Background: We have previously reported that the ClC-2 chloride channel has an important role in regulation of tight junction barrier function during experimental colitis, and the pharmaceutical ClC-2 activator lubiprostone initiates intestinal barrier repair in ischemic-injured intestine. Thus, we hypothesized that pharmaceutical ClC-2 activation would have a protective and therapeutic effect in murine models of colitis, which would be absent in ClC-2−/− mice.

Methods: We administered lubiprostone to wild-type or ClC-2−/− mice with dextran sulfate sodium (DSS) or 2, 4, 5-trinitrobenzene sulfonic acid–induced colitis. We determined the severity of colitis and assessed intestinal permeability. Selected tight junction proteins were analyzed by Western blotting and immunofluorescence/confocal microscopy, whereas proliferative and differentiated cells were examined with special staining and immunohistochemistry.

Results: Oral preventive or therapeutic administration of lubiprostone significantly reduced the severity of colitis and reduced intestinal permeability in both DSS and trinitrobenzene sulfonic acid–induced colitis. Preventive treatment with lubiprostone induced significant recovery of the expression and distribution of selected sealing tight junction proteins in mice with DSS-induced colitis. In addition, lubiprostone reduced crypt proliferation and increased the number of differentiated epithelial cells. Alternatively, when lubiprostone was administered to ClC-2−/− mice, the protective effect against DSS colitis was limited.

Conclusions: This study suggests a central role for ClC-2 in restoration of barrier function and tight junction architecture in experimental murine colitis, which can be therapeutically targeted with lubiprostone.

Key Words: ClC-2 chloride channel, lubiprostone, tight junctions, inflammatory bowel diseases, intestinal barrier function

Intestinal homeostasis requires an epithelial barrier that separates the luminal contents from the interstitium. Increased intestinal paracellular permeability or barrier dysfunction is believed to be a major factor in the pathogenesis of inflammatory bowel diseases (IBD), which include ulcerative colitis (UC) and Crohn’s disease (CD). Disruption of intestinal barrier function has been detected in experimental IBD models. The intestinal epithelial barrier consists of a highly organized complex of intercellular junctions within which the tight junction is the most critical for regulating barrier function. The tight junction is the most apical component of the epithelial junctional complex acting as a barrier (gate) to the paracellular pathway by regulating passive diffusion of solutes and macromolecules and as a fence that restricts the movement of lipids and membrane proteins between the apical and the basolateral membrane of polarized intestinal epithelial cells. Moreover, the intestinal tight junctions are believed to play a role in the regulation of epithelial proliferation and differentiation and overall mucosal architecture. In addition, goblet cells produce the mucin covering the entire length of the gastrointestinal tract, which further protect the mucosal surface from harmful molecules and bacteria and reinforce the overall intestinal barrier. Colonic mucosa from patients with IBD and from animals with experimental colitis have shown structural alteration of the epithelial barrier, including a reduced number of horizontal tight junction strands on freeze-fracture electronmicroscopy, altered tight junction protein expression and subcellular distribution, and a decreased number of goblet cells resulting in the reduction of mucin secretion.

The ClC-2 chloride channel is expressed in the plasma membranes of epithelial cells from many mammalian tissues. The physiological contribution of CIC-2 in the intestine is not completely understood. However, in our previous work, we have demonstrated a critical role for CIC-2 in epithelial repair. Localization of CIC-2 to the region of the tight junction in porcine and murine intestine is associated with its regulatory interactions
In previous studies, we have shown that CIC-2 knockout mice have retarded recovery of barrier function after small intestinal ischemic injury, and knockdown of CIC-2 in Caco-2 cells reduces the ability of cultured cells to form tight junction complexes.

More recently, we reported that lubiprostone initiates intestinal barrier repair in ischemic-injured intestine through its principal target, CIC-2. In this study, we investigated the possible therapeutic effects of oral treatment with lubiprostone in murine experimental colitis and determined whether this effect was dependent on CIC-2. Our findings indicate that oral administration of lubiprostone dramatically improves mucosal and tight junction architecture with associated benefits on barrier function in a CIC-2–dependent manner. In addition, we also noted a novel action for lubiprostone; that of an increased proportion of goblet cells, which occurred in a CIC-2–independent manner.

