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Basic Study

Diabetes exacerbates inflammatory bowel disease in mice with diet-induced obesity

Diabetes exacerbates inflammatory bowel disease

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Abstract

BACKGROUND

The increased prevalence of inflammatory bowel disease among patients with obesity and type 2 diabetes suggests a causal link between these diseases, potentially involving the effect of hyperglycemia to disrupt intestinal barrier integrity.

AIM

We investigated whether the deleterious impact of diabetes on the intestinal barrier associates with increased inflammatory bowel disease severity in a murine model of colitis in mice with and without diet-induced obesity.

METHODS

Mice were fed chow or high-fat diet and subsequently received streptozotocin to induce diabetic-range hyperglycemia. Six weeks later, dextran sodium sulfate was given to induce colitis. In select experiments, a subset of diabetic mice was treated with the antidiabetic drug dapagliflozin prior to colitis onset. Endpoints included both clinical and histological measures of colitis activity as well as histochemical markers of colonic epithelial barrier integrity.

RESULTS

In mice given a high-fat diet, but not chow-fed animals, diabetes was associated with significantly increased clinical colitis activity and histopathologic markers of disease severity. Diabetes was also associated with a decrease in key components that regulate colonic epithelial barrier integrity (colonic mucin layer content and epithelial tight junction proteins) in diet-induced obese mice. Each of these effects of diabetes in diet-induced obese mice was ameliorated by restoring normoglycemia.

CONCLUSION

In obese mice, diabetes worsens clinical and pathologic outcomes of colitis *via* mechanisms that are reversible with treatment of hyperglycemia. Hyperglycemia-induced intestinal barrier dysfunction offers a plausible mechanism linking diabetes to increased colitis severity. These findings suggest that effective diabetes management may decrease the clinical severity of inflammatory bowel disease.

Key Words: Inflammatory bowel disease; Type 2 diabetes; Obesity; Intestinal barrier; Hyperglycemia; Colitis in mice; Tight junction proteins

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Core Tip: Metabolic syndrome affects many patients with inflammatory bowel disease (IBD). This study used mouse models of colitis to investigate how diabetes and obesity interact to impair intestinal barrier function and exacerbate IBD outcomes, highlighting the deleterious impact of sustained hyperglycemia on intestinal barrier integrity. We show that diabetic hyperglycemia impairs the colonic mucin barrier and tight junction protein abundance in the setting of diet-induced obesity, which corresponds to worse clinical and histopathological IBD outcomes. These findings are important because as more patients with IBD are affected by obesity and/or diabetes, it is imperative to understand how these disease processes interact.

INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), and type 2 diabetes (T2D) are among the most challenging and costly medical disorders in modern society. Each is a chronic condition with no permanent medical cure that is increasing in prevalence globally and is associated with significant patient morbidity and economic cost[1,2]. An autoimmune condition affecting the

gastrointestinal tract, the pathogenesis of IBD is multifactorial, involving genetic predisposition, immunologic abnormalities, alterations in gut microbiota, and environmental factors, particularly exposure to a Western diet[3–5]. The pathogenesis of T2D involves insulin resistance and loss of pancreatic β cell function related to risk factors including obesity, visceral adiposity, and exposure to high-fat and high-sugar diets[6,7].

There is increasing evidence that these two disease processes may be linked. Recent national cohort studies have shown that patients with IBD are at increased risk of developing T2D, even after controlling for multiple risk factors including steroid exposure, age, and body mass index[8,9]. Furthermore, the development of T2D in patients with IBD is a predictor of poor disease-related outcomes, with several studies showing higher rates of IBD-related hospitalizations in patients with IBD[10,11], as well as disease flares in patients with either CD or UC, and increased IBD-related surgeries in patients with CD[11].

Obesity also constitutes a potential link between T2D and IBD. T2D is strongly associated with obesity, with the majority of T2D patients being either overweight or obese[12,13]. Obesity also affects up to 40% of adult IBD patients, with an additional 20%-40% of patients being overweight[14]. Comorbid obesity in IBD patients has been associated with higher rates of surgical complications and more severe disease that may be less responsive to standard medical therapies[15,16]. Consumption of obesity-inducing high-fat diets (HFDs) is a well-known risk factor for developing IBD[17–19], and in preclinical rodent studies, diet-induced obesity (DIO) caused by consuming a HFD worsens clinical and histological IBD outcomes[20,21]. However, the mechanisms underlying the relationship among DIO, T2D, and the development of IBD are unknown.

