

The effects of parenteral glutamine on intestinal adaptation in a rat model of short bowel syndrome

Research Article

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Abstract: Short bowel syndrome (SBS) is characterized by the malabsorption of nutrients and fluids that occurs after major intestinal resection, resulting from an adaptation process that begins immediately to increase the mucosal surface area and absorption. Certain nutrients and trophic factors are widely used to increase intestinal adaptation following massive intestinal resection. The efficacy and benefits of glutamine on the intestinal adaptation process is still controversial. This study was conducted to determine the effects of parenteral glutamine administration on intestinal adaptation in a rat model of SBS. Fourteen male Wistar rats were divided into two groups; all 14 underwent 75% small bowel resection. Within each group, rats were assigned to 14 days of treatment with subcutaneous glutamine (0.3 g/kg/day) or isotonic saline daily. Weight changes and histological intestinal adaptation parameters (mucosal thickness, villus height, and crypt depth) were measured. Non-mucosal intestinal changes were evaluated by intestinal fractionated collagen analysis. All rats initially lost weight and began to gain weight postoperatively; however, they did not reach their preoperative weights during the experiment and there was no significant difference between the groups. Histological adaptation parameters were significantly increased after 75% intestinal resection in both groups compared to paired native samples ($P < 0.01$); although the percent of increase was slightly higher in Gln group, no significant difference was detected between the two groups. Fractionated-collagen amounts were found to be similar between groups. The results indicated that parenteral glutamine administration alone does not improve the intestinal adaptation process after massive intestinal resection in rats.

Keywords: Short bowel syndrome • Glutamine • Intestinal adaptation • Collagen • Malabsorption

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Abbreviations

SBS - Short bowel syndrome,
TPN - Total parenteral nutrition,
GH - Growth hormone,
IGF-I - Insulin-like growth factor-I,
EGF - Epidermal growth factor,
GLP-2 - Glucagon-like peptide,
HGF - Hepatocyte growth factor,

Gln - Glutamine,
SCFA - Short-chain fatty acids,
GALT - Gut-associated lymphoid tissue.

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1. Introduction

Short bowel syndrome (SBS) occurs when resection of small intestine results in the malabsorption of nutrients and fluids that is characterized by severe diarrhea, dehydration, electrolyte disturbances, progressive malnutrition, and weight loss. Mesenteric ischemia, trauma, inflammatory bowel diseases, necrotizing enterocolitis and intestinal volvulus are well-known etiological factors [1]. The adaptation process starts in the remnant small intestine immediately after resection; the success of the adaptation process is the most important factor determining the clinical outcome and survival. However, this process is commonly insufficient for body requirements after massive intestinal resection [2]. Total parenteral nutrition (TPN) is the single treatment option until the sufficient absorption surface is achieved. However, during TPN treatment, central line complications, multiple systemic infections, cholestasis, liver failure, and long-term hospitalization, which seriously affect the quality of life of the patient, have been observed [3]. Thus, enhancing the intestinal adaptation process is important after major intestinal resection. Some trophic factors (i.e. growth hormone [GH], insulin-like growth factor-I [IGF-I], epidermal growth factor [EGF], glucagon-like peptide 2 [GLP-2], hepatocyte growth factor [HGF], and nutrients (i.e. glutamine [Gln], short-chain fatty acids [SCFA]) have been suggested to increase intestinal adaptation in SBS [4-10].

The amino acid Gln is essential for the synthesis of nucleic acids and proliferation of cells [11] and provides a major portion of the energy required by the rapidly dividing cells of the gastrointestinal tract (enterocytes and colonocytes) [12-15]. On the other hand, gut-associated lymphoid tissue (GALT) plays a critical role in the maintenance of gut structures, function, metabolism, and immunity [16]. It has been shown that glutamine supplementation protects the GALT atrophy and IgA depletion that result from long-term TPN and enhances nutrient absorption [17, 18]. However, conflicting results have been reported in terms of enteral or parenteral glutamine administration in animal studies, as well as clinical trials that evaluated intestinal adaptation after massive intestinal resection [4, 5, 19-27]. Therefore, the efficacy of Gln administration in the management of SBS is currently controversial. The aim of the present study was to determine the effects of parenteral Gln administration on intestinal adaptation in a rat model of SBS.

