BOLUS ORNITHINE AND ARGinine-KETOGLUTARATE SUPPLEMENTATION IN DISTAL INTESTINE AFTER 65% RESECTION IN RATS

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ABSTRACT

Introduction: Enteral feeding has been reported to increase intestinal mucosa proliferation after resection. Dietary components influence the intestinal adaptive response. The purpose of this study was to evaluate the effect of ornithine- (OKG) or arginine-ketoglutarate (AKG) bolus supplementation on intestinal postresectional adaptation in the rat. Methods: Male Wistar rats underwent 65% small-bowel resection and received either OKG 3 g/kg/day, isonitrogenous AKG or saline by gavage once daily. The animals had free access to rat chow. Sampling was done 10 days after resection. Fed animals without surgery or specific treatment served as controls. Results: Mucosal wet weight, DNA, RNA, protein content and sucrose activity of the mucosa, as well as villus height were significantly increased in all resected animals compared to controls. No significant differences in body weight or intestinal adaptation could be found between the three dietary groups. Conclusion: Postoperative enteral bolus feeding supplemented with OKG or AKG did not significantly enhance the adaptation of the remnant small bowel 10 days after massive intestinal resection when rats had free access to rat chow.

Key words: Adaptation, Resection, Ornithine-ketoglutarate, Arginine, Alpha-ketoglutarate, Short bowel syndrome

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INTRODUCTION

Short-bowel syndrome (SBS) can occur as a result of massive surgical resection, surgical bypass or intrinsic disease, and it can lead to malabsorption of nutrients, diarrhea and weight loss (1). Post-resectional hyperplasia is associated with hypercellularity, increased crypt depth, villus height, and increased mucosal mass, as well as an increase in the total activity of brush border enzymes, leading to enhanced segmental absorption of many nutrients. The larger and more proximal the resection is, the greater are the adaptive changes (2). Post-resectional intestinal hyperplasia is influenced not only by the time interval and the amount of gut resected, but also by the route, amount and quality of nutrients. It has been widely accepted that three main factors are responsible for promoting intestinal adaptation, i.e. pancreatobiliary secretions, hormonal factors, and luminal nutrients. Clearly, enteral nutrition has the capacity to influence all of these factors.

Current research supports the view that enteral nutrition is the most clearly defined stimulus for adaptive intestinal growth and regeneration after bowel resection (3, 4). It has been generally accepted that intestinal adaptation occurs transiently after the resection stimulus. This implies that failure to provide the appropriate luminal nutrition during this period may permanently impair the adaptive response (5). Early enteral feeding has been reported to increase gut mucosa integrity by stimulating intestinal mucosa proliferation (6). A number of factors regulate the proliferative response of the intestinal tract, and specific nutrients in the diet for optimizing intestinal absorption and maximizing adaptation of the intestinal remnant need to be investigated.

A high protein diet leads to increased peptide hydrolase and alkaline phosphatase activities, and dietary starch leads to increased disaccharidase activities (7). Long chain triglyceride seems to be a more potent trophic stimulus than protein, starch or medium chain triglyceride (8). Short chain fatty acids (9) also stimulate adaptation. Recently, the role of specific nutrients and growth factors have been examined. Hormonal substances such as insulin-like growth factor (IGF-1) (10) and epidermal growth factor (EGF) (11), as well as exogenous growth hormone (12) have been shown to stimulate intestinal adaptation after massive intestinal resection.

Glutamine has been studied in this field and different but mostly negative results have been reported regarding the intestinal adaptation after intestinal resection (13-15). However, little is known about the relative trophic effect of ornithine-ketoglutarate and arginine-ketoglutarate after massive intestinal resection. Ornithine α-ketoglutarate (OKG) and arginine have been suggested as particularly potent in stimulating intestinal proliferation (16, 17). OKG is a precursor of glutamine when given by the oral enteral route and alpha-ketoglutarate is the carbon skeleton of glutamine. Ornithine is a precursor of polyamines. The bolus feeding of OKG has been suggested to be more effective to increase the glutamine and arginine plasma concentration than constant administration in treatment of burn patients (18). Also, arginine plays an important role in intestinal function through the nitric oxide pathway. For these reasons, the aim of this study was to investigate the effects of oral supplementation of ornithine or arginine in combination with α-ketoglutarate on the early adaptive response to massive small bowel resection in the rat.