Notably, both IBD and metabolic syndrome (of which T2D and DIO comprise two of the principle components) are associated with altered gut microbiome, chronic systemic inflammation, and intestinal barrier dysfunction[4,22,23]. Specifically, the role of increased gut permeability leading to enhanced influx of microbial products from the gut lumen

into systemic circulation has been implicated in the pathogenesis of metabolic syndrome and its many complications[22]. Increased gut permeability is also associated with active IBD, as confocal laser endomicroscopic assessment of fluorescent leakage across the intestinal barrier is increased in symptomatic patients with IBD (both CD and UC) compared to healthy controls and asymptomatic IBD patients[24].

Currently, the mechanisms that link intestinal barrier dysfunction in diabetes, obesity, and IBD remain poorly understood. Recent work indicates that diabetic hyperglycemia may drive intestinal barrier impairments resulting in increased risk for enteric infections[25]. However, the extent to which intestinal barrier dysfunction contributes to the increased risk of IBD complications in patients with comorbid T2D—and what role diet-induced obesity may play in this effect—is unknown.

The current work was undertaken to determine the pathogenic role of diabetes to exacerbate intestinal inflammation in a mouse model of IBD with or without co-existing DIO, highlighting intestinal barrier disruption as a mechanism that links diabetes and IBD.

MATERIALS AND METHODS

Animals

Eight-week-old C57BL/6J male mice were purchased from Jackson Laboratory (Bar Harbor, ME, United States). All animals were group-housed under specific pathogen-free conditions in a temperature-controlled environment (14:10 h lights on/off cycle; lights on at 7:00 am) in cages containing a maximum of five animals. Mice were either fed standard laboratory chow (5053 PicoLab® Rodent Diet 20; LabDiet, St. Louis, MO, United States) throughout the study or placed on an HFD (D12492; Research Diets, New Brunswick, NJ, United States) at 8 wk of age for a period of 9 subsequent weeks to induce DIO. All procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Washington. Body

weight (BW) and food intake were recorded at least twice weekly throughout the experiment.

Streptozotocin-induced diabetes

Mice were randomly chosen to be placed on HFD for 9 wk to induce DIO (n = 30) or maintained on standard chow (n = 20), and then received five consecutive daily intraperitoneal injections of streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO, United States) at a low dose (40 mg/kg BW) to induce diabetes-range hyperglycemia (STZ-diabetes; random blood glucose levels ≥ 250 mg/dL) or sodium citrate vehicle control (Veh) at an equivalent dose (40 mg/kg)[26]. Mice were chosen randomly for the treatment groups, anesthetized with isoflurane during STZ injections, and placed immediately back into their home cage afterwards. Following STZ administration, random blood glucose levels were recorded at least twice weekly throughout the experiment.

Dextran sodium sulfate-induced colitis

Six weeks following STZ or Veh administration, 2% dextran sodium sulfate (DSS) (36-50 kDa; MP Biochemicals, Santa Ana, CA, United States) dissolved in autoclaved water, or untreated autoclaved water (Veh), was provided in drinking water for 7 d, with 4-8 animals per experimental group. Animals were acclimated to the medicated water delivery system for 1 wk prior to DSS course in their home cage, with daily water and food intake recorded. To control for the observation that diabetic mice consumed more water leading up to the DSS course, paired water administration was performed during the DSS course. Disease activity index (DAI) scores were calculated daily based on the sum of the percentage of BW lost (0-4), degree of rectal bleeding (0-4), and consistency of stools (0-4)[27].

Diabetes treatment with a sodium-glucose cotransporter-2 inhibitor

A subset of C57BL/6J male mice (n = 23) was placed on an HFD to induce DIO and then received either STZ or sodium citrate Veh as described above. After 6 wk of sustained hyperglycemia, the sodium-glucose cotransporter-2 inhibitor (SGLT2i) dapagliflozin was added to the drinking water for randomly selected groups at a dose of 25 mg/kg based on ideal BW (0.03 kg) to ameliorate hyperglycemia for 3 wk in total[28]. During the last week, 2% DSS was added to drinking water for select experimental groups as detailed above. Results were compared to mice that received drinking water without dapagliflozin. There were 7-8 animals in each experimental group.