2. Material and Methods

2.1. Animals and surgical model

Fourteen male Wistar rats weighing 180-190 g were included in our study. The rats were housed in a room maintained at $22\pm 2^{\circ}\text{C}$, $55\pm 5\%$ humidity, under a 12-hour day/night cycle through all the experiments, and were fed with a standard rat chow and water *ad libitum*. Before surgery, rats were fasted overnight and then weighed. After general anesthesia with ketamine and xylazine, the abdomen was incised under sterile conditions, and all rats underwent 75% small bowel resection (leaving 5 cm of jejunum from the ligament of Treitz and 10 cm of ileum from the ileocecal valve) followed by one-layer end-to-end jejunoileal anastomosis with interrupted stitches of 6-0 polipropylene suture. The abdomen was closed and 5 mL saline was injected subcutaneously. Full-thickness biopsies from the resected distal ileal end were collected in 10% formalin and preserved for histopathological analysis.

2.2. Experimental groups and post-operative care

During the first three postoperative days, the animals received 10 mL of Ringer lactate and 5% dextrose solutions subcutaneously, tap water on the second through fifth days, and standard rat chow thereafter. After the surgical procedure the rats were randomly assigned into two groups: each animal to receive the same regimen with 0.3 mL/day saline solution (control group), or the same regimen supplemented with 0.3 g/kg/day Gln (Dipeptiven®, Fresenius Kabi, GB) (Gln group), subcutaneously from day 0 to 14. Fourteen days after resection, the rats were reanesthetized and laparotomy was performed. The first 3 cm of ileum distal to the anastomosis was flushed with normal saline and fixed in 10% formalin for histopathological evaluation. The rats were sacrificed by intracardiac puncture and hypovolemia.

2.3. Histopathological evaluation

Sections obtained from paraffin embedded tissues were prepared with hematoxylin and eosin dye. Images at 10x magnification were obtained under a light microscope (Nikon® Labophot-2) and photographs of 640x480 pixels were produced by using a camera (Hitachi® Color Video Camera VK-C220E) with a image analyzer computer program (BS Image System® BS 200 v2.0). The longest 10 villi of each specimen were detected and total number of goblet cells, crypt depth, villus height, and mucosal thickness were recorded.

2.4. Fractioned Intestinal Tissue Collagen Analysis

Intestinal tissue collagen was fractioned into two soluble collagen components (salt and acid soluble) by using the previously described method [28]. Briefly, 100 mg tissue was transferred into 5 mL 0.05 M Tris-HCl (pH: 7.2) and sonicated (Potter S Homogenizer B. Braun®, Australia) into the ice. Following 2x24-hour shaker-incubation at +4°C, the homogenate was centrifuged (2K15C Sigma®, Germany) at 9000xg for 75 minutes at +4 °C. The first supernatant was analyzed for salt-soluble collagen concentrations (newly-synthesized tissue collagen). Then, the pellet was mixed with 0.5 acetic acid and kept in a shaker (Thermolyne® Cimarec 3, USA) during 24-hour re-incubation at +4 °C. The mixture was centrifuged at 9000xg for 75 minutes at +4 °C. Second, the supernatant was analyzed for acid-soluble collagen amount. Salt-soluble and acid-soluble extracts were evaporated to dryness in an oven at 110°C (Heraeus® Thermo). These dried extracts were determined as an index of collagen concentration as described by Reddy and Enwemeka [29]. The salt-soluble collagen amount was defined as “newly-synthesized collagen” and acid-soluble component that contained higher cross-linked fibers was regarded as less-mature or “structural” collagen of the intestinal wall.

2.5. Evolution of body weight

Animals were weighted during the daily visits (day 0 to day 14 postoperatively).

2.6. Statistics

Results are expressed as means±SEM. Comparisons of data from basal conditions with postoperative day14 specimens in each group were performed with paired samples t test and between groups with independent samples t test. The differences were considered statistically significant at $P<0.05$.

