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Insulin-like Growth Factor-I (IGF-I) in Malnourished Rats Following Major Hepatic Resection

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Summary. **We examined the effect of malnutrition on liver regeneration and changes in plasma insulin-like growth factor-I (IGF-I) concentrations, and the effect of the administration of IGF-I to rats following hepatic resection. Twenty-nine male Sprague-Dawley rats were allocated to one of three groups. Group I (n=11) consisted of normally nourished rats which each underwent a 70% partial hepatectomy by the method of Higgins and Anderson.[1] Group II (n=8) was composed of malnourished rats which also underwent partial hepatectomies. Group III (n=10) consisted of malnourished rats which each underwent a partial hepatectomy and received 200 μg of exogenous IGF-I for 24 h following surgery. Group I rats were permitted to eat *ad libitum*., rats in Groups II and III received 50% of their normal intake for one week prior to and following hepatectomy. The rats were killed after 48 h of hepatic regeneration, and the livers were weighed and prepared for hamatoxylineosin staining to facilitate an assessment of the number of mitotic cells.**

Decreases in the remnant liver weights and mitotic indices were more extensive in the malnourished rats. Additionally, malnutrition was associated with low plasma IGF-I concentrations following partial hepatectomy. IGF-I administration was not associated with an improved nitrogen balance following hepatectomy.

These results suggest that an adequate nutritional status is essential for liver regeneration, and that malnutrition is associated with lower plasma IGF-I concentrations following partial hepatectomy. An inadequate nutrient supply may abolish the anabolic effect of the administration of IGF-I on nutritional repletion postoperatively.

INTRODUCTION

The prolonged catabolism and protein wasting that accompanies critical illness are associated with increased morbidity and mortality. Although nutritional support attenuates this catabolic process, it is very difficult to maintain or increase protein mass in severely stressed patients. Various investigators have administered growth hormone, which enhances protein synthesis and nitrogen retention to provide a supplement to conventional nutritional therapy. The administration of growth hormone to patients recovering from gastrointestinal surgery has improved their nitrogen balance in some cases.[2,3] The protein anabolic properties of growth hormone are thought to be mediated by the insulin-like growth factor-I (IGF-I). Growth hormone stimulates IGF-I synthesis in the liver that exerts its anabolic effect on target tissues. Growth hormone therapy results in a marked increase in IGF-I and its carrier protein. However, this response is inhibited by starvation and malnutrition. Dahn and his colleagues have reported that growth hormone treatment in critically ill and/or septic patients does not affect nitrogen balance or serum IGF-I levels.[4] In contrast, Voerman has demonstrated that growth hormone administration reduces nitrogen excretion in patients with severe sepsis.[5] Although this lack of response may reflect insufficient growth hormone dosage, the data suggest that the anabolic effects of growth hormone can not be observed during periods of limited IGF-I production. Thus, the effect of IGF-I on catabolic patients remains unclear. We attempted to clarify the effect of malnutrition on liver regeneration and changes in plasma IGF-I levels, and to determine the effect of IGF-I administration on the nutritional status of malnourished rats following major hepatic resection, testing the hypothesis that IGF-I administration is associated with improved nitrogen balance during a period of surgical stress.

MATERIALS AND METHODS

Experimental animals

Male Spraugue-Dawley rats weighing approximately 180-200 g (Charles River Laboratories, Atsugi) were housed for 1 week in a room at 23±2℃ with a light-dark cycle of 12 : 12, and light onset at 07.00 h. They received standard laboratory chow (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

Twenty-nine rats were separated into one of three groups. Group I (n=11) rats received standard chow *ad libitum* for 1 week prior to and following liver resection. Group II (n=8) rats received 50% of their normal intake of standard rat chow for 1 week prior to and 2 days following liver resection. Group III (n=10) rats received 50% of their normal intake of standard rat chow for 1 week prior to the surgery. Postoperatively, the rats received 50% of their normal intake of standard rat chow and 200 μg of exogenous IGF-I (Fujisawa Co., Ltd., Osaka) administered continuously for 24 h.