Tissue collection

Mice were sacrificed on day 7 of the DSS course after euthanasia with an anesthetic overdose. Blood, colon, small intestine, liver, mesenteric fat, spleen, and fecal samples were collected. The spleen weight was recorded. Sections of liver, spleen, and mesenteric fat were stored either fresh frozen at -80 °C or placed into 10% zinc-buffered formalin (ZBF) for fixation and histological processing. The entire colon was removed from the surrounding mesentery and excised, and the length was measured from the end of the cecum to the end of the rectum. The colon was flushed with 0.1 M phosphate-buffered saline (PBS). A small section of proximal colon was cut and flash frozen at -80 °C, and the remaining colon was fixed in ZBF for histological examination. The small intestine was divided in half, flushed with PBS, and pieces of proximal and distal small bowel were flash frozen at -80 °C, and the remaining tissue was placed into ZBF. Tissue collection and assessment of intestinal barrier integrity were performed 7 wk after STZ administration, limiting any direct toxic effect of intraperitoneal injection of STZ on the colon.

Histopathology

After 3 d fixation in ZBF, the intestines were cut longitudinally, swiss-rolled, pinned, and placed in 70% ethanol. Colon, proximal small bowel, and distal small bowel were paraffin embedded, sectioned at 4 μm thickness, de-paraffinized, and stained with

either hematoxylin and eosin, or Alcian blue (AB) to stain mucins by the University of Washington Diabetes Research Center Cellular and Molecular Imaging Core. Using a modified protocol from Wirtz *et al*[29], histopathologic scoring of the extent of tissue damage was performed by a blinded pathologist, taking into account distribution of tissue damage (0 = none, 1 = focal [less than 3 sites], 1 = moderate [3-5 sites], 3 = diffuse [> 5 sites]), severity of tissue damage (0 = none, 1 = isolated focal epithelial damage, 2 = mucosal erosions and ulcerations, 3 = extensive damage deep into the bowel wall), lamina propria inflammatory cell infiltration (0 = infrequent, 1 = increased, some neutrophils, 2 = submucosal presence of inflammatory cell clusters, 3 = transmural cell infiltrations), and presence of chronic or active inflammation (0 = none, 1 = active, 2 = chronic).

Brightfield microscopy of AB-stained colonic tissues was performed using the Keyence BZ-X800 microscope (Keyence Corp. of America, Itasca, IL, United States). Four random images at 20x magnification were taken from each animal of intact colonic tissue, avoiding any ulceration of the epithelium, from a blinded independent investigator. Percent area of AB staining was performed using Fiji open source imaging software specific for AB imaging analysis (Color Deconvolution, Vector: Alcian Blue & H, Color 1). Threshold limits were set (0, 207) and used to measure the percent area of staining across the region of interest selected (4 representative crypts per image). This process was repeated for four distinct images per animal and values were averaged per animal.

Immunofluorescence

Colon sections mounted on slides were de-paraffinized with Xylene and antigen-retrieval in 10 mmol/L sodium citrate, pH 6.0 was performed. Sections were incubated in 0.1 M PBS followed by 0.2% Triton X-100 in PBS, then blocked in 2% donkey serum in 0.05% Triton X-100 at 37 °C for 1 h, and finally incubated overnight with rabbit anti-E-cadherin (24E10; Cell Signaling Technology, Danvers, MA, United States). Sections were

washed, incubated for 2 h with Alexa 555-conjugated donkey anti-rabbit antibody, and then stained with DAPI.

Immunofluorescence images were captured using the Keyence BZ-X800 microscope, and four random images of intact colonic tissue at 20x magnification were taken from each animal. Percent area of E-cadherin staining in the epithelium was performed using Fiji open source imaging software, building on prior published immunohistochemistry protein quantification methods[30]. Fluorescent images (E-cadherin: 555) were converted to 8-bit images, threshold staining limits were set (E-cadherin: 9, 255), and the region of interest outlining the entire epithelium captured in the 20x image was selected. Percent area values were recorded for each image, repeated for a total of four images per animal, and then averaged.

Statistical analysis

Data from individual experiments including blood glucose, DAI, colon length, and spleen length data are shown as dot plots representing data from individual animals, and bar graphs represent mean ± standard error of the mean (SEM). AB and E-cadherin data are also presented as mean values ± SEM. Student's *t*-test was used to compare the means in two groups, and one-way analysis of variance was used to compare multiple groups, using GraphPad Prism 5 (GraphPad software, La Jolla, CA, United States). Animals were not excluded from the study unless otherwise indicated. All differences were considered statistically significant at $P < 0.05$.