MATERIAL AND METHODS

Study design:
Animals that were starved for 12 hours were submitted to 65% mid-small-bowel resection and randomized to receive OKG, isonitrogenous AKG or saline. The resected rats were given amino acids once a day, and the chow ad libitum. for ten days. Rats fed ordinary rat chow without
surgery or specific treatment served as control (n=6). Mucosa from the ileum was taken for analysis of weight, protein, DNA and RNA content, sucrase activity and morphology ten days after resection.

Animal and surgical model:
Male Wistar rats (B&K Universal AB, Stockholm, Sweden), weighing ~ 200 g were used for studies. The rats were housed individually in a room maintained at 23°C, under a 12-h day/night cycle through all the experiments, and were fed a normal diet with no restriction on food or water supply during 2 weeks prior to surgery. Before surgery, rats were fasted over night, and were weighed. Anesthesia with ketamine [Ketalar®, 80 mg/kg] and xylazine [Rompun©, 8 mg/kg] was given intraperitoneally. The abdomen was opened under sterile conditions by a full-length midline incision. The small intestine was located, and the mesenteric vessels were ligated with 5-0 ligature. Sixty five percent small-bowel resection was performed, i.e. removing all bowel between the point 5 cm distal to the ligament of Treitz and 25 cm proximal to the ileocecal valve. After resection, an end to end jejunileum anastomosis was performed with six interrupted stitches of 6/0 absorbable polyglactin 910 (Vicryl©) suture. The abdomen was closed, 5 ml saline was infused subcutaneously.

Animal groups and nutrition:
After surgery, all rats had free access to water for 12 hours, but food was withdrew during that time. No antibiotics were used. All the rats were randomized in three groups (each group, n=7). The three groups were treated as follows:
OKG group: OKG (3.0 g/kg body weight/day).
AKG group: isonitrogenous to OKG
Saline group: 50 ml saline/kg body weight /day

The resected rats were given amino acids dissolved in water, 50 ml/kg once a day for 10 days by gavage, and were allowed to eat rat chow (R36, Lactamin AB, Stockholm, Sweden) ad libitum. OKG and AKG were provided by Gramineer AB in Sweden.

Observation and analysis:
Each morning before feeding rats, the rats and remaining chow were weighed, in order to evaluate the body weight and calculate the consumption of chow. On the tenth day after resection, animals were reanesthetized, and laparotomy was performed. The ileum was rapidly dissected free from its mesentery and rinsed with ice-cold saline. It was suspended with a 5 g weight attached to allow measurement of a standard length of intestine. The animals were killed by heart cut. The intestine was weighed. The first cm of ileum 1 cm away from the anastomosis was fixed in 10% formalin for histologic sectioning. The fixed samples were dehydrated in progressive concentrations of ethanol, and were embedded in 7100 technovin. 2.5 μm sections were stained with hemotoxylin and eosin. Measurements of mucosal villus height and crypt depth were analysed using an imager analyser (Scion Image).

Mucosa was scraped using a microscope slide over ice, and the scrapings were weighed, homogenized, and stored frozen for biochemical analysis. Intestinal mucosal RNA was determined by spectrophotometry using a modified Schmidt-Tannhauser method as described by Munro & Fleck (19). DNA was analysed fluorimetrically using the method by Prasad et al (20). Protein was analysed according to the method of Lowry (21). RNA, DNA are expressed in μg per centimeter segment of intestine. Protein results are expressed in mg per centimeter segment of intestine. The specific activity of sucrase was measured according to the method of Dahlqvist (22), and specific activities are expressed as mU/cm.min.
Statistics:
Data are presented as mean±standard error (SEM). Comparison between groups was done with the Kruskal-Wallis test. Differences were considered significant at the p<0.05 level.

