Abstract. Dipeptidyl-peptidase IV (DPP-IV) inhibitors are expected to prolong the half-life of Glucagon-like peptide (GLP-2) as well as GLP-1, and may result in the promotion of epithelial cell proliferation and regeneration. The aim of this study was to investigate whether a DPP-IV inhibitor can promote epithelial proliferation and attenuate dextran sodium sulfate (DSS)-induced colitis. Nine-week-old female C57/B6 mice were given a single dose of ER-319711 to assess the changes in plasma GLP-2 concentrations. Ten mice were divided into two groups: a vehicle group and an ER-319711 group. ER-319711 was administered orally for 7 days. The mice were then given bromodeoxyuridine (BrdU) intraperitoneally 2 h before sacrifice on day 7. Twenty-six mice were divided into three groups: a vehicle group, a DSS-induced colitis group and a DSS-induced colitis treated with ER-319711 group. The mice were given DSS for 5 days and sacrificed on day 14. Plasma GLP-2 levels were elevated in response to ER-319711. The ER-319711 group had a significantly decreased body weight from days 1 to 3. The number of BrdU positive cells per crypt and the crypt height were increased in the ER-319711 group. The DSS + ER-319711 group had a decreased body weight transition. The disease activity index and colon length showed an amelioration of colitis in the DSS + ER-319711 group. DPP-IV inhibitors are thought to promote the proliferation of the intestinal epithelium. However, the amelioration of DSS-induced colitis was only partial.

Introduction

Inflammatory bowel diseases, such as Crohn’s disease and ulcerative colitis, are characterized by chronic inflammation of the intestinal mucosa. Repeated damage and injury of the intestinal surface are key features of inflammatory bowel disease, and require the constant repair of the epithelium. In the process of epithelial regeneration, humoral factors such as fibroblast growth factor (FGF)-7, keratinocyte growth factor (KGF) (1), FGF-2 (b-FGF; basic-FGF), prostaglandins (2,3), and interleukin (IL)-11 (4,5) have been reported to attenuate epithelial injury. Several growth factors such as transforming growth factor (TGF)-β (6) and platelet-derived growth factor (PDGF) (7) play an important role in epithelial restoration. Moreover, glucagon-like peptide (GLP)-2 has been reported to attenuate indomethacin-induced colitis, and also promotes the proliferation and differentiation of long-lived progenitor cells (8).

GLP-1 and GLP-2 are both secreted from enteroendocrine L-cells and are rapidly degraded by dipeptidyl peptidase IV (DPP-IV) (9). Although GLP-1 is mainly involved in glucose metabolism, GLP-2 has several actions on the intestine: the promotion of epithelial proliferation, the inhibition of epithelial apoptosis, the stimulation of enterocyte glucose transport and glucose transporter 2 (GLUT2) expression, and the inhibition of gastric emptying and gastric acid secretion (10-12). In a clinical setting, GLP-2 has been administered to patients with short bowel syndrome. GLP-2 improved energy absorption, increased body weight and lean body mass, decreased fat mass, and increased crypt and villus height (13).

ER-319711 is a well-characterized DPP-IV inhibitor (14). ER-319711 inhibited human DPP-IV with an IC_{50} value of 0.089 µM, whereas its IC_{50} values toward human DPP8 and DPP9 were >100 µM. Antihyperglycemic activity of ER-319711 was similar compared with vidagliptin, a slow-binding and long-acting DPP-IV inhibitor, at the same dose (14). DPP-IV inhibitors, which are among the newest drugs for the treatment of type 2 diabetes mellitus (T2DM), are expected to prolong the half-life of GLP-2 as well as GLP-1, thus resulting in the promotion of epithelial cell proliferation.
and regeneration. The aim of this study was to investigate whether a DPP-IV inhibitor can promote epithelial proliferation and attenuate dextran sodium sulfate (DSS)-induced colitis.

Materials and methods

Animals. Nine-week-old female C57/B6 mice were purchased from Charles River Japan (Kanagawa, Japan). They were housed in a room maintained at 22°C under a 12-h day-night cycle throughout the experiments. The mice were fed normal chow (MF; Oriental Yeast Co., Ltd, Tokyo, Japan) and tap water ad libitum. All procedures were conducted according to the Animal Care and Use Committee of the Shiga University of Medical Science.