RESULTS

Effects of STZ-diabetes and DSS-induced colitis on BW, blood glucose, food intake, and water consumption in DIO mice

As a first step, we characterized the effects of STZ and DSS administration either alone or in combination on BW, blood glucose, food intake, and water intake in DIO mice. Mice were fed an HFD for 9 wk to induce DIO, and then given either Veh or STZ to induce diabetes. After 6 wk, both STZ and Veh groups were subsequently exposed to

either DSS or control drinking water (Veh), generating four study groups (Veh/Veh, STZ/Veh, Veh/DSS, and STZ/DSS). In terms of BW, all mice developed DIO after 9 wk of HFD leading up to STZ administration, with no significant differences in BW between each group (mean BW pre-STZ Veh/Veh 45.9 ± 3.9 g, Veh/STZ 45.4 ± 1.6 g, Veh/DSS 45.02 ± 5.0 g, STZ/DSS 44.4 ± 5.2 g; $P = 0.924$) (Figure 1A). Following STZ administration, BW decreased relative to Veh-treated controls but stabilized prior to DSS administration (Figure 1A). While BW was maintained in Veh-treated non-diabetic (Veh/Veh) and STZ-diabetic (STZ/Veh) mice, BW declined in both Veh/DSS and STZ/DSS groups throughout the 7-d DSS course, but to a greater extent in STZ/DSS mice (Figure 1A, B).

As expected, STZ administration induced diabetes-range hyperglycemia across all groups relative to Veh-treated controls (30-d pre-DSS random blood glucose mean level: STZ 333 ± 31 mg/dL *vs* Veh 167 ± 10 mg/dL; $P < 0.0001$) (Figure 1C). Notably, the effect of DSS administration to reduce food and water intake in the STZ/DSS group (Figure 1E, F) was associated with significant reductions of the blood glucose level during DSS course (Figure 1C, D), an effect less pronounced in non-diabetic DSS-treated mice (Veh/DSS, Figure 1C-F). Paired water administration between Veh/DSS- and STZ/DSS-treated groups ensured that hyperglycemic mice did not consume more DSS-treated water (Figure 1F).

Diabetic hyperglycemia worsens clinical and pathological outcomes of DSS colitis in DIO mice

We next determined the effects of STZ-hyperglycemia on clinical and histopathological outcomes of DSS colitis in DIO mice. Diabetic STZ/DSS mice had a more rapid onset of DSS colitis, preceding the onset of clinical colitis symptoms in Veh/DSS mice by 2 d (day 2 *vs* day 4 following DSS) (Figure 2A, B), and their DAI scores were significantly higher throughout the entire course of DSS (from days 2-7; Figure 2A, B, $P < 0.05$ on days 2 and 5, $P < 0.0001$ on days 3, 4, 6, and 7), such that by the end of the DSS course (day 7), the mean DAI score was significantly higher in diabetic STZ/DSS mice

compared to normoglycemic Veh/DSS controls (Figure 2B). As disease activity was undetectable in groups that did not receive DSS (Veh/Veh and STZ/Veh), we conclude that STZ-diabetes does not independently cause colitis symptoms (Figure 2A).

In murine models of IBD, colonic length serves as a pathologic marker of disease severity, as it shortens in response to mural inflammation in DSS colitis[20]. In STZ/DSS mice, the mean colon length at time of sacrifice on day 7 was significantly shorter compared to Veh/DSS mice (Figure 2C). Spleen weight serves as another pathologic marker of disease severity in DSS colitis, as its weight increases in response to systemic inflammation[20]. Our finding that the spleen-to-BW ratio was significantly higher in STZ/DSS mice than Veh/DSS mice (Figure 2D) indicated greater systemic inflammation in the former group. Consistent with this interpretation, histologic damage scores assessing the degree of colonic inflammation and tissue injury were also significantly higher in STZ/DSS mice than in Veh/DSS mice (Figure 2E-G). Collectively, these findings indicate that in DIO mice, STZ-diabetes both hastens the onset of and worsens the clinical and histopathological severity of DSS-induced colitis.