2.7. Ethics

Experimental procedures were approved by the Ethical Committee of Animal Research of Dokuz Eylul University.

3. Results

No operative and perioperative mortality was observed. There were no anastomotic complications in either group. We observed mild diarrhea in all rats.

Figure 1. Changes in body weight of rats after massive intestinal resection.

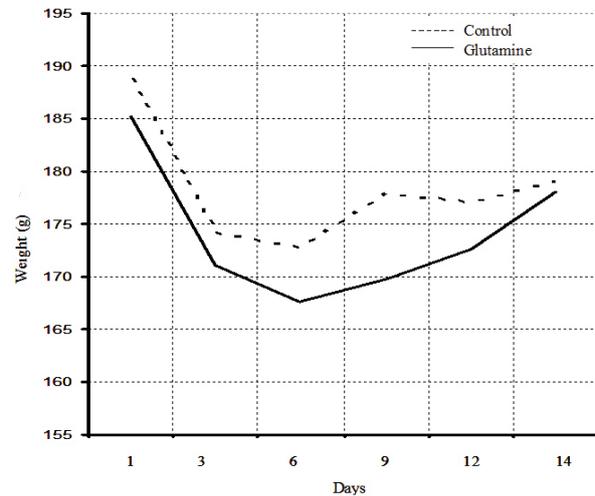


Table 1. Change in histopathological adaptation parameters in 75% small intestine resected rats 14 days after surgery.

	Control	Gln	P*
Mucosal thickness (% increase)	27.5	34.4	0.095
Villus height (% increase)	27.9	24.1	0.158
Crypt depth (% increase)	28.5	36.4	0.091

*Independent samples t-test.

3.1. Body weight

All rats initially lost weight and began to gain weight after postoperative day 6; however, they did not reach their preoperative weights on postoperative day 14. There was no statistical significant difference between the groups in terms of body weight during the experiments (Figure 1).

3.2. Intestinal mucosal morphology

Mucosal thickness, villus height, and crypt depth were significantly increased after 75% intestinal resection in both groups compared to paired native samples ($P=0.001$, $P=0.000$, $P=0.000$, respectively). Although the percent of increase was slightly higher in Gln group (Table 1), no significant effect was observed in intestinal mucosal morphology in the Gln group on postoperative day 14 when compared with the control group (Table 2).

3.3. Fractioned Intestinal Tissue Collagen Amounts

No significant difference was observed between two groups, in the aspects of the both collagen fractions (Table 3).

Table 2. Intestinal morphology after 75% small intestine resection.

	Control	Gln	P*
Mucosal thickness (μm)	576.2 ± 7.7	563.5 ± 8.1	0.275
Villus height (μm)	377.7 ± 9.2	355.1 ± 8.0	0.088
Crypt depth (μm)	203.9 ± 3.8	196.7 ± 1.4	0.111

Values are mean ± SEM
*Independent samples t-test.

4. Discussion

Once small intestinal resection is indicated, the aim should be to preserve as much intestinal tissue as possible. After massive intestinal resection, TPN treatment is mandatory at early stages; however, long term TPN (hospitalized or outpatient setting) treatment has several complications with a high cost. An adaptation process to increase mucosal absorption surface begins soon after intestinal resection and may take several weeks or years to complete [30]. If the adaptation process is inadequate to meet the demands of the organism, patients will become dependent upon TPN support [31]. Thus, successful efforts to increase intestinal absorption surface will decrease the need for long term TPN treatment.

In this study, the effects of parenteral Gln support on intestinal adaptation after 75% intestinal resection was investigated. We observed a significant increase in mucosal thickness, villus height, and crypt depth in both groups after an extensive intestinal resection, which shows that 75% intestinal resection triggers intestinal adaptation. The results of this study demonstrated

that after massive intestinal resection, parenteral administration of Gln alone did not have significant trophic effect on intestinal adaptation, since it did not improve the postoperative weight gain.