Ethics:
The study was approved by the Animal Ethics Committee of the Faculty of Health Sciences, Linköping University.

RESULTS
Rats recovered rapidly from anesthesia, and no operative mortality was observed. One rat died due to hemorrhage after surgery, prior to receiving food. All animals in the three study groups developed mild diarrhea after receiving oral food during the first three days. There were no signs of peritonitis or obstruction in any of the animals.

Food intake and body weight:
The intake of chow was similar in all study groups (10 g/day on postoperative days 1-4, and 21 g/day on postoperative days 5 through 10). Rat growth curves are shown in Figure 1. All rats initially lost weight, and returned to the preoperative weight by postoperative day 6. Body weight at day 4 after surgery decreased 12.3 ± 4.4 g in the OKG group, 15.9 ± 3.1 g in the AKG group and 16.2 ± 2.3 g in the saline group. Ten days after resection, the OKG group had gained 19.3 ± 5.8 g, the AKG group 15.7 ± 5.9 g and the saline group 13.5 ± 7.8 g compared to the preoperative body weight. The body weight changes were however not significantly different (figure 1).

Figure 1. Mean body weight after massive intestinal resection, receiving oral food, and supplemented with amino acids or saline during 10 days after surgery.
Mucosal wet weight of the ileum (mg/10 cm) was significantly increased in all the study groups compared to unoperated controls, but there were no significant differences between the resected groups (table 1). Ileal mucosal DNA results (μg/cm) are shown in table 1. They were significantly higher than control, but there was no significant difference between the refeeding groups. The changes of ileal mucosal protein and RNA contents (table 1) were similar to the changes in DNA contents and there were no significant differences in RNA or protein contents between the resection groups. No significant difference of mean sucrase activity were found between the resection groups; OKG 125 (15) vs AKG 115 (22) vs saline 118 (10) mU/cm.min (SEM) 10 days post resection.

The villus height and crypt depth were observed in the remnant ileum on postoperative day 10. Villus heights were significant increased in resection groups compared with control, but there was no significant difference between the three groups (figure 2).

Figure 2. Histology of the distal small bowel 10 days after 65% resection in rats supplemented with OKG, AKG or saline compared to unoperated control animals. Data are expressed as mean ± SEM. *compared to all resected groups, p<0.01

DISCUSSION

In the study presented, bolus supplementation of ornithine- or arginine-ketoglutarate had no apparent benefical effect on the intestinal adapation 10 days after massive intestinal resection. The supplements did not improve the total mucosa weight, ileal DNA, RNA, protein concentration, sucrase activity or intestinal morphology. These markers of intestinal adaptation were significantly enhanced 10 days after resection, in agreement with previous studies (23, 24).

Since intravenous feeding results in mucosal atrophy (25), enteral refeeding is probably the most important stimulus for intestinal adaptation (1, 26). Many experimental models have been utilized to demonstrate the physiologic importance of specific factors in controlling intestinal cell growth.
Among the tested amino acids, glutamine, arginine as well as ornithine have emerged as particularly potent to the maintenance of intestinal integrity (27-29). A number of studies suggest that glutamine is conditionally essential for gut metabolism, structure, and function in the stressed animal (30, 31).

Due to a metabolic interaction between ornithine and α-ketoglutarate in the splanchnic area, ornithine-ketoglutarate (OKG) is regarded to be a very potent precursor of glutamine when given by the oral or enteral route (32). Also, OKG stimulates growth hormone and is a precursor of polyamines (putrescine, spermine, spermidine) necessary for proliferation and of arginine which has an important role in intestinal function through the nitric oxide pathway (33). The small intestine is a major site of metabolism for ornithine. It has been shown that OKG supplemented diet stimulates protein synthesis in vivo in the small intestine and that oral supplementation with OKG improves protein/DNA contents of intestinal mucosa after small bowel transplantation in rats (34).