Effects of ER-319711 on plasma GLP-2 concentration. Fifteen mice were divided into three groups: i) a vehicle group (carboxymethylcellulose: CMC alone); ii) an ER-319711 (14) (DPP-IV inhibitor) (50 mg/kg) group; and iii) an ER-319711 (100 mg/kg) group. The ER-319711 was generously donated from Eisai Co., Ltd. (Ibaraki, Japan). Food was withheld for 12 h, and then the mice were given CMC or ER-319711 orally. Three hours later, plasma samples were taken. The GLP-2 levels were determined by a GLP-2 ELISA kit (Yanaihara Institute, Fujinomiya, Japan).

Effects of ER-319711 on epithelial proliferation. Ten mice were divided into two groups: i) a vehicle group (carboxymethylcellulose: CMC alone); and ii) an ER-319711 group (CMC + ER-319711). The ER-319711 was given orally via a gastric tube twice daily (100 mg/kg/day) for 1 week. The mice were fed normal chow (MF; Oriental Yeast Co.) and water ad libitum. The mice were then sacrificed on day 7. Two hours before sacrifice, the mice were given bromodeoxyuridine: BrdU (50 mg/kg) intraperitoneally.

Effects of ER-319711 on DSS-induced colitis. Twenty-six mice were divided into three groups: i) a vehicle group (CMC alone, n=6); ii) a DSS-induced colitis group: DSS group (n=10); and iii) a DSS-induced colitis treated with ER-319711 group: DSS + ER-319711 group (n=10). The ER-319711 was given orally via a gastric tube twice daily (100 mg/kg/day) for two weeks. The mice were given 2.0% (wt/wt) DSS (MP Biomedicals, OH, USA) added into their drinking water ad libitum for 5 days, then changed to normal water, and finally sacrificed on day 14.

Assessment of inflammation in DSS-induced colitis. Daily food and water intake was measured for each cage. The disease activity index (DAI) score of the DSS-induced colitis was assessed, including the body weight, an evaluation of stool consistency, and the presence of blood in the stools by a guaiac paper test. The stool consistency was assessed using the following four point scale: 0, normal; 1, soft; 2, very soft but formed; and 3, liquid. The intensity of the guaiac test was scored by the following scale: 0, negative; 1, faintly blue; 2, moderately blue; 3, dark blue; and 4, blood visible. A validated clinical disease activity index ranging from 0 to 4 was then calculated using the following parameters: stool consistency, presence of fecal blood, and changes in body weight (15). The mice were sacrificed at day 14, and the length and weight of their colons were measured.

Histological assessments. A histological examination was performed on three samples of the distal colon from each animal. The samples were fixed in 10% buffered formalin, dehydrated in ethanol, and then embedded in paraffin. Four micrometer-thick sections were then prepared and stained with hematoxylin and eosin (H&E). All histological evaluations were performed in a blinded fashion using a validated scoring system (16). BrdU was detected using a BrdU immunohistochemistry system (Oncogene Research products, San Diego, CA), and the number of BrdU positive cells per crypt were counted. The crypt height was measured on cross sections stained with H&E, and an average of 10 well-oriented crypts from 3 different cross sections were analyzed per mouse.

Statistical analysis. The statistical significance of the differences was determined by the Mann-Whitney U test. Differences resulting in P-values <0.05 were considered to be statistically significant.

Results

Effects of ER-319711 on plasma GLP-2 levels. As shown in Fig. 1, the plasma GLP-2 levels were elevated after 3 h of administration in the ER-319711 (50 mg/kg) group without statistical significance. In the ER-319711 (100 mg/kg) group, the plasma GLP-2 levels were significantly increased as compared to vehicle. Although the administration of ER-319711 at a dose of 50 mg/kg was not enough to significantly elevate the plasma GLP-2 concentration, the average plasma GLP-2 concentration tended to increase. The dose of 50 mg/kg was used for the following experiments.

Effects of ER-319711 on epithelial proliferation. As shown in Fig. 2a, the ER-319711 group had a significantly decreased body weight from days 1 to 3 as compared to the vehicle group. Food and water intake in the ER-319711 group tended to be less than in the vehicle group (Fig. 2b). However, the body weight of the ER-319711 group gradually increased, and caught up with the vehicle group after day 5 (Fig. 2a).
The colon length, weight and weight/length ratio were all nearly identical (Fig. 3). The number of BrdU positive cells per crypt increased in the ER-319711 group without statistical significance (P=0.052, Fig. 4a). Moreover, the crypt heights were significantly increased in the ER-319711 group (Fig. 4b). Therefore, the colon weight/length ratios were all nearly identical (Fig. 3). The number of BrdU positive cells per crypt increased in the ER-319711 group without statistical significance (P=0.052, Fig. 4a). Moreover, the crypt heights were significantly increased in the ER-319711 group (Fig. 4b).