Nygaard and co-workers reported that more than 70% of intestinal resection results in morphological and functional changes in the remnant intestine [7]. Liu and co-workers showed a trophic effect of standard rat chow on intestinal adaptation with 60% intestinal resection [5]. We performed 75% intestinal resection for the induction of adaptive response, and retention of both portions of the proximal jejunum and distal ileum was based on the nutritional implications of removing the specialized absorptive capacity of the terminal ileum for vitamin B₁₂ and bile acids [7,32].

As reported previously, the intestinal adaptation process in the rat begins as early as 24 hours after massive intestinal resection, and the adaptive response in metabolism can already be observed and trophic effects are almost complete by 14 days [33,34]. In our study all animals were sacrificed at postoperative day 14.

We performed a detailed analysis of collagen, which is a major component of extracellular matrix, for evaluation of the non-mucosal adaptation process. Logically, after massive intestinal resection, collagen production can be expected to play a role in intestinal elongation. Thus, the newly synthesized collagen content may reflect a non-mucosal adaptation process. In the present study, the acid-soluble form was analyzed for the establishment of less mature or nearly structured collagen content. Previously, investigators have been mainly focused on mucosal changes rather than other

Table 3. Intestinal newly synthesized and structural collagen fractions in two the groups at the end of the study.

Author	Route	Alone or in combination	% of intestinal resection	Result
Vanderhoof <i>et al.</i> [32]	Enteral	Gln + GH	70	No effect on intestinal adaptation
Zhou <i>et al.</i> [24]	Enteral	Gln + EN	85	Gln alone has no significant effect on intestinal adaptation; enhanced intestinal adaptation with combination.
Waitzberg <i>et al.</i> [47]	Enteral	Gln + GH	95	Enhanced intestinal adaptation
Spadoni <i>et al.</i> [48]	Enteral	Gln + GH	80	Enhanced intestinal adaptation
Ribeiro <i>et al.</i> [25]	Enteral	Alone	NA	Enhanced intestinal adaptation
Yang <i>et al.</i> [26]	Enteral	Alone	65	No effect on intestinal adaptation
Alavi <i>et al.</i> [9]	Enteral	Gln + HGF	80	No effect on intestinal adaptation and substrate absorption
Neves <i>et al.</i> [10]	Enteral	Gln + SCFA	70	Gln alone or in combination with SCFA has no significant effect on intestinal adaptation
Gu <i>et al.</i> [49]	Parenteral	Gln + GH	75	Gln alone has no significant effect on intestinal adaptation; enhanced intestinal adaptation with combination.
Liu <i>et al.</i> [5]	Parenteral	Gln + PN	60	Enhanced intestinal adaptation

Gln - glutamine; GH - growth hormon; EN - enteral nutrition; HGF - hepatocyte growth factor; SCFA - short-chain fatty acids; PN - parenteral nutrition

Table 4. Effects of enteral or parenteral Gln administration alone or in combination with growth factors, nutrients in experimental model of SBS.

Author	Route	Gln alone or in combination	% of intestinal resection	Result
Ribeiro <i>et al.</i> [25]	Enteral	Gln	NA	Enhanced intestinal adaptation
Yang <i>et al.</i> [26]	Enteral	Gln	65	No effect on intestinal adaptation
Zhou <i>et al.</i> [27]	Enteral	Gln + EN	85	Gln alone has no significant effect on intestinal adaptation; enhanced intestinal adaptation with combination.
Vanderhoof <i>et al.</i> [33]	Enteral	Gln + GH	70	No effect on intestinal adaptation
Waitzberg <i>et al.</i> [48]	Enteral	Gln + GH	95	Enhanced intestinal adaptation
Spadoni <i>et al.</i> [49]	Enteral	Gln + GH	80	Enhanced intestinal adaptation
Alavi <i>et al.</i> [9]	Enteral	Gln + HGF	80	No effect on intestinal adaptation and substrate absorption
Neves <i>et al.</i> [10]	Enteral	Gln + SCFA	70	Gln alone or in combination with SCFA has no significant effect on intestinal adaptation
Gu <i>et al.</i> [50]	Parenteral	Gln + GH	75	Gln alone has no significant effect on intestinal adaptation; enhanced intestinal adaptation with combination.
Liu <i>et al.</i> [5]	Parenteral	Gln + PN	60	Enhanced intestinal adaptation