A 70% liver resection was performed according to the method of Higgins and Anderson[1] using ether as an anesthetic. Briefly, the liver was mobilized and large median and left lateral lobes were ligated and excised following a midline incision. The rats were killed 48 h following the hepatectomy after abdominal aortic blood samples were obtained.

Administration of IGF-I

Group III rats received 200 μg of IGF-I continuously for 24 h immediately following hepatectomy by means of a subcutaneous osmotic pump (Alzet®, CA, U. S. A.) which was implanted during the hepatic resection. In contrast, Group II rats received saline *via* a similar osmotic pump arrangement.

Laboratory methods

All blood samples were centrifuged immediately, and the plasma was removed and stored at −80℃ until analysis. Following the hepatic resection, urine specimens were collected in acidified bottles for 48 h for the analysis of urinary nitrogen excretion. Plasma IGF-I concentrations were measured using a commercially available kit (Eiken Chemical Inc., Tokyo) employing radioimmunoassay. Plasma glucose concentrations were measured using the mutarotase-glucose oxidase method. Measurements of nitrogen excretion in the urine were performed with chemiluminescence.[6] The nitrogen balance was calculated by subtracting urinary nitrogen excretion from nitrogen intake from the rat chow.

Hepatic regeneration

The remnant liver was removed immediately and weighed. A tissue sample was fixed with 10 % formalin solution in preparation for hematoxylin and eosin staining. The fixed liver tissue sample was then embedded in paraffin, sectioned and mounted on glass slides. Deparaffinized sections were stained with hematoxylineosin and viewed with light microscopy to facilitate an assessment of the number of mitotic cells per 1000 hepatocytes.

Statistical analysis

Duplicate determinations of each parameter were averaged. Data are presented as the mean and standard error of the mean. ANOVA was used to compare data. A Student's unpaired t test also was used to detect differences within groups. Ap value of less than 0.05 was considered significant.

RESULT

Fig. 1 shows changes in the rats body weights during the 9-day study. Rats that were food restricted sustained significant decreases in body weight during the study compared with animals who ate *ad libitum* ($p<0.05$). Fig. 2 illustrates the liver weights of rats in the three groups two days following hepatectomy. A significant difference in liver weight between Groups I, II and III was observed ($p<0.0001$). However, treatment with IGF-I did not result in a difference in liver weights between Groups II and III. The mitotic indices of Groups I, II and III were 8.8±1.0, 3.1±0.9, and 3.6±1.1, respectively, and did not differ between Groups II and III. Figure 3 shows rat plasma IGF-I concentrations two days following hepatectomy. The plasma concentrations of IGF-I were significantly lower in the malnourished animals following hepatic resection compared with the rats that ate *ad libitum* ($p<0.0001$). Fig. 4 illustrates the nitrogen balance for 48 h following hepatectomy. No difference in nitrogen balance between Groups II and III was observed.

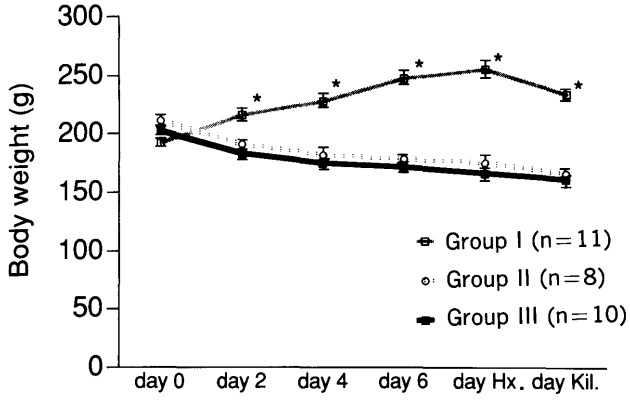

Fig. 1. Changes in body weights of rats in the three subject groups. Group I rats received standard chow *ad libitum* for 1 week prior to and following liver resection. Group II rats received 50% of their normal intake of standard rat chow for 1 week prior to and 2 days following liver resection. Group III rats received 50% of their normal intake of standard rat chow for 1 week prior to surgery. Postoperatively, the rats received 50% of their normal intake of standard rat chow and 200 μg of exogenous IGF-I. *$p<0.05$ compared with groups II and III. Hx, hepatectomy; Kil, killed.