**MATERIALS AND METHODS**

**Animals**

Studies were approved by the North Carolina State University Institutional Animal Care and Use Committee. Female C57BL/6 (6 weeks old) and BALB/c (7 weeks old) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and breeding pairs of heterozygous mice (CIC-2+/−) were a kind gift of Dr. James E. Melvin (University of Rochester, NY). The generation of CIC-2 null (CIC-2−/−) mice and genotyping PCR has been previously described.

**Induction and Assessment of Murine Colitis**

DSS colitis was induced in C57BL/6 mice by offering 3% (wt/vol) DSS (36,000–50,000 daltons; MP Biomedicals, Santa Ana, CA) in autoclaved drinking water for 5 days. At the end of day 5, mice were returned to normal drinking water until day 8 when they were euthanized. TNBS colitis was induced by the intrarectal administration of 2.5% TNBS (Sigma-Aldrich, St. Louis, MO) in 50% ethanol to BALB/c mice. The mice were lightly anesthetized with isoflurane (Aerme; Baxter Healthcare Corporation, Deerfield, IL), after which a 3.5 Fr umbilical catheter (Utah Medical Product, Inc., Midvale, UT) lubricated with the lubricating jelly was inserted into the colon through the anus. An enema of 100 μL of 2.5% (vol/vol) TNBS was injected when the tip of the catheter was 4 cm inside the anus. The mice were held in a vertical position for 1 minute after the intrarectal injection. The control group received 100 μL of 50% ethanol in a similar manner. Body weight and clinical signs of colitis were monitored daily, and the disease activity index (DAI) was determined, as previously described. The DAI scores were graded as follows: weight loss: 0 (within 1% of the baseline), 1 (1%–5% loss), 2 (5%–10% loss), 3 (10%–15% loss), or 4 (loss >15%); stool consistency: 0 (no diarrhea), 2 (loose stool that did not stick to the anus), and 4 (liquid stool that did stick to the anus); fecal blood: 0 (none), 2 (moderate), and 4 (gross bleeding). At the end of the experiments, the colons were excised and colon length measurements were performed. In addition, the distal portion of each colon was immediately fixed in 10% neutral buffered formalin solution, embedded in paraffin wax, and then sectioned before being deparaffinized. Slides were stained using H&E standard techniques, and the microscopic score was determined as described previously.

**Pharmacological Treatments**

The following series of treatments were designed to investigate the preventive and/or the therapeutic properties of lubiprostone (Sucampo Pharmaceuticals, Inc., Bethesda, MD) in DSS- and TNBS-induced colitis in mice. DSS colitis mice were orally treated by gavage with 1 (low), 10 (middle), or 100 (high) μg/kg lubiprostone from days 0 to 8 (preventive treatment) or with 10 or 100 μg/kg from days 3 to 7 (therapeutic treatment). To determine the therapeutic dose of lubiprostone, the animal equivalent dose (10 μg/kg) was calculated using the following formula: Animal equivalent dose = Human dose × Human K<sub>m</sub>/Animal K<sub>m</sub>. TNBS colitis mice were also orally administered lubiprostone by gavage with 100 μg/kg from days 0 to 3. To explore the dependency of lubiprostone on CIC-2, another set of experiments was performed in CIC-2−/− mice gavaged with 100 μg/kg of lubiprostone from days 0 to 8. Negative control and colitis control groups received only medium chain triacylglyceride (Sucampo Pharmaceuticals, Inc.) vehicle solution.

**In Vivo Intestinal Permeability Assay**

In vivo intestinal permeability was assessed by luminal enteral administration of FITC-dextran (4000 daltons; Sigma-Aldrich), a nonmetabolizable macromolecule that is used as a permeability probe. Food was withdrawn for 3 hours, and mice were gavaged with FITC-dextran (6 mg/10 g body weight) 2 hours before euthanasia. Whole blood was collected by cardiac puncture at the time of euthanasia. Fluorescence intensity in serum was analyzed using a plate reader (excitation, 492 nm; emission, 525 nm). The concentration of FITC-dextran was determined from FITC-dextran standard curve generated by serial dilution. Permeability was calculated by linear regression of sample fluorescence.

**Real-time PCR for Cytokine Expression**

Total RNA was isolated from distal colonic tissues from mice with DSS colitis in the preventive lubiprostone treatment group using an RNeasy kit (Qiagen, Valencia, CA) and quantified spectrophotometrically. Equal amounts of RNA were used for complementary DNA synthesis (SuperScript III First-Strand...
Synthesis; Invitrogen, Carlsbad, CA). Real-time PCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), the ABI StepOnePlus system (Applied Biosciences, Carlsbad, CA), and PCR primers obtained from the Integrated DNA Technology, Inc. (Coralville, IA) (see Table, Supplemental Digital Content 1, http://links.lww.com/IBD/B50).

