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Effect of Intraluminal Administration of Insulin-like Growth Factor-I on Rats with Methotrexate-induced Enterocolitis

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Summary. Background: **Insulin-like growth factor-I (IGF-I) is a polypeptide hormone that is an important mediator of cellular growth, replication, and differentiation. IGF-I provides a major stimulus for enterocyte proliferation and the absorptive function. The aim of this study was to determine the effect of the intraluminal administration of IGF-I on the gastrointestinal tracts of rats with methotrexate (MTX)-induced enterocolitis.**

Study design: Male Sprague-Dawley rats weighing 140 to 205 g were intraperitoneally implanted with osmotic minipumps that delivered either 0.9% NaCl (saline group; n=8) or 10 nM IGF-I (IGF group; n=9) to the ileal lumen through a short silastic catheter at a rate of 1 μL/h. Rats received 20 mg/kg intraperitoneal MTX at the time of catheter insertion. Forty-eight hours after the MTX infusion, the small intestine was excised to measure mucosal protein and DNA content in jejunal and ileal segments. Additional measurements during the study period included changes in body weight and urinary nitrogen loss.

Results: No significant difference in plasma IGF-I levels was seen between the groups. The saline group lost more body weight than the IGF group ($p<0.05$). In the IGF group, neither protein nor DNA content of the jejunum differed from those measured in the ileum. However, the ratio of the ileal to the jejunal protein content was significantly higher in the IGF group than in the saline group.

Conclusion: Intraluminal administration of IGF-I may play a role in changing the distribution of gut mucosal proteins, in this rat model of MTX-induced enterocolitis.

Key words—insulin-like growth factor I, IGF-I, intraluminal administration, methotrexate, enterocolitis.

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INTRODUCTION

Insulin-like growth factor-I (IGF-I) is a polypeptide hormone that is an important mediator of cellular growth, replication, and differentiation.[1] IGF-I is primarily produced in the liver, and is also synthesized in other organs, including the intestine.[2] IGF-I provides a major stimulus for enterocyte proliferation and the absorptive function. It has been shown that the adaptive proliferative responses seen in residual small and large bowels following massive small bowel resection are associated with significant increases in intestinal IGF-I mRNA and altered expressions of mRNA transcripts for IGF-I.[3] Systemic administration of IGF-I exerts a potent trophic effect on postresection intestinal growth and function in the rat.[4] IGF-I also reduces gut atrophy and bacterial translocation to the mesenteric lymph nodes after severe burn injury[5] or in sepsis induced by cecal ligation.[6] Recent clinical reports, however, have identified complications of systemic IGF-I administration, including intracranial hypertension (pseudotumor cerebri syndrome),[7,8] altered mental status, and hypoglycemia.[9] Local delivery of IGF-I to the gut lumen could reduce systemic side effects of IGF-I while locally stimulating intestinal trophism.[10] Intraluminal IGF-I infusion of rats can produce an approximate doubling of mucosal wet weight and total mucosal RNA, DNA, and protein content as compared with rats infused with NaCl.[11]

Chemotherapy is an important part of te armamentarium used to treat various cancers. However, chemotherapy commonly produces structural and functional damage to the intestinal mucosa in cancer patients, a majority of whom are already malnourished. A common side effect of many chemothera-

peutic agents, including methotrexate (MTX), is severe enterocolitis. MTX produces severe mucosal injury characterized by denudation of villi, intraluminal bleeding, and necrosis of villi that line the small intestine.[12,13] Few studies have examined the effects of intraluminal administration of IGF-I on chemotherapy-induced enterocolitis.

In this study, we examined the effects of intraluminal gut infusions of IGF-I on intestinal injury in a rat model of MTX-induced enterocolitis.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats weighing 140 to 205 g (Charles River Laboratories, Atsugi, Japan) were housed for 1 week in a room at 23±2°C with a light-dark cycle of 12 : 12 h, light onset at 0700 h. They received a standard laboratory diet (MF, Oriental Yeast, Tokyo, Japan) and water ad *libitum* prior to the experiments. All studies were approved by the Committee for the Use and Care of Laboratory Animals at Niigata University.

