Correlation between Plasma Glucagon-Like Peptide 2 Levels and Proliferative Makers in Small Intestinal Injury in Rats Induced by Methotrexate Administration

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MATERIALS AND METHODS

Animals Male Wistar rats (Clea Japan Inc., Tokyo, Japan) weighing 220—250 g were used in all experiments.

GLP-2, a 33-amino acid peptide member of a family of proglucagon-derived peptides (PGDP) that also includes enteroglucagon, is secreted from L-type entero-endocrine cells of the distal small intestine and colon.12) GLP-2 increases crypt cell proliferation and decreases the rate of apoptosis in small intestinal epithelium.13) Acute or chronic administration of GLP-2 induces functional changes in the intestine, improving its capacity for nutrient absorption.13—15)

In addition, GLP-2 treatment has been shown to enhance intestinal adaptation following major small bowel resection,16,17) to prevent TPN-induced atrophy,18) and to reduce the severity of dextran sulfate-induced colitis19) and indomethacin-induced enteritis20) in animal models. On the other hand, plasma GLP-2 levels appear to vary with intestinal mucosal condition and injury. However, although changes in various conditions of the intestinal mucosa are now being widely studied,21—23) the relationship between plasma GLP-2 levels and intestinal mucosal injury induced by various drugs, including antineoplastic agents, has yet to be clearly determined. Moreover, no standard methods of diagnosis or therapeutic protocols exist for chemotherapy-induced enteritis, and further research into new therapeutic avenues for treatment or prevention of this intestinal injury is needed.24)
Animals were acclimated to laboratory conditions for 5 d before experiments. They were housed under a 12-h light–dark cycle at 23 °C, with free access to standard rat chow and water. All experiments were performed in accordance with the Guidelines for Animal Experiments of Osaka University School of Medicine.

Administration of MTX MTX (Lederle Japan Ltd., Tokyo, Japan) was dissolved in distilled water before administration. MTX or vehicle alone was administered orally for 6 consecutive days at doses of 1.25, 2.5, and 5.0 mg/kg body weight per day (MTX(1.25)-G, MTX(2.5)-G and MTX(5.0)-G) on each of the 8 d. Animals were sacrificed on the morning of day 8, and body weight of each rat was monitored daily.

Mucosal Analysis Animals were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg body weight). At laparotomy, sections of duodenum, jejunum, and the middle portion of the ileum were quickly excised, and flushed with iced saline solution. Excess liquid was removed by blotting the tissue segments with filter paper, and segments were then immediately frozen at −80 °C until assay for DNA and protein content. Blood was taken directly from the vena cava for GLP-2 assay. Blood samples were drawn into vials on ice containing EDTA (2 mg) and aprotinin (400 KIU) per 1 ml of blood. After immediately centrifuging the blood, plasma samples were stored at −30 °C until assayed.

Each segment of rat intestine was then thawed and opened longitudinally, gently patted dry and weighed. The mucosa was scraped off with a glass slide, and weighed. Mucosal samples of each segment were homogenized on ice using a homogenizer (Model BM-1, Nihon Seiki Seisakusho, Tokyo, Japan) in a five-fold weight of ice-cold saline. The homogenate was used to assess DNA content and total protein after dilution with saline. Mucosal DNA was measured by the method of LePecq and Paoletti using polymerized calf thymus DNA (Sigma Chemical Co., MO, U.S.A.) as a standard.25) Total protein in the mucosa was measured by the method of Lowry et al. using bovine serum albumin as a standard.26)

Assay of Rat GLP-2 The EIA system for measurement of rat plasma GLP-2 levels was developed using synthetic rat GLP-2 (1—33) as a standard antigen (0.137—100 ng/ml), biotin-GFP (32) as a labeled antigen (2.42 ng/ml), and anti-rat GLP-2 serum Y652 (1 : 150000 dilution) as an antibody (YK140 Rat GLP-2 EIA, Yanaihara Inc., Shizuoka, Japan).

RESULTS

Rat Body Weight In all MTX-treated groups, a significant decrease in rat body weight was observed on the final day of the experiment when compared with the control group (123.6 ± 10.9%): MTX(1.25)-G, 105.5 ± 5.6% (p<0.01); MTX (2.5)-G, 91.3 ± 3.7% (p<0.01); MTX(5.0)-G, 83.9 ± 3.2% (p<0.01) (Fig. 1). Weight gain in the MTX (2.5)-G and MTX(5.0)-G groups was markedly suppressed from the 6th day after administration. No weight changes were observed in MTX (1.25)-G.

Plasma GLP-2 Levels Plasma GLP-2 levels were significantly increased on the final day after MTX administration compared with the control group (2.03 ± 0.16 ng/ml): MTX (2.5)-G, 3.44 ± 1.06 ng/ml (p<0.05); MTX(5.0)-G, 9.75 ± 4.04 ng/ml (p<0.01) (Fig. 2). Plasma GLP-2 levels in MTX(1.25)-G were 1.99 ± 0.26 ng/ml.

Mucosal Weight, DNA and Protein Content after MTX Administration Changes in rat mucosal weight, DNA and protein after administration of MTX are shown in Fig. 3. In each MTX-treated group, the ileum exhibited the highest values for all indices, with the order of indices being
ileum>duodenum>jejunum. The values of all indices tended to decrease in all segments with increased dose of MTX, with MTX (5.0)-G exhibiting the lowest values in all indices in each segment.