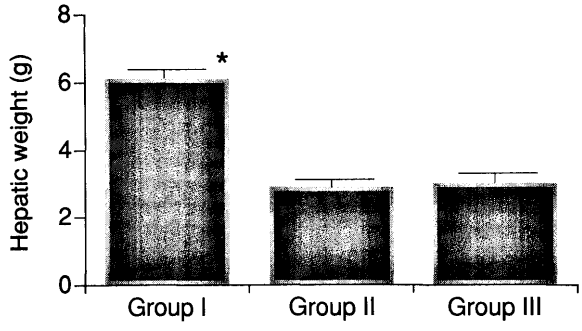

Fig. 2. Hepatic weights of rats in the three subject groups 2 days after hepatectomy. Group I rats received standard chow *ad libitum* for 1 week prior to and following liver resection. Group II rats received 50% of their normal intake of standard rat chow for 1 week prior to and 2 days following liver resection. Group III rats received 50% of their normal intake of standard rat chow for 1 week prior to surgery. Postoperatively, the rats received 50% of their normal intake of standard rat chow and 200 μg of exogenous IGF-I. *$p<0.0001$ compared with Groups II and III.

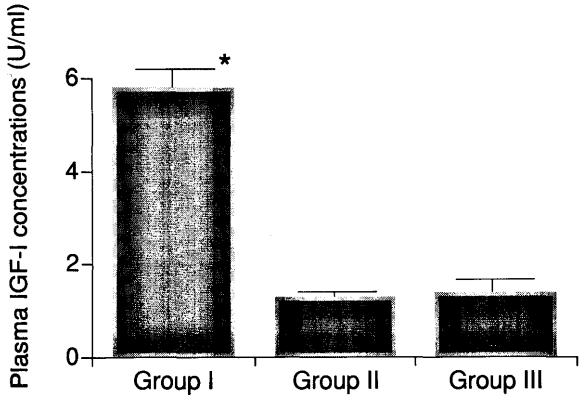

Fig. 3. Plasma IGF-I concentrations of rats in the three subject groups 48 h after hepatectomy. Group I rats received standard chow *ad libitum* for 1 week prior to and following liver resection. Group II rats received 50 % of their normal intake of standard rat chow for 1 week prior to and 2 days following liver resection. Group III rats received 50% of their normal intake of standard rat chow for 1 week prior to surgery. Postoperatively, the rats received 50% of their normal intake of standard rat chow and 200 μg of exogenous IGF-I. *$p<0.0001$ compared with Groups II and III.

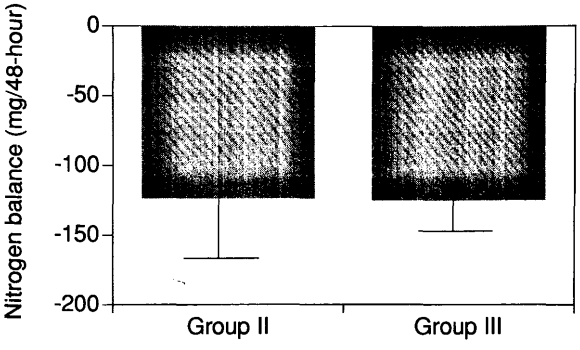

Fig. 4. Nitrogen balance of rats from subjects Groups II and III 48 h after hepatectomy. Group II rats received 50% of their normal intake of standard rat chow for 1 week prior to and 2 days following liver resection. Group III rats received 50% of their normal intake of standard rat chow for 1 week prior to surgery. Postoperatively, the rats received 50% of their normal intake of standard rat chow and 200 μg of exogenous IGF-I. The data from Group I rats is not displayed here. Group I rats received standard chow *ad libitum*. We did not calculate the nitrogen balance in Group I rats.