**Gel Electrophoresis and Western Blotting**

Distal colonic tissues from DSS colitis WT and CIC-2+/− mice were lysed in Tissue Extraction Reagent I (Invitrogen), homogenized, and centrifuged. Supernatants were quantified applying a BCA protein assay kit (Thermo Scientific, Rockford, IL). Proteins were separated 4% to 12% Bis-Tris gels with XT MOPS SDS running buffer (both Bio-Rad Laboratories, Inc.). After protein transfer to a polyvinylidene difluoride membrane, the membranes were probed using anti-ZO-1, anti-occludin, anti-claudin-1, and anti-claudin-2 (Invitrogen) antibodies. Horseradish peroxidase–conjugated secondary antibodies were used (Santa Cruz Biotechnology, Inc., Dallas, TX).

**Immunofluorescence Analyses**

Distal colonic cryosections from WT and CIC-2+/− mice were fixed for 10 minutes with cold acetone at −20°C. Immunostaining was performed using primary antibodies: anti-ZO-1, anti-occludin, anti-claudin-1, anti-claudin-2 (Invitrogen), and FITC-EpCAM (BioLegend, San Diego, CA). Cy3-conjugated anti-occludin, anti-claudin-1, anti-claudin-2 (Invitrogen) antibodies. Horseradish peroxidase–conjugated secondary antibodies were used (Santa Cruz Biotechnology, Inc., Dallas, TX).

**Proliferative/Differentiated Cell Analyses**

Colonic crypt Height quantification was performed using the Image J software. Twenty random colonic crypts were measured from each H&E-stained section to make n = 1. All measurements were performed in a blinded fashion.

Immunohistochemistry for proliferating cell nuclear antigen (PCNA), carbonic anhydrase II (CA II; colonocyte), and chromogranin A (CgA; enterochromaffin cell) on murine colonic tissues was performed by standard methods. Heat-activated antigen retrieval was performed in sodium citrate buffer (pH6). Paraffin sections were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched by incubation in 3% H2O2 in methanol for 10 minutes. After blocking in protein block serum-free (DakoCytomation), the sections were incubated in primary antibodies; mouse anti-PCNA (Abcam, Cambridge, MA), goat anti-CALII (Santa Cruz Biotechnology, Inc.), and rabbit anti-CgA (ImmunoStar, Inc., Hudson, WI). After washing, the EnVision system (DakoCytomation) was used to reveal the reaction. After washing slides were developed for 5 minutes with 3,3′-diamino-benzindin chromogen and counterstained with hematoxylin. Ten intact crypts were randomly analyzed for PCNS or CgA-positive cells among 3 to 4 colonic cross sections from each mouse to make (n = 1). The results were calculated as the percentage of total crypt cells.

WT and CIC-2+/− mice sections were incubated with alcian blue solution (1% wt/vol alcian blue, 8GX in 3% vol/vol acetic acid, pH2.5) and washed with diH2O. Sections were counterstained with nuclear fast red (0.1% wt/vol nuclear fast red, 5% wt/vol aluminum sulfate in diH2O). Slides were dehydrated and mounted. Goblet cell numbers were quantified from 15 random colonic crypts glands from each mouse.

**Statistical Analyses**

Data are reported as mean ± SE. Differences between groups were tested with one-way analysis of variance and LSD post hoc testing (IBM SPSS Statistics 21, IBM, Armonk, NY). Where appropriate, differences between 2 groups were tested with a Student’s t test (Excel, Microsoft 2013).