DISCUSSION

GLP-2 exerts its actions through a distinct and specific receptor expressed in its principal target tissue, the gastrointestinal tract. GLP-2 receptor RNA levels were highest in jejunum, followed by duodenum, ileum, colon and stomach, whereas expression was undetectable in other tissues. GLP-2 also is produced and secreted in a nutrient-dependent fashion by the enteroendocrine L cells of the small and large intestine. Thus, GLP-2 has a highly tissue-specific trophic effect on the small intestine compared with various cytokines and growth factors with the intestinotropic and protective properties. The circulating levels of GLP-2 have been reported to be altered in the adapting or injured intestine. For example, the circulating levels of GLP-2 were increased in patients with active inflammatory bowel disease. Therefore, circulating levels of GLP-2 may reflect in the pathophysiological states of adapting or injured intestine. We examined in this study whether circulating levels of GLP-2 might be a potential index for chemotherapy-induced intestinal mucositis.

GLP-2 has been identified as a proglucagon-derived peptide with intestinotrophic activity in the intestinal mucosa. GLP-2 may be useful in adjunctive therapy in human diseases characterized by intestinal epithelial injury and nutrient malabsorption, as several studies have confirmed the beneficial effects of GLP-2 in preclinical experimental models of intestinal injury. Furthermore, findings have been obtained regarding circulating levels of GLP-2 in patients with intestinal diseases. Patients with mild to moderate intestinal inflammation exhibited increased circulating levels of bioactive GLP-2 due in part to decreases in levels of circulating dipeptidyl peptidase IV (DPPIV), a key determinant of GLP-2 (1—33) degradation. In contrast, patients who have undergone major small bowel resection or who have inflammatory bowel disease exhibited reduced level of the circulating GLP-2 secreted from L-cells of the intestine. Following initial nutrient-stimulated increases in circulating levels of GLP-2, levels of the bioactive form of GLP-2 fell rapidly.

No reports are available on the relationship between circulating levels of GLP-2 and the extent of injury in the intestinal mucosa induced by chemotherapeutic agents or by drugs such as indomethacin. In the present study, plasma levels of GLP-2 were significantly increased in rats in MTX(2.5)-G (p<0.05) and MTX (5.0)-G (p<0.01), but not in MTX(1.25)-G, when compared with control rats (Fig. 2). However, we did not observe an adaptive response of the intestine accompany with increasing plasma GLP-2 levels, because GLP-2 did not ameliorate the weight loss and intestinal damage induced by MTX treatment (Figs. 1, 3). We also found that the order of severity of mucosal injury was jejunum>duodenum>ileum, along with marked decreases in body weight in rats treated with MTX (Figs. 1, 3). Furthermore, administration of MTX resulted in a dose-dependent reduction in mucosal weight, DNA, and protein content in each segment of the small bowel (Fig. 3). These results agree with the finding that GLP-2 receptor RNA levels are highest in the jejunum, followed by the duodenum, ileum, colon and stomach. In addition, the decrease in body weight may result from malabsorption of nutrients caused by intestinal mucosal injury. Thus, intestinal side effects are the most common complications of MTX administration, which injures immature intestinal crypt cells.

We then statistically examined the results of the present study to determine whether any relationships between plasma GLP-2 levels and mucosal injury in rat intestine after MTX treatment. Plasma GLP-2 levels were significantly correlated with mucosal weight, DNA and protein content in the duodenal mucosa (correlations with mucosal weight: r = −0.644; p<0.01, mucosal DNA: r = −0.630; p<0.05, mucosal protein: r = −0.677; p<0.01) and the jejunal mucosa (correlations with the mucosal weight: r = −0.584; p<0.05, mucosal DNA: r = −0.561; p<0.01, mucosal protein: r = −0.597; p<0.01), but not in the ileal mucosa, indicating that plasma GLP-2 levels reflect the degree of mucosal injury induced by MTX treatment in the duodenum and jejunum. In addition, the reason these indices for mucosal injury are a higher correlation in duodenum than jejunum is thought that a dose-dependent reduction of these indices is comparatively strong in duodenum and jejunal injury is more severe even in low dose of MTX. It is thus likely that GLP-2 is responsible for up- or down-regulation of epithelial cell growth following mucosal injury, and that plasma GLP-2 levels depend on the relative

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**Fig. 3. Changes in Mucosal Weight, DNA, and Protein Content in Segments of Rat Intestine after Administration of MTX**

Segments of the small intestine taken from each rat on day 8 after administration of MTX indicated duodenum (○), jejunum (△), and middle portion of ileum (□). Values are the mean±S.D. of five rats. *p<0.05, **p<0.01, compared with control rats.
abundance of GLP-2 producing entero-endocrine L cells in the ileum. These findings can be applied to the clinical diagnosis of intestinal status and absorptive function, and suggest that plasma levels of GLP-2 in vivo may be useful as a marker of intestinal injury when the small intestine sustains major injury during chemotherapy.

The intestinotrophic and anti-apoptotic properties of GLP-2 on the small and large bowels of rodents\(^{12,34}\) suggest that exogenous GLP-2 administration may prevent or ameliorate chemotherapy-induced intestinal injury. We previously showed that administration of GLP-2 to rats following major small bowel resection enhanced endogenous intestinal adaptation.\(^{17}\) Because of decreases in GLP-2 receptors as a result of severe injury to the jejunum and duodenum by MTX treatment, GLP-2-induced proliferation was probably less active in these segments than in the ileum. Whether GLP-2 may be useful for the prevention of chemotherapy induced mucositis, will require further study.

In conclusion, correlations were found between plasma GLP-2 levels and mucosal weight, DNA and protein content, indicating that plasma GLP-2 levels reflect the degree of intestinal injury following MTX administration in the preclinical model we examined. Whether measurement of GLP-2 may be useful for diagnosing chemotherapy-induced mucositis requires further study.

REFERENCES

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