THE EFFECTS OF GLUTAMINE-ENRICHED FEEDING ON ILEAL ANASTOMOSE HEALING IN RATS

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ABSTRACT

Objective: In this experiment, supplementation of the diet with increased levels of glutamine was used to determine glutamine enriched diet effect on the healing of ileal anastomosis.

Methods: Forty albino Wistar rats, in four groups, had a special diet 7 days prior to surgery and 7 days after surgery. On the 7th day of study a complete cut of the ileal wall was carried out and an anastomosis was performed using single layer interrupted inverting sutures. Group 1 received a normal diet for all 14 days; group 2 received a glutamine enriched diet 7 days prior to surgery and a normal diet 7 days after surgery; group 3 received a normal diet prior to surgery and a glutamine enriched diet after surgery; group 4 received a constant glutamine enriched diet. On the 7th postoperative day, tensile strength measurements and hydroxyproline level analyses were done.

Results: A preoperative glutamine enriched diet did not show any significant improvement in the bursting pressure and hydroxyproline levels on the 7th postoperative day, but pre and postoperative, and postoperative glutamine enriched diet significantly improved when compared with the normal diet group ($p<0.05$).

Conclusion: These findings show that when a glutamine enriched diet was used, in the postoperative or both pre and postoperative period, hydroxyproline levels and bursting pressures increased, indicating a good anastomotic healing.

Key Words: Anastomotic healing, Glutamine, Bursting pressure

INTRODUCTION

The healing of intestinal anastomoses remains a topic of ongoing research because clinical practice shows it occur rather frequently. Malnutrition increases the risk of wound-related complications, and correction of malnutrition, either parenterally or enterally during the preoperative or perioperative period, reduces these complications (1). Glutamine is the most abundant free amino acid in the circulation, it is also the primary fuel for rapidly dividing cells and plays a key role in the transport of nitrogen between organs (2). High glutaminase activity is characteristic of many rapidly dividing cells such as fibroblasts (3). On the other hand, the use of nutritional supplementation for the modulation of wound healing, especially in well nourished patients or animals mainly in the experimental stage, has shown only sporadic evidence of clinical efficacy (1).

In this experiment, supplementation of the diet with increased levels of glutamine was used to determine glutamine enriched diet effect on the healing of ileal anastomosis.

MATERIALS AND METHODS

Animals and surgical technique

Forty albino Wistar rats, weighing 200-250 g were randomly assigned to four groups. All groups had a special diet 7 days prior to surgery and 7 days after surgery. On the 7th day of study the animals were anesthetized with an intramuscular ketamine (40 mg/kg), xylazine (2.5 mg/kg) combination anesthesia. The abdomen was shaved and entered through a 5 cm midline incision. 10 cm proximal to cecum, a complete cut of the ileal wall was carried out and an

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anastomosis was performed using 8, end-to-end, one layer of interrupted inverting sutures with 5/0 polypropylene (Prolene-Ethicon Co). The abdominal wall was closed in two layers.

Feeding protocol

Group 1 received a normal diet for all 14 days; group 2 received a glutamine enriched diet 7 days prior to surgery and a normal diet 7 days after surgery; group 3 received a normal diet prior to surgery and a glutamine enriched diet after surgery; group 4 received a constant glutamine enriched diet. A normal diet consists of rat chow and water; a glutamine enriched diet consists of rat chow and specialized elemental nutrition with 70 g glutamine enriched powder (AlitraQ, Abbott) in 250 cc water. The rat's mean daily intake of the solution was 240±60 mL/kg throughout the study period. The nutritional composition of the glutamine enriched solution is listed in Table I. and the amino acid composition is shown in Table II. There was no significant difference among the groups concerning the daily rat chow intake.

Bursting pressure measurements

On the 7th postoperative day, the bursting pressure of the anastomoses were measured in vivo by infusion of normal saline (2 ml/min.) under pressure, until a leak was noted by an infusion pump with a pressure monitor (4,5).

Tissue hydroxyproline levels

One cm ileal samples were frozen immediately in liquid nitrogen and stored at -70°C until hydroxyproline levels were determined according to the method of Weesner (6). Briefly, the samples were sealed in small pyrex test tubes and hydrolized for 3 hours at 130°C in the presence of 5ml of 6N HCl. The hydrolyzed samples were neutralized by 2.3 N NaOH. The pH of the solution was adjusted to 6-7 using either HCl or NaOH. Hydroxyproline oxidation was initiated by adding 1ml chloramine T, for 20 min at room temperature. The chloramine T was then destroyed by adding 1ml of 3.15 M perchloric acid. After 5 min, 1 mL of Ehrlich's reagent was added, the mixture shaken, and tubes were placed in a 60°C water bath for 20 min. They were then cooled in tab water for 5 min and the absorbancy of the solutions were determined at 557 nm. The hydroxyproline values were determined using a standard curve.

Statistical analysis

The bursting pressure measurements and tissue hydroxyproline levels were expressed as the mean±standard error of the mean (SEM) and data were compared using the paired Student's t-test with alpha level of 0.05 to establish significance.

RESULTS

Bursting pressure measurements

The preoperative glutamine enriched diet did not show any significant improvement in the bursting pressure on the 7th postoperative day. The pre and postoperative, and postoperative glutamine enriched diets significantly improved the bursting pressure when compared with the normal diet group ($p<0.05$). When the pre and postoperative, and postoperative glutamine enriched diet groups were compared there was a minor difference in the bursting pressure values, but this was not statistically significant (Table III).