Gln - glutamine; *GH* - growth hormon; *EN* - enteral nutrition; *HGF* - hepatocyte growth factor; *SCFA* - short-chain fatty acids; *PN* - parenteral nutrition

parts of intestinal wall for the quantization of adaptive responses. Although tissue collagen contents are not involved among intestinal adaptation parameters, and the data regarding the non-mucosal changes following massive small bowel resection is extremely limited, the analysis of soluble collagen fractions is considered a reliable indicator of intestinal wound healing [34,35]. On the other hand, the “glutamate family” of amino acids plays an important role on fibroblastic growth, and thus, tissue collagen production [36]. Glutamine is also utilized for the synthesis of several compounds, which include hydroxyproline, a major amino acid substrate in the hepatic cells and mucosal metabolic pathways [37, 38]. Therefore, following intestinal resection, glutamine-induced collagen production can be expected in the remnant intestine.

The rationale for using Gln as a trophic factor on intestinal adaptation after massive intestinal resection was based on its importance as a major energy source for enterocytes; therefore, it has been postulated to be an essential amino acid for intestinal health and to have crucial importance for intestinal mucosal growth and for attenuation of mucosal atrophy [38-43]. Additionally, it has important functions in amino acid, nucleotide, and protein synthesis [44]. Several studies suggest an increase in glutamine use in the remnant intestinal tissue immediately after massive intestinal resection [45,46]. Glutamine uptake by intestinal epithelial cells occurs from the brush border [45]. In SBS, decrease in intestinal absorption causes a malabsorptive state for luminal contents. The rate of intestinal adaptation after glutamine administration may be related to the length of

the remnant intestine; therefore, we used the parenteral route rather than the enteral route for glutamine administration in our study.

Contrary to our initial expectations, no beneficial effects of glutamine were demonstrated regarding intestinal adaptation parameters, collagen fractions, and weight changes of the rats. These results may be related to the lack of additional growth factors, nutrients, and supportive enteral or parenteral nutrition. To remedy this problem, a glutamine and GH combination seems to be the most frequently studied combination, although conflicting results are reported (Table 4) [5,9,10,25-27,33,48-50]. Although some authors report enhanced intestinal adaptation either with enteral [25] or parenteral [5] glutamine supplementation alone, others report no effect on intestinal adaptation [9,10,26,27,33,50]. Several studies report that parenteral or enteral Gln in combination with GH [48-50], growth factors [9], EN [27], or PN [5] enhances adaptation after massive intestinal resection in experimental models. Controversy in the literature may involve several factors, such as the amount of intestinal resection, the route of glutamine administration (enteral or parenteral), and the additional combinations with TPN or enteral nutrition.

Luo and co-workers investigated the role of plasma citrulline (Cit) and Gln as a biomarker for intestinal absorptive function [51]. They studied 24 patients with SBS receiving chronic PN ± recombinant human GH in a double-blind, randomized trial. They performed intestinal nutrient absorption studies during PN weaning. No significant correlation was observed between the Cit and Gln concentration and percent of absorption

of nutrient substances. They observed a positive correlation between residual small bowel length and plasma Cit levels.

There are many clinical studies investigating the physiological effects of Gln treatment in patients with short bowel syndrome [24]. The amount of available clinical data is still limited and the results of these trials are controversial. Combination therapies, especially with GH, seem to be more effective. Byrne and co-workers investigated the clinical endpoints of a number of variables (body weight, urine volume, enteral balance, change in body water, renal function, electrolyte concentrations, and average infusion days per week) [52]. They observed a gradual reduction in PN dependence, which was more significant in GH + enteral Gln group.

Our results demonstrated that parenteral glutamine administration had no apparent beneficial effect on

the intestinal adaptation after 75% intestinal resection. Further experimental and clinical studies, particularly with combination of different growth factors and nutrients, are required in order to evaluate their effects on the intestinal adaptation process.

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