Experimental preparation

The rats were housed in individual metabolic wire-bottom cages to limit coprophagy and to allow urine and stool collection. After an overnight fast, the animals were anesthetized by ether inhalation, weighed, and a silastic catheter was placed in the ileum. Osmotic minipumps (Alzet®, Palo Alto, CA, USA) that delivered either 0.9% NaCl (saline group; n=8) or 10 nM IGF-I (Fujisawa Co., Osaka, Japan) (IGF group; n=9) to the ileal lumen through the silastic catheter were intraperitoneally (IP) implanted. This dose of IGF-I was chosen because it has been shown to have trophic effects.[10] Rats were then injected with 20 mg/kg MTX (Methotrexate-LPF, Lederle, Pearl River, NY, USA) IP and returned to their cages. During the 48-h study period, the animals were only provided water. The rats were killed by abdominal aortic puncture 48 h following the MTX injection. The intestine from the jejunum to the colon was rapidly excised, freed of mesenteric fat, and rinsed in ice-cold saline. Two 5-cm segments, measured under fixed tension (10 g), located immediately distal to the jejunal transection site and proximal to the ileocecal junction, were obtained. The intestinal segments were longitudinally opened and rinsed in ice-cold saline. The mucosa was completely removed by scraping with a glass slide and then weighed. Two 1-cm segments taken from either the proximal end of the jejunum or the distal end of the terminal ileum were removed for histologic examination.

Laboratory methods

All blood samples were immediately centrifuged and the removed plasma was stored at −80°C until analysis. Following MTX injection, urine was collected in acidified bottles for 48 h to determine urinary nitrogen excretion. Plasma IGF-I concentrations were measured using a commercially available radioimmunoassay (Eiken Chemical, Tokyo, Japan). Protein content was determined by the method described by Lowry, which uses bovine serum albumin as a standard.[14] DNA content was measured by the Burton modification of the diphenylamine procedure using calf thymus DNA as a standard.[15] Urinary nitrogen excretion was determined by chemiluminescence.[16]

Histologic examination

A 1-cm segment of small intestine was fixed with a 10% formalin solution, embedded in paraffin, sectioned, and mounted on glass slides. Deparaffinized sections were stained with hematoxylin and eosin and viewed by light microscopy to determine the histologic severity of MTX enterocolitis. Severity of MTX-induced enterocolitis was evaluated by decreases in the villus and crypt areas.

Statistical analysis

Data are expressed as the mean ± SD. Group differences were analyzed using the Mann-Whitney U test, with a p value less than 0.05 considered significant.

RESULTS

No significant differences in plasma IGF-I levels were seen between the study groups. All animals that received MTX lost weight, and those in the saline group lost more body weight than the IGF group ($p < 0.05$) (Table 1). Urinary nitrogen excretion tended to be smaller in the IGF group than the saline group, but the difference did not reach statistical significance ($p = 0.12$). In the IGF group, neither protein nor DNA content in the jejunum differed from those measured in the ileum (Table 2). However, the ratio of the protein content in the ileum to that of the jejunum was significantly higher in the IGF group than the saline group (Fig. 1). The ratio of the DNA content in

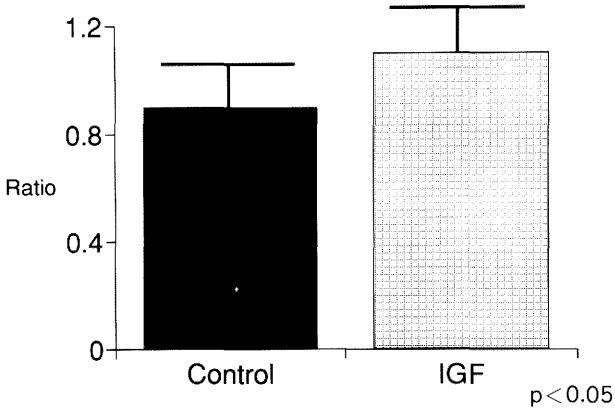

Fig. 1. The ratio of ileal to jejunal protein contents. Values are the means ± SDs.