Effect of hyperglycemia on intestinal barrier in IBD in DIO mice

To investigate the mechanism by which STZ-hyperglycemia mitigates IBD outcomes in the setting of DIO, we examined the effect of STZ with and without DSS exposure on intestinal barrier function. The mucous layer of the colon and the tight junction proteins in the colonic epithelial layer are key components of the barrier that defend against pathogen entry into the bloodstream. The former is composed of mucins secreted by goblet cells in colonic crypts, and by coating the colonic epithelial layer, it limits exposure of the epithelium to luminal contents[31,32]. When this mucous layer is thinned in disease states, its protective properties are diminished, placing the epithelium at greater risk of injury[31,32]. To assess the degree of mucous layer thinning, we stained postmortem colonic tissue for mucins and goblet cells with AB. Our findings showed that in DIO mice, hyperglycemia (STZ/Veh) was associated with significantly reduced AB staining compared to non-diabetic controls (Veh/Veh) (Figure 3A-C). We further

showed that at the end of the DSS course in DSS-treated mice, STZ-diabetes was associated with significantly decreased colonic AB staining, and sampled from areas of the colon with intact epithelium (Figure 3C). Therefore, diabetes was independently found to impair the colonic mucin barrier, with no significant effect of DSS alone when looking at areas of intact bowel without active colonic inflammation. Loss of the colonic mucin barrier, therefore, could play a causal role in the effect of diabetes to increase the rapidity of onset and severity of DSS colitis in the setting of DIO.

Tight junction proteins are an additional key component of the colon's protective barrier, as they help to regulate the permeability of the epithelial layer[33]. Changes in the composition and concentration of tight junction proteins that occur in many disease states including IBD are associated with a more permeable epithelial barrier[33–35]. In the setting of DIO, we found that the abundance of the colonic epithelial tight junction protein E-cadherin, a key determinant of epithelial barrier integrity, was significantly decreased in hyperglycemic mice (STZ/Veh)[36], compared to non-diabetic controls (Veh/Veh) (Figure 3D-F). In the presence of DSS exposure, STZ-diabetes was also associated with a significant decrease of colonic E-cadherin staining (Figure 3F) when superimposed on DIO. Combined with the decrease of the protective mucous layer noted above, these data suggest that epithelial barrier integrity is impaired by diabetic hyperglycemia. Importantly, the degree of hyperglycemia correlated inversely with both AB staining and E-cadherin staining in all DIO mice regardless of exposure to STZ or DSS (Figure 3G, H). Furthermore, in DSS-treated groups, the amount of AB and E-cadherin staining correlates inversely with DAI scores on day 7 (Figure 3I, J). Finally, the degree of hyperglycemia correlates directly with IBD activity (Figure 3K). Taken together, these findings support a mechanism whereby hyperglycemia increases the onset and severity of DSS colitis by decreasing the expression of key tight junction proteins and diminishing the colonic mucins that help maintain gut epithelial barrier integrity.

Hyperglycemia fails to worsen DSS-colitis symptoms or impair the intestinal barrier in chow-fed mice

To investigate the contribution made by DIO to the deleterious effects of STZ-diabetes on IBD outcomes in mice, we repeated the above experiments in chow-fed mice. Mice were given low-dose STZ (or Veh) to induce T2D-range hyperglycemia 6 wk prior to DSS. In contrast to HFD-fed mice (mean BW 45.1 ± 4.1 g) in the above experiments, chow-fed mice maintained normal BWs leading up to STZ administration (Figure 4A; mean BW 30.1 ± 2.1 g). STZ administration induced hyperglycemia leading up to the DSS course, which was comparable to that observed in mice fed an HFD (Figure 4B). During the course of DSS, however, in contrast to STZ-diabetic DIO mice, STZ-diabetic chow-fed mice (Chow/STZ/DSS) did not exhibit a more rapid onset or greater degree of severity of colitis following DSS administration compared to chow-fed mice without STZ-diabetes (Chow/Veh/DSS) (Figure 4C). Chow/Veh/DSS mice had a slightly shorter colon length on day 7 of DSS compared to Chow/STZ/DSS mice (Figure 1D), but there were no significant differences in spleen weight between both DSS-treated groups (Figure 1E). Notably, neither AB mucin staining (Chow/Veh/Veh *vs* Chow/STZ/Veh, $P = 0.761$; Chow/Veh/DSS *vs* Chow/STZ/DSS, $P = 0.994$) (Figure 4) nor E-cadherin tight junction protein staining (Chow/Veh/Veh *vs* Chow/STZ/Veh, $P = 0.863$; Chow/Veh/DSS *vs* Chow/STZ/DSS, $P = 0.999$) (Figure 4G) was impacted by STZ administration among chow-fed mice, irrespective of DSS administration. Thus, non-obese, chow-fed mice were protected from the deleterious effects of STZ-diabetes on both colitis severity and markers of gut epithelial permeability. These findings indicate that hyperglycemia exacerbates DSS-induced colitis only in the setting of DIO.