**RESULTS**

**CIC-2 Activator Lubiprostone Reduces DSS-induced Colitis in a Preventive and Therapeutic Manner**

The administration of DSS solution resulted in reproducible colitis in mice, which resembles the acute phase of human UC, and is characterized by increased DAI and colonic damage.24 To assess the potential effect of the CIC-2 activator lubiprostone on DSS-induced colitis, mice were treated orally by gavage with 3 different doses (1, 10, and 100 μg/kg) once a day from days 0 to 8 (preventive treatment) to detect potential dose-dependent effects. DSS-treated C57BL/6 mice started to lose weight on day 6 and lost ~11% of their body weight by day 8. No weight loss was seen in the control groups, which had access to tap water without DSS. Preventive oral treatment with 100 μg/kg of lubiprostone but not 1 or 10 μg/kg significantly protected from marked body weight loss (Fig. 1A). Alternatively, all dosages of lubiprostone significantly reduced the DAI in a dose-dependent manner (Fig. 1B). To determine if lubiprostone had an effect on intestinal paracellular permeability because we have shown in previous studies, in vivo FITC-dextran permeability assays were performed. These experiments revealed that DSS colitis mice receiving oral treatment with 100 μg/kg of lubiprostone had significantly decreased intestinal permeability to FITC-conjugated dextran compared with untreated DSS colitis mice (Fig. 1C). Administration of 10 or 100 μg/kg of lubiprostone dose dependently prevented reductions in colon length (P < 0.05, Fig. 1D, E). Histological evaluation of colonic mucosa from mice subjected to DSS in their water revealed transmural infiltration with inflammatory cells, associated with epithelial erosions and severe edema in the submucosa. Histological injury scores were also dose

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dependently reduced by administration of lubiprostone at doses of 10 or 100 μg/kg with reduced granulocyte infiltration and submucosal edema (Fig. 1F).

In further experiments designed to test the potential therapeutic effects of lubiprostone, we noted that mice with DSS-induced acute colitis had body weight loss and marked diarrhea with bloody stools resulting in a sharp increase of the DAI from day 3 onwards. Therefore, therapeutic treatment was instituted from days 3 to 8 with lubiprostone doses of 10 and 100 μg/kg. These studies revealed that the high-dose lubiprostone significantly reduced clinical and pathological evidence of DSS colitis, such as body weight loss, DAI, colon shortening, histologic score, and intestinal permeability (Fig. 2). These effects were not apparent at the lower lubiprostone dose of 1 μg/kg. Taken together, these data indicate that oral treatment of mice with lubiprostone has preventive and therapeutic effects on experimental colitis although preventive effects were noted at lower dosages as compared with therapeutic dosages.

Preventive Administration of Lubiprostone Decreases Colonic Inflammatory Mediators in Mice with DSS Colitis

Various inflammatory mediators have been associated with the pathogenesis of human IBD. Similarly, DSS-induced colitis is also associated with release of colonic mucosal inflammatory mediators.23 For example, in a previous study on DSS colitis, proinflammatory mediators and anti-inflammatory mediators were elevated during colitis, with reduction in proinflammatory cytokines preceding reductions in anti-inflammatory mediators after DSS withdrawal.30 To examine whether the protection from DSS-induced colitis in mice pretreated with lubiprostone was associated with a decrease in the production of inflammatory mediators, the mRNA levels of the cytokines TNF-α, IFN-γ, and IL-1β were assessed in colonic tissues using real-time PCR (see Table, Supplemental Digital Content 1, http://links.lww.com/IBD/B50). Mice with DSS-induced colitis had significant increases in the proinflammatory cytokines TNF-α, IFN-γ, and IL-1β. In mice receiving preemptive oral lubiprostone (100 μg/kg), there were...
significant reductions in expression of TNF-α and IL-1β mRNA but not of IFN-γ mRNA (see Fig., Supplemental Digital Content 2, http://links.lww.com/IBD/B51). In addition, we measured the colonic mRNA expression of the anti-inflammatory mediators IL-10 and TGF-β and showed significantly increased IL-10 but not TGF-β levels in DSS colitis tissues compared with the control group. Interestingly, lubiprostone significantly decreased both IL-10 and TGF-β levels in the distal colonic tissue of mice with DSS-induced colitis (see Fig., Supplemental Digital Content 2, http://links.lww.com/IBD/B51), which may have been attributable to an overall reduction in inflammation in the presence of lubiprostone, possibly reducing the impetus for anti-inflammatory signaling.

**Preventive Treatment with Lubiprostone Protects Tight Junction Structure in DSS-induced Colitis**

Tight junctions are assembled at the apical-lateral cellular domain, where they form the most critical component of the paracellular barrier.14 In prior experiments, we had already shown that this paracellular barrier was disturbed in DSS colitis, based on changes in permeability assays. Therefore, to determine if the action of lubiprostone in DSS-induced colitis mice is linked to the tight junction, we initially determined expression of the tight junction proteins ZO-1, occludin, claudin-1, and claudin-2 by Western blot. Expression of ZO-1, claudin-1, and claudin-2 was not different in the distal colon of colitis mice as compared with control mice. However, occludin expression was decreased in colonic mucosa from mice with DSS-induced colitis. Alternatively, gavaging mice with lubiprostone (100 μg/kg) before the administration of DSS resulted in significant increases in occludin and claudin-1 expression. Furthermore, expression of claudin-2, a pore-forming tight junction protein, was significantly reduced in colitis mice receiving lubiprostone as compared with controls (Fig. 3A, B).