A study from Le Bricon et al (18) showed that when OKG was infused continuously over 21 h in burn patients, plasma glutamine and arginine concentration lower than after a 10 g bolus. This suggests that the bolus mode generate higher levels of metabolically available glutamine and arginine. The present study was therefore designed to evaluate the effect of adding bolus doses of OKG or arginine-alphaketoglutarate on the early adaptation of intestine after massive intestinal resection. However, we found that bolus OKG supplementation had no significant beneficial effect on body weight, ileal mucosal weight, protein, DNA, RNA contents and sucrase activity, or on villus height and depth of crypt compared to arginine or saline. Recently, a study by Dumas et al (35) found that enteral OKG supplementation for seven days resulted in an increase in villous/crypt ratio in the jejunum after intestinal resection in rats. It had however no apparent effect on body weight or on proliferation of the ileal mucosa which is where adaptation occurs. In the study by Dumas OKG was given continuously but the daily dose was slightly lower than in the study here presented (5 mmol/kg vs. 7 mmol/kg B.W.).

In contrast to these results are the data from another study on OKG in short-bowel syndrome by Czemichow et al (36). Wistar rats were fed intragastrically with a nutritive mixture supplemented either with casein hydrolysate or OKG (1 g/kg body weight) after resection of the proximal jejunum. They found, as expected, that OKG supplementation caused a increase in putrescine content as well as in the amount of ornithine decarboxylase mRNA in the mucosa, but also an increase in the height of the ileal villi. The body weight, mucosal weight and crypt depth were however not significantly different between the OKG and casein supplementation groups. Sampling and resection area were nearly the same (50% in their study, 65% in our study). The difference in experimental design where Czemichow et al used OKG 1 g/kg body weight continuously for 7 days whereas we gave OKG 3 g/kg bolus once daily added to chow ad lib for 10 days could probably explain the difference in results. Looking at body weight development there seems to be an effect in favour of OKG at day four but no intestinal sampling to examine villus height in the ileum was performed at that time point in the study presented here.

Arginine is also a interesting amino acid which is mainly absorbed in the small bowel (37). It has been demonstrated that arginine has hormonal secretagogic effects, i.e. stimulation the secretion of growth hormone, insulin and glucagon; prevents protein hypercatabolism and weight loss in trauma situations; maintains normal growth; improves gut function; accelerates wound healing and displays immunological action (38). Arginine is the precursor of nitric oxide, and dietary enrichment with arginine improves the survival of septic mice (39) and attenuates the damage of
intestinal barrier function after mesenteric ischaemia (40). However, arginine had no beneficial effect on adaptation of intestine after massive intestinal resection the study here presented. One explanation could be that the inflammatory challenge is of less magnitude in this surgical model. Also, an isonitrogenous dose of arginine represents much less of a dose than ornithine on a molar basis.

Furthermore, it has been difficult to demonstrate beneficial effects of adding glutamine to the diet after intestinal resection. Vanderhoof et al. found that glutamine supplementation resulted in significantly lower mucosal weight, protein, DNA contents and sucrase activity in the duodnum when 5% glutamine was added to rat chow diet after small-bowel resection in the rat. Moreover, the addition of 2% glutamine to a glutamine-free elemental diet did not enhance intestinal adaptation when compared with glucose (41, 42). The paradoxical lack of effect of gut specific nutrients in a situation where proliferation is very intensive could be related to the decrease in the absolute amount of "gut nutrients" needed when a major part of the intestine is absent. Another possibility is that the growth stimulation by growth factors is maximal and the benefit of specific nutrients is marginal compared to the effect of nutrients provided intraluminally by the diet. If the GI tract tolerates food, there is today only limited evidence of clinical benefits of gut specific supplementations.

In conclusion, bolus ornithine- or arginine-ketoglutarate supplementation did not enhance the adaptation of the remnant intestine or the body weight development 10 days after massive intestinal resection in the rat when animals had free access to rat chow.

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