Restoring normoglycemia attenuates the deleterious effect of DSS treatment on colitis severity and intestinal barrier integrity

To determine the impact of hyperglycemia *per se*, independent of other elements of the diabetic state generated by STZ, on intestinal barrier function and colitis outcomes, we repeated the study with DIO mice treated with STZ and DSS, with the additional step of

administering the SGLT2i dapagliflozin at a dose that normalizes glycemia to a subgroup of these mice prior to DSS administration[28]. As before, mice were given an HFD to induce DIO (mean BW 49.7 ± 6.2 g) (Figure 5A) and were then given either vehicle or STZ to induce diabetic-range hyperglycemia (mean post-STZ blood glucose levels: Veh 173.6 ± 15.8 *vs* STZ 282.5 ± 68.6; $P = 0.0007$) (Figure 5B). After 6 wk of sustained hyperglycemia post-STZ treatment, the SGLT2i dapagliflozin (or Veh) was added to the drinking water, which resulted in euglycemic blood glucose levels equivalent to non-STZ treated control levels (mean 10 d pre-DSS blood glucose levels: Veh/Veh/DSS 156.8 ± 11.7 mg/dL *vs* STZ/SGLT2i/DSS 172.8 ± 17.5 mg/dL; $P = 0.793$) (Figure 5B, C) and significantly lower than STZ-treated mice who did not receive dapagliflozin (STZ/Veh/DSS 242.1 ± 72.8mg/dL *vs* STZ/SGLT2i/DSS 172.8 ± 17.5 mg/dL; $P = 0.022$).

After 2 wk of SGLT2i (or Veh) exposure, DSS was added to drinking water for the last week to induce colitis in each group. Similar to our earlier observations in DIO mice (Figure 1), DSS administration caused a significant reduction in BW (Figure 5A) and blood glucose levels in all groups (Figure 5B, C). Paired DSS water administration was performed, and total DSS consumption did not differ significantly between groups (Figure 5D). As previously noted (Figure 2A), the onset of colitis symptoms was accelerated in hyperglycemic STZ/Veh/DSS mice with DIO compared to normoglycemic Veh/Veh/DSS mice, becoming evident by day 2 compared to day 3 (Figure 5E, F). This more rapid onset of symptoms was mitigated by SGLT2i treatment, as STZ/SGLT2i/DSS mice manifested clinical colitis on day 3 instead of day 2 (Figure 5E, F). While symptoms of clinical colitis severity were comparable between groups from days 3-5 (Figure 5E), STZ/Veh/DSS mice demonstrated significantly worse DAI scores on both days 6 and 7 of DSS administration than either non-STZ treated mice (Veh/Veh/DSS) or STZ-treated mice receiving SGLT2i (STZ/SGLT2i/DSS) (Figure 5F).

Similar outcomes were observed when histochemical parameters of epithelial barrier were assessed in areas of intact colon in DSS-treated mice. The effect of untreated

hyperglycemia to decrease AB mucins in DIO mice (compared to non-diabetic controls) in intact areas of colonic epithelium was fully reversed by dapagliflozin treatment (Figure 6A, B), and a similar trend was observed for E-cadherin abundance, although these differences did not achieve statistical significance (Figure 6D, E). Moreover, the amount of colonic AB staining correlated inversely with the degree of hyperglycemia across the 3 groups (based on 1-wk average blood glucose levels prior to DSS) (Figure 6C). The abundance of the colonic tight junction protein E-cadherin reached near significance with an inverse correlation to blood glucose levels as well (Figure 6F). These findings strengthen the conclusion that in DIO mice, hyperglycemia is the primary driver of both STZ-induced colonic epithelial barrier disruption and colitis severity.