Given the importance of the cellular localization of tight junctions in the regulation of barrier function, the distribution of tight junction proteins was visualized by immunofluorescent confocal microscopy. In the control group, immunolocalization of tight junction proteins was observed primarily in the intercellular junctions of the epithelial cells adjacent to the apical membrane. The distribution of tight junction proteins was altered by DSS administration, including an apparent reduction in occludin fluorescence, claudin-1 endocytosis, and increased claudin-2 fluorescence consistent with our findings on Western analyses. The distribution of ZO-1 and EpCAM (a membrane marker) was maintained in colitis mice. Interestingly, although claudin-2 fluorescence was primarily localized to the crypt in control colon, its expression was increased in both the mucosal surface and crypt epithelium in DSS colitis mice. Preemptive administration of lubiprostone resulted in recovery of tight junction immunolocalization of occludin, claudin-1, and claudin-2 in DSS-induced colitis (Fig. 3C). Therapeutic use of 100 μg/kg of lubiprostone also induced similar beneficial changes to the distribution of tight junction proteins (see Fig., Supplemental Digital Content 3, http://links.lww.com/IBD/B52). Collectively, these data indicate that treatment with lubiprostone results in maintenance of tight junction protein expression and distribution in DSS-induced colitis.

**Lubiprostone Has Limited Protective Effects in DSS-induced CIC-2−/− Mice Colitis**

Considering the purported action of lubiprostone as a CIC-2 activator, we next investigated the effect of lubiprostone on colitis in CIC-2 knockout mice. Cuppoletti et al15 showed that...
Lubiprostone is a selective and dose-dependent activator of ClC-2 with no observable effect on CFTR. However, other studies have suggested alternate actions of lubiprostone, clouding the issue of the mechanisms of action for this prostone.

To clarify the mechanism of action of lubiprostone in our murine DSS colitis model, we performed a study using lubiprostone pretreatment with 100 μg/kg lubiprostone in ClC-2/−/− mice. Oral pretreatment with lubiprostone did not protect against colon shortening or histological evidence of colitis as had been the case in lubiprostone-treated WT mice with DSS-induced colitis. However, pretreatment with lubiprostone did provide some protection in ClC-2/−/− mice from clinical signs of colitis, including less...
marked reductions in body weight, amelioration of increases in DAI, and reductions in intestinal permeability (Fig. 4). However, the level of protection was notably less than that noted in lubiprostone-treated WT mice. In addition, preemptive treatment with lubiprostone did not change expression and distribution of tight junction proteins (ZO-1, occludin, claudin-1, and claudin-2) in DSS-induced CIC-2−/− mice colitis model (see Fig., Supplemental Digital Content 5, http://links.lww.com/IBD/B54). To determine the effect of an intermediary dose of lubiprostone (10 μg/kg) given preemptively, we performed an additional trial on CIC-2−/− mice with DSS-induced colitis. However, 10 μg/kg lubiprostone did not protect against clinical signs, colon shortening, and histological score in CIC-2−/− mice with colitis (see Fig., Supplemental Digital Content 5, http://links.lww.com/IBD/B54), whereas 10 μg/kg lubiprostone had protective effects on WT mice with DSS-induced colitis (Fig. 1). These results indicated that the full spectrum of beneficial effects of lubiprostone was only noted in the presence of CIC-2.