**Effect of ER-319711 on DSS-induced colitis.** The DSS + ER-319711 group had a decreased body weight transition as compared to the DSS group. The difference between the two groups was significant on days 2, 4, 10 and 12 (Fig. 5). However, on day 14, there was no significant difference between these two groups. The DAI and colon length showed an amelioration of the colitis in the DSS + ER-319711 group as compared to the DSS group, which was statistically significant (Fig. 6a and c). However, the colon weight was significantly heavier in the DSS + ER-319711 group as compared to the DSS group (Fig. 6b). Therefore, the colon weight/length ratios were all nearly identical (Fig. 3). The number of BrdU positive cells per crypt increased in the ER-319711 group without statistical significance (P=0.052, Fig. 4a). Moreover, the crypt heights were significantly increased in the ER-319711 group (Fig. 4b).
were nearly identical between the DSS group and the DSS + ER-319711 group (Fig. 6d). In addition, the histological score showed a slight amelioration in the DSS + er-319711 group as compared to the DSS group, but without statistical significance (Fig. 7).

Discussion

The administration of ER-319711 for one week did not cause any changes in the length or weight of the colon, but the body weight was significantly decreased in the early days. There are some reports accounting for the mechanism of body weight loss after the administration of DPP-IV inhibitors. The plasma concentration of GLP-1 was increased as well as GLP-2, thus resulting in a delayed gastric emptying time and suppressed appetite (17). In addition, Deacon et al showed a decreased body weight loss in response to GLP-1 analogs in humans (18,19). However, the body weight after DPP-IV administration has been reported to decrease (20) or stay unchanged in humans (18). Our results showed a decreased tendency for food and water intake in the ER-319711 group. However, the body weight loss recovered quickly, and thus the body weight loss after the administration of ER-319711 may be a temporary effect. DPP-IV inhibitors have also been reported to increase glucose-dependent insulinotropic peptide (GIP) and to induce body weight gain (21). Furthermore, Kos et al reported that DPP-IV inhibitors augmented the anti-lipolytic effect of neuropeptide Y in adipose tissues, supporting the lack of weight loss in T2DM (22). These mechanisms may take part in the recovery of the body weight during the later part of the experiment.

In the ER-319711 group, the elongation of the crypt length and an increased tendency towards BrdU positive cells were observed. These findings were thought to be mediated by an elevation in plasma GLP-2 levels. Cheng et al reported that GLP-2 attenuates indomethacin-induced colitis, and also promotes the proliferation and differentiation of long-lived progenitor cells. Interestingly, the receptor for GLP-2 was located on enteric neurons (8), and the GLP-2 activation of these neurons produced a rapid induction in c-Fos expression which signals the growth of columnar epithelial cell progenitors and stem cells.

In line with a previous report by Yazbeck et al (23), ER-319711 did not exhibit a strong amelioration of DSS-induced colitis in our experiments. However, despite the body weight loss in the early days, the disease activity index and colon length suggested an amelioration of the colitis. These results may reflect the increased proliferation and regeneration of epithelial cells rather than anti-inflammatory effects. The

Figure 6. Effects of ER-319711 on disease activity index (a), colon weight (b), colon length (c), and colon weight/length ratio (d) in DSS-treated mice. The data represent the means ± SD. *P<0.05, **P<0.01.

Figure 7. Histological findings for ER-319711 in DSS-induced colitis. The colon was excised after 14 days and stained with hematoxylin and eosin (x100). The data represent the means ± SD. *P<0.01; vehicle vs. DSS alone and DSS + ER-319711.
substrates of the DPP-IV inhibitors include not only peptides which mediate nutrition metabolism and neuropeptides (e.g. GLP-1, GLP-2, GIP, substance P), but also peptides which mediate immunity (e.g. lymphotxin, exotxin, RANTES, monocyte chemoattractant protein (MCP) and interferon-inducible protein 10 (IP-10). Therefore, inhibition of DPP-IV may result in increased pro-inflammatory chemokines.

Our results indicate that the ER-319711 showed proliferative effects on the colonic epithelium. However, the amelioration of DSS-induced colitis was only partial. As described above, GLP-2 agonists are given for short bowel syndrome. DPP-IV inhibitors have relatively fewer adverse effects, and can be used in patients with short bowel syndrome. The accumulation of more preclinical data is expected.

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References