DISCUSSION

In the current work, we determined whether diabetic hyperglycemia exacerbates colitis severity in a murine model of IBD. Our findings demonstrate that diabetic hyperglycemia both accelerates the onset and worsens the clinical and pathological outcomes of DSS colitis. Interestingly, each of these effects of diabetes on IBD outcomes was detected in mice with DIO, but not in chow-fed, non-obese mice. Furthermore, we show that the severity of IBD increases directly in relation to the degree of hyperglycemia, and that reversal of hyperglycemia with an antidiabetic medication eliminated this effect of diabetes. Taken together, these findings support a model whereby the combination of obesity and hyperglycemia predisposes to more severe outcomes of intestinal inflammation in IBD.

As a first step towards understanding the mechanisms by which diabetes and DIO influence IBD outcomes, we sought to investigate the effect of diabetes on the intestinal barrier, focusing in particular on the colonic mucin layer and tight junction proteins. Previous work had suggested that diabetic hyperglycemia impairs intestinal barrier function and increases intestinal permeability, although how this might impact IBD pathology had not been studied[25]. Importantly, it has been reported that symptomatic

patients with both UC and CD have increased *in vivo* intestinal permeability[24] and decreased intestinal mucins and goblet cell depletion compared to healthy controls[37]. Additionally, in IBD colon organoid cultures, tight junction proteins are significantly reduced[38]. In our current work, we report that STZ-induced diabetic hyperglycemia significantly impairs these two components of intestinal barrier integrity in the setting of DIO. Furthermore, the amount of mucins and tight junction protein staining correlates inversely with colitis disease activity. This supports a model in which the diabetic state acts injuriously on the intestinal barrier, making the intestines more susceptible to DSS-induced colitis. It is notable that diabetes on its own is insufficient to cause IBD pathology, but when exposed to a chemical colitic agent, the presence of diabetes and associated impaired intestinal barrier function significantly worsens IBD outcomes.

Our next goal was to determine the contribution of DIO on the effect of diabetes to exacerbate DSS-induced intestinal inflammation. Obesity is present in the vast majority of T2D patients[12,13], and patients with IBD have similar rates of obesity compared to the general population[39]. Comorbid obesity has been associated with higher hospitalization rates, more active disease, and a higher prevalence of perianal disease in patients with CD[40]. In patients with UC, elevated BMI is associated with increased risk of biologic therapy treatment failure[41]. Furthermore, HFD exposure exacerbates IBD outcomes in preclinical rodent models[20,21]. Conversely, severe hyperglycemia seen in uncontrolled, insulin-deficient T1D has a deleterious effect on intestinal barrier function independent of obesity[25]. To determine the contribution of DIO on the ability of a more modest, physiologic degree of hyperglycemia, mimicking T2D, to impact intestinal barrier pathology and IBD outcomes, we tested whether low-dose STZ would worsen DSS colitis in DIO mice fed an HFD compared to non-obese, chow-fed mice. We report here that the effect of modest hyperglycemia to impair intestinal barrier function and therefore influence IBD outcomes is dependent on co-existing DIO. One potential explanation for these findings involves an effect of HFD exposure on the gut microbiome, shifting its composition to a more mucin-degrading and pro-inflammatory

profile[42]. When superimposed on this change of gut flora and the state of chronic inflammation in obesity, we observed clear-cut, deleterious effects of diabetes on both intestinal permeability and colitis disease activity. Further studies are warranted to determine the exact contribution that HFD consumption makes to worsen IBD outcomes independent of obesity.

Next, we determined the specific contribution made by hyperglycemia *per se* to the effect of diabetes on intestinal barrier pathology. To this end, we sought to normalize the blood glucose level without reversing other aspects of the uncontrolled diabetic state (*e.g.*, insulin deficiency, elevated circulating levels of ketone bodies and free fatty acids). This goal was achieved by administering an SGLT2i, which selectively normalizes glycemia without impacting insulin levels or other aspects of the diabetic state. We report that restoring normoglycemia *via* SGLT2i administration after STZ treatment modestly improved clinical colitis outcomes. Notably, the onset of clinical colitis was delayed in mice treated with SGLT2i compared to hyperglycemic mice, and the severity of colitis at the end of DSS course was significant improved compared to hyperglycemic mice. Reversal of hyperglycemia after STZ treatment with an SGLT2i resulted in significant improvement in the colonic mucin barrier, with no difference between non-STZ treated normoglycemic mice and mice treated with STZ who then received SGLT2i. The abundance of tight junction proteins also tended to improve with normalization of the blood glucose level, although it did not reach statistical significance. From these findings, we infer that the effect of diabetes to influence intestinal barrier pathology and IBD outcomes appears to be dependent, or at least heavily reliant, on hyperglycemia. This conclusion is further supported by our findings that the degree of intestinal barrier dysfunction and colitis disease severity varied directly with the degree of hyperglycemia. These findings heighten the importance of studies to evaluate the impact of effective diabetes treatment in patients with IBD, especially given recent evidence that patients with IBD are at increased risk of developing T2D[8,9], that comorbid T2D predicts poorer IBD outcomes[11], and that high-fat, obesogenic diets associate with higher incidence of IBD[17–19].