Alternate Protective Effects of Lubiprostone Pretreatment

Although CIC-2−/− colitis mice treated with 100 μg/kg lubiprostone did not differ from untreated colitis mice in their immune cell infiltration, epithelial damage, and tight junction structure, histological evaluation suggested changes in the goblet cell number. Interestingly, mice with DSS colitis have been reported to have evidence of increased crypt cell proliferation with concurrent reductions in the number of differentiated epithelial cells. Therefore, to find alternative protective mechanisms for lubiprostone, we performed immunohistochemistry for proliferating and differentiated cells, as well as Alcian blue staining for mucins in colonic tissues of CIC-2−/− colitis mice. There were no differences in crypt height (Fig. 5A), the number of proliferative cells (PCNA+, Fig. 5B), enterochromaffin cells (CgA+, Fig. 5C), and colonocytes (CAII+, Fig. 5E) between the 2 groups. However, the number of goblet cells was significantly increased in lubiprostone-treated colitis mice compared with CIC-2−/− colitis mice (Fig. 5D). These findings suggested that the modest protective effects of 100 μg/kg lubiprostone in CIC-2−/− mice might be attributable to a change in the mucus-secreting capacity of the mucosa. However, the effects of high-dose lubiprostone in CIC-2−/− mice were far less than WT mice treated with high-dose lubiprostone (see Fig. 1 for comparison) and limited only to a modest reduction of loss of body weight, DAI, and intestinal permeability with no differences in histological appearance of the intestine. An additional finding was that there were no changes in the number of goblet cells when comparing untreated CIC-2−/− and WT mice (data not shown). To further explore these findings, additional analyses of tissues from WT colitis with DSS colitis were performed. In these mice, DSS colitis was associated with an increase in crypt height, proliferation index (increased PCNA+ cells), and a reduction in CAII+, CgA+ cells, and goblet cells (Fig. 6A–E). Treatment of WT DSS colitis mice with 100 μg/kg lubiprostone significantly reversed all of these trends. Thus, high-dose lubiprostone has significant effects on goblet cell numbers as had been seen in CIC-2−/− mice. However, in WT mice, there was also an effect of lubiprostone on epithelial proliferation/differentiation, which had not been seen in the absence of CIC-2.

Lubiprostone Ameliorates Murine Colitis Induced by TNBS

To extend our observations on the beneficial effects of lubiprostone in DSS colitis, a TNBS-induced model of CD in BALB/c mice was used. TNBS-induced colitis mice had progressive reductions in body weight and increases in DAI that were maximal at day 3, associated with marked histological pathology, including mucosal infiltration with neutrophils and lymphocytes, ulceration, and severe submucosal edema. However, mice treated orally with lubiprostone (100 μg/kg) showed significantly less loss of body weight and a significantly reduced DAI (Fig. 7A, B). As had

![FIGURE 4. Effect of high-dose lubiprostone in CIC-2−/− mice DSS-induced colitis. A and B, Lubiprostone reduced clinical signs of DSS colitis, demonstrated by weight-loss curve (A) and DAI (B) (n = 5). C and D, Effect of lubiprostone on intestinal permeability (C) and colon shortening (D) in DSS-induced colitis (n = 5). E and F, Histological scoring (E) and representative H&E-stained sections (F) from colonic tissue of each groups (n = 5) (Bar = 100 μm). #P < 0.05 and ##P < 0.01, versus DSS without lubiprostone; Student’s t test.](https://academic.oup.com/ibdjournal/article-abstract/21/12/2747/4579121/2753)
FIGURE 5. Oral treatment with lubiprostone increased goblet cell differentiation in DSS-induced colitis in ClC-2−/− mice. Histological analyses were performed to evaluate colonic epithelial proliferation and differentiation in DSS colitis in the absence of lubiprostone (DSS) or the presence of 100 μg/kg lubiprostone (DSS + Lubi). A, Crypt height (as an indicator of crypt proliferation) in H&E-stained colonic cross sections, showing no significant difference between DSS and DSS + Lubi-treated mice. B, The ratio of proliferating cells per crypt, quantified using PCNA staining, was also not significantly different in sections from DSS or DSS + Lubi mice. C, The ratio of enteroendocrine cells per crypt was quantified using CgA staining and showed no significant difference between mice subjected to DSS alone versus those treated with DSS + Lubi. Arrow heads indicate CgA-positive cells. D, The number of goblet cells per crypt was quantified using Alcian blue staining and showed dramatic and significant (**P < 0.001) increases in the number of goblet cells in the DSS + Lubi group as compared with mice subjected to DSS alone. E, Immunohistochemical staining for CA II as a marker of mature colonocytes. The brackets indicate highly expressed CAII in the crypts, but there was no perceptible difference between the DSS and DSS + Lubi groups (no quantification shown) (Bar = 50 μm). Data are represented as mean ± SEM (n = 5 or 6 for each group).