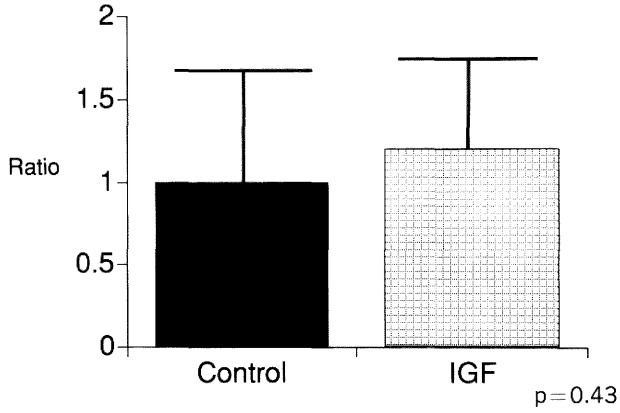

Fig. 2. The ratio of ileal to jejunal DNA contents. Values are the means ± SDs.

Table 1. Effect of intraluminal administration of IGF-I on rats with MTX-induced enterocolitis

	Plasma IGF-I (ng/mL)	Change in body weight (gm)	Urinary nitrogen excretion (mg)
IGF	191±168	−23±2.4#	239±39
Saline	164±103	−27±4.0	291±70

IGF, insulin-like growth factor #p<0.05
MTX, methotrexate

Table 2. Effect of intraluminal administration of IGF-I on mucosal injury of MTX-induced enterocolitis in rats

	Jejunal mucosa		Ileal mucosa	
	Protein content (mg/cm)	DNA content (μg/cm)	Protein content (mg/cm)	DNA content (μg/cm)
IGF	3.8±0.6	380±170	3.8±1.6	331±103
Saline	4.6±1.8	380±203	4.1±2.1	291±146

IGF, insulin-like growth factor
MTX, methotrexate

the ileum to that of the jejunum tended to be higher in the IGF group than the saline group (p=0.43) (Fig. 2). Fig. 3 shows characteristic histologic findings in the jejunums and the ileums of rats injected with MTX. Specifically, MTX produced a significant shortening of villi. No difference in the severity of the resulting enterocolitis was found between the groups.

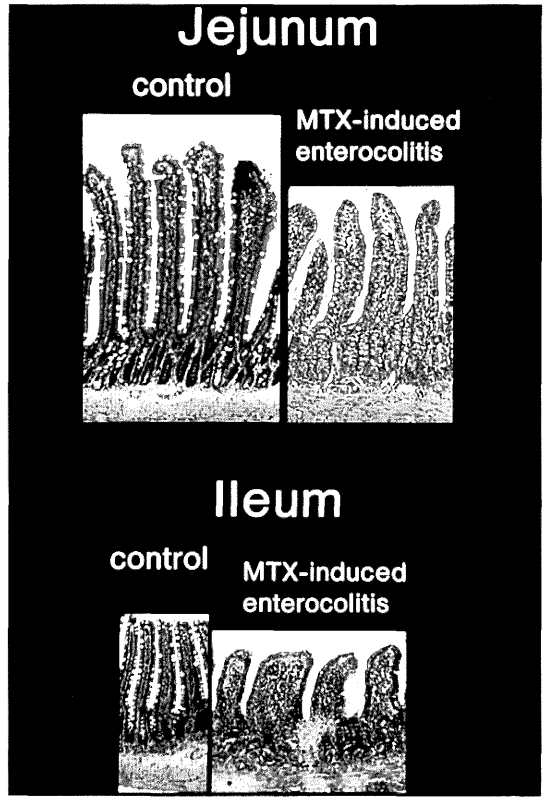

Fig. 3. Histologic changes seen in the small intestine of rats 48-h after methotrexate injection. (H & E; ×25). Villus heights were as follows: jejunum of control rat, 467±22; jejunum of rat with Methotrexate (MTX)-induced enterocolitis, 298±39; ileum of control rat, 293±13; ileum of rat with MTX-induced enterocolitis 216±6 μm. Values are the means ±SDs.