The growing patient population affected by T2D, obesity, and IBD strengthens creates a compelling rationale for continued efforts to understand shared mechanisms between these disease processes, particularly in light of evidence that comorbid T2D or obesity negatively affects IBD outcomes in patients[10,11,40,41]. It is also imperative to understand how treatments for each of these conditions affect the others, particularly as corticosteroids are a mainstay of treatment to induce remission in both UC and CD and are known to both exacerbate hyperglycemia in patients with pre-existing T2D and to precipitate hyperglycemia in patients with no prior diabetes diagnosis[43]. These considerations underscore the need for clinicians to consider how these disease processes and their respective treatments affect their patients and highlight the need to investigate the potential role of antidiabetic medications in IBD management prior to hyperglycemia onset or exacerbation among those at risk.

CONCLUSION

In mice with DIO, diabetic hyperglycemia disrupts the intestinal barrier integrity and is associated with more severe clinical and pathological outcomes of colitis, highlighting the potential translational importance of ensuring optimal diabetes management in IBD patients.

ARTICLE HIGHLIGHTS

Research background

Emerging epidemiologic evidence links type 2 diabetes (T2D) and obesity to inflammatory bowel disease (IBD). However, evidence to determine the exact mechanisms by which obesity and/or diabetes influence IBD outcomes is limited. This study uses mouse models of colitis to investigate how diabetes and obesity interact to impair intestinal barrier function and exacerbate IBD outcomes, highlighting the deleterious impact of sustained hyperglycemia on intestinal barrier integrity.

Research motivation

Patients with IBD are at increased risk of developing T2D, which serves as a predictor of poor outcomes in IBD. The rates of comorbid obesity in IBD are increasing as well, and obesity is related to more a severe IBD phenotype. As more patients with IBD are affected by obesity and/or T2D, it is imperative to understand how these disease processes interact and how treatments for each condition may impact the other.

Research objectives

In this study, we used murine models of colitis to determine the effect of T2D-range hyperglycemia on IBD outcomes and intestinal barrier function with and without coexisting diet-induced obesity (DIO).

Research methods

Mice were fed standard chow or high-fat diet (HFD) to induce DIO, and then given streptozotocin (STZ) to induce sustained T2D-range hyperglycemia. Mice were then given dextran sodium sulfate (DSS) to induce colitis. Body weight and blood glucose levels were compared, as well as clinical colitis scores and histopathologic assessment of intestinal injury. The effects of hyperglycemia and DIO on intestinal barrier function were interrogated by comparing colonic mucins and tight junction protein abundance. To highlight the role of hyperglycemia itself, a sodium-glucose cotransporter-2 inhibitor (SGLT2i) was subsequently used to selectively reverse hyperglycemia prior to DSS course.

Research results

In the setting of DIO, STZ-diabetes significantly worsened clinical and histopathological outcomes of DSS colitis in mice. This effect was associated with a significant reduction in the colonic mucin barrier and tight junction protein abundance and was ameliorated by the use of an SGLT2i to reverse hyperglycemia prior to colitis onset. Together, these findings highlight the deleterious effect of diabetic hyperglycemia on the intestinal barrier as a mechanism by which diabetes and obesity interact to affect IBD outcomes.

Research conclusions

This study reports the novel finding that diabetic hyperglycemia disrupts intestinal barrier integrity in the setting of DIO and exacerbates DSS colitis outcomes in mice. Given the increased prevalence of T2D in patients with IBD and the negative impact of comorbid obesity on IBD outcomes, it is imperative to understand how these disease processes interact.

Research perspectives

These findings have significant translational relevance, and future research can expand on them by determining whether strict glycemic control in patients with T2D and IBD is associated with improved IBD outcomes.

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