FIGURE 6. Oral treatment of lubiprostone decreases cellular turnover and induces goblet cell differentiation. Histological analyses were performed to evaluate colonic epithelial proliferation and differentiation in control (CT), DSS colitis (DSS), and 100 μg/kg of lubiprostone-treated DSS colitis WT mice (DSS + Lubi). A, Crypt height in H&E-stained colonic cross sections was significantly elevated in the DSS group versus control (CT) mice, whereas the DSS + Lubi group had a midrange crypt height significantly different from the CT and DSS alone groups. B, The ratio of proliferating cells per crypt was quantified using PCNA staining, showing the same pattern of significant changes as the crypt height data. C, The ratio of enteroendocrine cells per crypt was quantified using CgA staining (arrowheads indicate CgA-positive cells). D, The number of goblet cells per crypt was quantified using Alcian blue staining, showing significant reductions in goblet cells in the DSS group as compared with CT and DSS + Lubi groups. E, Immunohistochemical staining of CA II for differentiated colonocytes showing the same pattern of change as the enteroendocrine and goblet cells. Brackets indicate highly expressed CAII in the crypts (Bar = 50 μm). Data are represented as mean ± SEM (n = 4 or 5 for each group). **P < 0.01 and ***P < 0.001 versus CT; #P < 0.05; ##P < 0.01, and ###P < 0.001 versus DSS; one-way analysis of variance.
been shown in mice with DSS colitis, high-dose lubiprostone also significantly ameliorated colon shortening induced by TNBS (Fig. 7C) and restored the histological score compared with vehicle control mice (Fig. 7E). In terms of barrier function, lubiprostone also significantly reduced TNBS-induced increases in intestinal permeability (Fig. 7D). However, treatment of TNBS colitis in mice with low-dose (10 μg/kg) lubiprostone did not show any beneficial effects on the loss of body weight and DAI (see Fig., Supplemental Digital Content 6, http://links.lww.com/IBD/B55).

DISCUSSION

In this report, pharmaceutical targeting of the chloride channel ClC-2 appears to reduce symptoms in experimental colitis, associated with the restoration of barrier function. The expression of ClC-2 in the apical lateral membrane raises questions as to its regulatory interactions with signaling molecules in the tight junctions.\(^5\),\(^3\),\(^3\),\(^9\) Previously, we have demonstrated that the severity of colitis and intestinal permeability is increased in ClC-2;\(^{-/-}\) mice, associated with perturbed mucosal epithelial tight junctions.\(^1\),\(^5\) In a detailed mechanistic study, we have demonstrated that ClC-2 plays a critical role in shuttling the tight junction protein occludin to the apical lateral membrane through an interaction with caveolin-1 and the small GTPase Rab5.\(^1\),\(^6\) We have also shown that the ClC-2 activator lubiprostone accelerates barrier repair in ischemic-injured porcine intestine by regulating tight junctions in a ClC-2-dependent manner.\(^1\),\(^9\),\(^4\) Interestingly, expression of other members of the ClC family of chloride channels, including ClC-3 and ClC-5, have been shown to be reduced in colonic tissues from patients with IBD as compared with normal tissues.\(^4\),\(^1\) Furthermore, genetic deletion of ClC-3 increased the susceptibility of animals to experimental colitis by promoting intestinal epithelial cell apoptosis and Paneth cell loss.\(^4\) In addition, ClC-5 may be involved in the reduction of intestinal Na\(^+\) absorption by inducing trafficking of Na\(^+\)/H\(^+\)-exchanger 3 (NHE-3), thereby contributing to diarrhea in IBD.\(^4\) Although the mechanisms of each of the ClC channels in IBD seem to be distinct, these data suggest that chloride channels have a critical role in IBD pathogenesis beyond their role as ion transporters.

In this study, we used 2 distinct murine models of IBD: DSS- and TNBS-induced colitis. Oral administration of lubiprostone in a preventive manner before administration of DSS reduced mucosal pathology and abnormal barrier function in DSS-induced colitis, a widely used model for UC-like intestinal inflammation and preserved intestinal barrier integrity by ClC-2-dependent tight junction reconstitution. Moreover, lubiprostone treatment suppressed the corresponding intestinal inflammation, leukocyte infiltration, and overproduction of proinflammatory cytokines observed during colitis. Lubiprostone also showed therapeutic effects when mice were treated after showing clinical signs of DSS-induced colitis. In addition, oral administration of a relatively high dose of lubiprostone (100 μg/kg) protected mice against TNBS-induced colitis, which more closely resembles human CD. Although DSS- and TNBS-induced colitis models have distinct differences from human IBD, these models share many clinical and pathological features with human IBD regarding loss of barrier function and inflammatory response.\(^2\),\(^8\),\(^4\)

