) developed fewer colonic adenomas than mice that have the mutant APC alone, suggesting that prostaglandins synthesized by Cox-2 are important mediators of adenoma formation or growth (19). However, Cox-1−/−/APC mutant mice also developed fewer adenomas than mice with only the APC mutation despite the presence of functional Cox-2 in these mice (1). When that finding is viewed in the light of the data presented here, it raises the possibility that Cox-1−/−/APC mutant mice had fewer adenomas in part because the diminished level of PGE₂ in these mice resulted in increased loss of damaged crypt epithelial cells and/or decreased numbers of surviving clonogenic stem cells with potential mutations leading to adenoma formation. However, the relationship between alterations in crypt epithelial cell fate after radiation-induced or carcinogen-induced epithelial injury and changes in the frequency of adenoma formation has not yet been determined.

Crypt survival is the result of the successful completion of a series of steps after irradiation (21). First, intestinal stem cells in the base of the crypt must survive radiation injury. These surviving stem cells must then give rise to a more actively proliferating transit cell population and form a regenerative crypt. Finally, the transit cells must proliferate to expand their numbers and give rise to all of the differentiated cell types found in the small intestinal or colonic epithelium. In the normal intestine, Cox-1 is expressed in all crypt epithelial cells, including stem cells, transit cells, and Paneth cells (3). After radiation injury, the transit cells cease replicating and are rapidly lost from the crypt via apoptosis or by migration onto the villus epithelium. Our previous studies (3) with indomethacin demonstrated that inhibition of prostaglandin synthesis had the greatest effect in decreasing crypt survival during the period from 24 to 48 h after radiation, when Paneth cells and replicating crypt transit cells have largely disappeared from the injured crypt. Thus one possible explanation for the decrease in crypt survival seen in the Cox-1−/−/mice is that Cox-1-mediated prostaglandin synthesis in crypt stem cells after irradiation plays an important role in maintaining maximal stem cell survival.

We found that production of PGE₂ by the intestinal epithelium was suppressed in Cox-1−/− mice both at baseline and after radiation injury. Previous studies also found that gastric PGE₂ levels in Cox-1−/− mice are <1% that of their wild-type littermates (13). Why, then, does disruption of the Cox-1 gene in mice recapitulate the effects of indomethacin in radiation injury although it is not associated with gastric ulceration in the unstressed condition? The most likely explanation is that prostaglandins produced through Cox-1 are not required for the maintenance of epithelial integrity in the absence of injury but are required for resistance of the epithelium to damage or for effective injury repair. A recent study that showed that absence of either functional Cox-1 or Cox-2 exacerbated dextran sodium sulfate-induced colitis is consistent with this possibility (18). An alternative explanation is that prostaglandins produced by Cox-1 are important for both the maintenance of epithelial integrity in the absence of injury and for the epithelial response to injury but that the Cox-1−/− mouse has developed a compensatory mechanism for the absence of prostaglandin synthesis by Cox-1 only in the unstressed condition and not in response to injury. Increases in Cox-2 activity or phospholipase A₂ activity, for example, could maintain prostaglandin levels, compensating for the loss of functional Cox-1 in Cox-1−/− mice. However, our data suggest that this latter possibility is unlikely because
prostaglandin levels in uninjured Cox-1−/− mice were markedly reduced compared with levels present in wild-type mice and prostaglandin levels failed to increase in the Cox-1−/− mice after radiation injury. The mechanism by which decreased crypt epithelial cell PGE2 production reduces crypt stem cell survival after irradiation is not yet clear. We found that Cox-1−/− mice had both increased crypt epithelial apoptosis and decreased crypt survival after radiation injury. These events could be related if one mechanism for the decrease in crypt survival in Cox-1−/− mice is enhanced loss of crypt stem cells through apoptosis. However, it should also be noted that Cox-1−/− mice had diminished intestinal PGE2 levels both before and after irradiation. We found that dimethyl-PGE2 suppressed apoptosis in both wild-type and Cox-1−/− mice when given either 1 h before or 1 h after irradiation. Furthermore, we observed significantly increased radiation-induced apoptosis in Cox-1−/− mice by 6 h after irradiation. Both of these observations demonstrate the existence of a prostaglandin-sensitive step important for regulation of apoptosis that occurs well before the critical time period identified in our previous study (3) for inhibition of crypt stem cell survival by indomethacin given after irradiation. Together with our previous study, these data suggest that prostaglandins synthesized by Cox-1 are important in regulating multiple, distinct events that determine the fate of damaged epithelial cells within the crypt after genotoxic and/or cytotoxic injury.

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REFERENCES