Tissue hydroxyproline levels

The preoperative glutamine enriched diet did not show any significant difference in the tissue hydroxyproline levels on the 7th postoperative day. The pre and postoperative, and postoperative glutamine enriched diets significantly improved the tissue hydroxyproline levels when compared with the normal diet group ($p<0.05$). When the pre and postoperative, and postoperative glutamine enriched diet groups were compared there was no statistically significant difference in tissue hydroxyproline levels (Table IV).

DISCUSSION

Healing of an anastomotic wound can be determined in many ways. One of them is the measurement of the bursting pressure of anastomoses. It is a direct method

Table I. Composition of the glutamine enriched solution.

	Glutamine enriched solution
Calories (kcal)	300
Amino acid (g)	15.80
Fat (g)	4.65
Carbohydrate (g)	49.50

Values are per 250 mL of the solution.

Table II. Amino acid composition of glutamine enriched solution.

Amino acid	g/100g of protein	Amino acid	g/100g of protein
Threonine	4.5	Glutamine	27.0
Valine	5.7	Metionin/Sistin	3.7
Isoleucine	4.8	Alanine	2.0
Leucine	8.0	Arginine	8.5
Lysine	6.2	Aspartic acid	5.0
Tryptophan	1.3	Glutamic acid	7.0
Histidine	2.0	Glycine	1.5
Fenilalanine/Tyrosine	8.3	Proline	2.4
		Serine	2.3

Values are in g/100g of protein

Table III. Bursting pressure measures (mean±SEM) (mmHg)

	Control (n=10)	Group2 (n=10)	Group3 (n=10)	Group4 (n=10)
Mean	79.20	85.50	145.00*	143.20*
SEM	±9.77	±11.93	±14.13	±16.64

* p<0.05, compared to control group

Table IV. Tissue hydroxyproline levels (microgram/gram) (mean±SEM)

	Control (n=10)	Group2 (n=10)	Group3 (n=10)	Group4 (n=10)
Mean	0.44	0.46	0.69 *	0.72 *
SEM	±0.052	±0.033	±0.060	±0.054

* p<0.005, compared to control group

in the investigation of a healing anastomosis and reflects the quality and speed of regeneration. On the other hand, the tissue hydroxyproline levels have been found to correlate directly with the collagen content of the wounds (7,8).

A significant number of studies have investigated the values of specific nutrients in the regulation of wound healing (1-3,9). Glutamine is one of these specific nutrients. It is the most abundant plasma amino acid and it provides energy for rapidly dividing cells such as fibroblasts, reticuloendothelial cells and gut epithelial cells (9-11). Glutamine is also an important respiratory fuel for lymphocytes, macrophages, and it is a nitrogen donor for the synthesis of nucleotides, purines and pyridines, necessary for cell replication (2,9). The primary fuel of the small intestine is glutamine; ketone bodies and glucose follows in descending order of uptake (9-12). Glutamine can be metabolized in many ways. One of metabolic pathways is through glutamate semialdehyde, especially in the jejunum to form proline, ornithine and citrulline; more than 23% of glutamine metabolized in the gut is released as citrulline (13). It has been shown that pharmacologic doses of arginine promotes wound healing, and proline is essential for the production of collagen (10,14,15). Although arginine and proline levels were not measured in our experiment, we measured the hydroxyproline levels.

Mc Cauley et al (16) found no significant difference in the bursting wall tension between rats receiving six days of postoperative support with either rat chow, parenteral nutrition or parenteral nutrition containing 1.2% glutamine, but they used undernourished rats in their experiment. In the rats, collagen deposition is rapid. Rodents also have a high metabolic rate and their tolerance for fasting is poor (16). Indeed, even short term starvation (less than one week) can be lethal for an adolescent rat. Additionally, total food intake decreases dramatically when rats are fed with a protein-free diet. Therefore, results of wound-healing studies using fasting or protein-free diets in rodents, may have limited correlation with events encountered in daily clinical practice (1). Polymorphonuclear leukocytes, macrophages and lymphocytes consume large amounts of glutamine. These cells use glutamine in similar or greater proportions compared to glucose. The increased utilization of glutamine by inflammatory cells within wounds can cause decreased tissue concentrations of glutamine (10). In the rats, a high rate of glutamine utilization may be present, not only for provision of energy, but also to supply several metabolic intermediates (17). Addition of 2% glutamine to TPN solution reversed jejunal permeability changes and improved mucosal atrophy induced by TPN (9). Glutamine increases the number of metaphase mitoses per crypt following radiation injury (18). Platell et al (19) demonstrated that only 0.5 g of glutamine per 100 g of parenteral nutrition significantly increased the weight of a small bowel. O'Dywer et al (20) found that 2% and 3% glutamine solutions significantly increased jejeunal mucosal weight, mucosal proline and villus height. 5-fluorouracil toxicity on intestinal mucosa, methotraxate induced colitis and radiation toxicity can be prevented by glutamine administration (18,21-23). O'Riordin et al (24) found that glutamine supplementation enhances T lymphocyte functions in patients undergoing colorectal surgery. Enhancement of T cell response is associated with reduced susceptibility to infections. Glutamine also exerts a trophic effect on the gut mucosa; it serves as an oxidative fuel and supports nucleotide biosynthesis as well as it acts as a secretogogue to stimulate the release of gut peptides (25).

Our results showed that pre and postoperative or only postoperative glutamine enriched diets significantly improved the bursting pressure and hydroxyproline content of ileal anastomoses in the rat. Preoperative administration did not show any beneficial effect. Our findings are explained by the essentiality of glutamine in stressful conditions. In these conditions glutamine is required in greater amounts than in normal conditions.

In conclusion, when a glutamine enriched diet was used postoperatively or pre and postoperatively, hydroxyproline levels and bursting pressures increased, indicating a good anastomotic healing. But we have the opinion that further experiments are required to show the exact role of glutamine and compare it with other nutritive substitutes.

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