Intestinal permeability has been proposed to be primarily under the control of interepithelial tight junctions.\(^1\) In this study, we show that preventive treatment with lubiprostone prevented DSS-induced alteration of tight junction proteins in WT mice but not ClC-2;\(^{-/-}\) mice. In a previous study examining human IBD tissues, a significant reduction in the expression of the tight junction molecule occludin was detected at the protein and mRNA levels by Western and northern blotting, respectively, in colonic epithelial cells.\(^4\) Our present results also showed that the total and

FIGURE 7. Preventive effect of lubiprostone in TNBS-induced colitis. A and B, Lubiprostone reduced clinical signs of DSS colitis, demonstrated by weight-loss curve (A) and DAI (B) (n = 9). C, Effect of lubiprostone on colon shortening in DSS-induced colitis (n = 9). D, In vivo permeability was evaluated using 4 kDa FITC-dextran (n = 6). E, Representative H&E-stained sections and histologic scoring from colonic tissue of each groups (n = 5). \(*P < 0.05, **P < 0.01, and ***P < 0.001\) versus controls (CT); \#P < 0.05 and \##P < 0.01, versus TNBS without lubiprostone; one-way analysis of variance.
This overexpression of claudin-2 is pharmacologically targeting ClC-2 also seems to
In 2005;17:453 Although total expression of
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mice resulting in limited protective effects on intestinal
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In 2007;87:545 Additionally, it has been reported that a number of clau-
In this study, claudin-1 endocytosis was detected by immuno
claudin-1 was not signi-
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orescence. However, lubiprostone treatment restored immunoloc-
this study, although the total expression of claudin-2 was not
significantly increased in tissues from mice with DSS-induced colitis, the apical membrane expression of claudin-2 was dramatically increased in the mucosal surface and crypts of colitis mice. Oral treatment with lubiprostone significantly reduced the total and apical membrane expression of claudin-2, suggesting a direct treatment effect relevant to human IBD. Regarding other tight junction proteins in IBD, one report indicated that total expression of the sealing junctional protein claudin-1 was significantly decreased in patients with UC. Although total expression of claudin-1 was not significantly changed in DSS-induced colitis in this study, claudin-1 endocytosis was detected by immunofluorescence. However, lubiprostone treatment restored immunolocalization of claudin-1 in mice with DSS-induced colitis as further evidence of a positive mechanistic effect of this CIC-2 activator. CIC-2−/− mice with DSS colitis also showed redistribution of tight junction proteins similar to WT mice with colitis. However, oral treatment with high-dose lubiprostone did not have any effect on tight junction proteins in these knockout animals. These data are therefore highly suggestive of the need for CIC-2 as a target for lubiprostone to repair the tight junction barrier in the setting of murine DSS colitis.

Although most studies concerning the role of tight junctions in IBD have focused on their critical role in regulating paracellular permeability, tight junctions are also mechanistically involved in regulation of cell proliferation and differentiation. Some of this knowledge has come from genetically modified mice. For example, claudin-15 deficiency leads to megaintestine caused by an increased number of transit-amplifying intestinal cells in the crypts. Additionally, it has been reported that a number of claudins are significantly altered in colitis-associated colorectal cancer, supporting the existence of a relationship between the tight junctions and intestinal epithelial cell proliferation. In this study, CIC-2–dependent tight junction reconstitution by lubiprostone reduced epithelial proliferation and induced differentiation in experimental colitis. The observed effects of lubiprostone-induced goblet cell proliferation have been not previously been noted to our knowledge, and although we speculate proliferation may relate to changes in tight junction structure, we have not investigated these mechanisms in detail. Overall, our data demonstrate that the CIC-2 activator lubiprostone ameliorates both DSS- and TNBS-induced colitis in mice. The beneficial effects of lubiprostone were associated with the protection of tight junctions and regulation of cellular differentiation that were dependent on the presence of CIC-2 (Fig. 8). Alternatively, lubiprostone promoted goblet cell proliferation in DSS-induced colitis in CIC-2−/− mice resulting in limited protective effects on intestinal barrier function. Although lubiprostone is presently indicated as a prosecretory drug in a number of human conditions involving constipation, pharmacologically targeting CIC-2 also seems to restore tight junction barrier function in experimental colitis, and therefore may provide a mechanistic basis for future exploration of alternative therapies of IBD.

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