DISCUSSION

We demonstrated that an adequate nutritional status is essential for hepatic regeneration, since decreases in the remnant liver weights and mitotic indices were more extensive in malnourished rats than in rats that ate *ad libitum*. Malnutrition following partial hepatectomy was associated with reduced plasma IGF-I concentrations. Administration of IGF-I to rats following hepatectomy did not contribute to an improved nitrogen balance in this study. Therefore, we suggest that a lack of adequate nutrients may abolish the beneficial effect of IGF-I administration on nutritional repletion and subsequent hepatic regeneration following partial hepatectomy.

We used malnourished, post-hepatectomy rats as a model to evaluate the effects of systemic IGF-I administration on nutritional status, because plasma IGF-I concentrations are low in a state of malnutrition and reduced liver masses are thought to be associated with further decreases in plasma IGF-I concentrations such as chronic liver disease.[7] We wanted to determine the effect of low dosages of administered IGF-I, since it is possible that IGF-I causes some undesirable effects. For example, hepatic IGF-I binding activity is diminished significantly in rats exposed to chronic growth hormone excess, suggesting the downregulation of IGF-I receptors by enhanced circulating levels of IGF-I.[8] It has been reported that IGF-I promotes the development of proliferative retinopathy,[9] and renal hypertrophy in patients with diabetes mellitus,[10] in addition to stimulating angiogenesis.[11] Thinking that the administration of IGF-I might be more effective at the nadir of the serum concentration of IGF-I, we administered IGF-I for 24 h immediately following hepatectomy, since serum IGF-I concentrations decrease 6 h after 70% partial hepatectomy and remain low for 48 h.[12,13] Insulin-like growth factor-I synthesis was not decreased in hepatocytes isolated 24 h after partial hepatectomy, suggesting that the decreases in the serum IGF-I concentrations were due to a reduction in liver mass. No differences were found in the plasma IGF-I and glucose concentrations between Groups II and III. The possibility exists that the amount of IGF-I administered was insufficient. Inaba et al.[14] reported that a decrease in weight was significantly less extensive in rats receiving 1 mg/kg/day of IGF-I when compared to receiving saline following gastrectomy.[14] We thought that the administered IGF-I had disappeared by the time the blood samples were drawn, since Group III rats received continuous IGF-I for only 24 h postoperatively.

Malnutrition is associated with a higher postoperative mortality and a reduced rate of hepatic regeneration in rats. Marked changes in liver composition consisting of severe fatty degeneration, decreased glycogen, and a rise in water content occur 48 h following regeneration. These results emphasize the importance of an adequate nutritional state for liver regeneration.[15] Other investigators have shown that nutritional intake is an important regulator of plasma IGF-I concentrations, and that these IGF-I concentrations are consistently correlated with nitrogen balance.[16–18] The results from our study are compatible with such findings.

Growth hormone stimulates IGF-I synthesis in the liver that exerts an anabolic effect on target tissues. Fat is oxidized in preference to protein during growth hormone administration that results in a reduction in the net rate of protein loss.[19,20] In this study, rats were food deprived and lost significant amounts of weight. It is likely that they lost both endogenous fat and protein, and then could not preferentially oxidize fat in order to spare protein. This is one of the possible mechanisms that might explain why inadequate nutrition may abolish the effect of postoperative administration of IGF-I on nutritional repletion. Mantell et al.[21] has shown that post-resection upregulation of colonic IGF-I mRNA occurs despite decreases in plasma IGF-I concentrations, suggesting that local IGF-I mediates the adaptive responses in rats with short bowel syndrome. Further studies are required to determine the expression of IGF-I and/or IGF-I receptor on the target tissue. In conclusion, the metabolic effect of exogenous IGF-I was abolished in malnourished rats that underwent hepatectomy, suggesting that the provision of protein and calories is mandatory for successful treatment with exogenous IGF-I.

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