DISCUSSION

We found that animals in the IGF group lost less weight than those in the saline group. In the IGF group, mucosal protein and DNA content were similar in the jejunum and the ileum. However, the ileal to jejunal protein content ratio was significantly higher in rats administered with IGF-I to the ileal lumen than those receiving saline. The ileal to jejunal DNA content ratio tended to be higher in the IGF group than in the saline group. We believe that these intraluminal doses of IGF-I were involved in gut trophism.

Although the ileal to jejunal protein content ratio was significantly higher in the IGF group than in the saline group, there was no significant difference in mucosal protein or DNA content between these two groups. One may argue that, rather than increases in ileal mucosal protein content, decreases in jejunal mucosal protein content are responsible for the increase in the ileal to jejunal protein content ratio seen in our IGF group.

Winesett and others have shown that during fasting, changes in jejunal mass correlate with changes in serum IGF-I and jejunal IGF-I mRNA levels. The availability of IGF-I for receptor binding at the cell surface is determined by both the concentration of the hormone and by the concentrations of IGF-I binding proteins (IGFBPs).[2] Several studies have suggested that serum and liver IGFBP-3 mRNAs are regulated by IGF-I, and increases in serum IGF-I promote increases in serum and liver IGFBP-3 mRNA.[17,18] IGF-I can increase jejunal IGFBP-3 and decrease jejunal mass and/or protein content. However, plasma IGF-I levels were not higher in the IGF-I group than the saline group in our study. Therefore, we thought that the intraluminal administration of IGF-I would result in a higher ratio of ileal to jejunal protein content, not by a decrease in jejunal mucosal protein content, but by the increase in ileal mucosal protein content seen in the IGF-I group.

We have previously shown that an inadequate supply of nutrients may abolish the anabolic effect of IGF-I administration on nutritional repletion.[19] One possible explanation for the lack of a trophic effect following intraluminal administration of IGF-I is that our protocol lacked nutrients.

It has been shown that increased plasma MTX levels 24 and 48 h after drug administration correlate with host toxicity in patients receiving chemotherapy. A malnutrition-related delay in plasma MTX clearance has been shown to be responsible for increased MTX toxicity in malnourished individuals.[20] Increased anabolic activity could not be demonstrated by nitrogen balance studies or 3-methylhistidine excretion in gastrectomy patients receiving 80 μg/kg body weight exogenous IGF-I. The time courses of serum IGF-I, IGFBP-1, and IGFBP-3 concentrations suggest that counterregulatory mechanisms may overcome the anabolic effects of recombinant human IGF-I.[21] Intraluminal administration could potentially be used to prevent the development of malnutrition and lessen the severity of enterocolitis in these patients.

One may postulate that the rapid degradation of IGF-I administered intraluminally may limit its efficacy.[22] However, it has been shown that milk-borne IGFs are stable in the neonatal gastrointestinal tract and remain biologically active for as long as 30 min postingestion.[23] Since we continuously administered IGF-I to the ileum, it is possible that IGF-I may have remained locally active in the ileal lumen.

From these results, we conclude that the intraluminal administration of IGF-I may be directly involved in changing the distribution of gut mucosal proteins in MTX-induced enterocolitis in rats. Intraluminal IGF-I infusions may explain the favorable nutritional effect which was reflected by smaller body weight losses in the IGF group. Further studies are needed to determine if the intraluminal administration of larger doses of IGF-I could reduce MTX gastrointestinal toxicity with concurrent enteral or parenteral feeding.

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