A Review of Glutamine's Function in the Intestinal Mucosa as a "Conditionally" Essential Amino Acid for Gut Health

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INTRODUCTION

Glutamine is the most abundant free amino acid synthesized in the body. Even though the body makes this amino acid, it is considered a "conditionally" essential amino acid because of its role in gut health. Glutamine was isolated in 1883 then identified in 1932. The importance of glutamine on gut health was not discovered until 1974 by Windmueller and Spaeth and then was considered "conditionally" essential in 1990. Glutamine is important because it performs many roles in the small intestine: it provides energy for enterocyte cells, regulates amino acid utilization, aids in nitrogen balance, and helps maintain gut barrier functions by providing immunity and increasing cell proliferation and repair. Many healthy adults and patients under stressed conditions undergo GI function problems. Glutamine supplementation may benefit individuals diagnosed with IBS or other gastrointestinal disorders. This paper will review the study that established the importance of glutamine as fuel in the gut, glutamine's role in regulating amino acid utilization, how glutamine maintains autophagy and inhibits apoptosis under stressed conditions, why glutamine is beneficial in enteral and parenteral nutrition and how glutamine decreases intestinal permeability to show why glutamine is a "conditionally" amino acid for gut health.

REVIEW OF LITERATURE

The Foundation

Windmueller and Spaeth provided the foundation for the increase interest of glutamine's role by investigating glutamine's metabolism and uptake in the small intestine (2). They wanted to identify the cells responsible for glutamine uptake, to determine the rate and end products of glutamine metabolism, and to understand the physiological role of glutamine in the small intestine. The study used nine foxhounds and three mongrel dogs, a mixed breed of cats, eight rhesus and one vervet monkey, and adult male Sprague-Dawley or Osbourne-Medel rats. The experiment lasted 4-10 days and used ion exchange chromatography, assays, spectrometer, and chromatographic amino acid analyses. They found the rat blood level fell from 600 micro moles to 200 micro moles in 60 minutes, but in the control group glutamine level only fell 8% in 5 hours. The blood with the glutamine inhibitor fell near zero. The end products were CO2 and ammonia from glutamine metabolism. The researchers concluded that glutamine primarily metabolizes in the mucosa and the uptake is concentration dependent. The study established the importance of glutamine as the primary energy source for enterocytes.

Glutamine's Role in Amino Acid Utilization

As well as providing fuel for the enterocytes, glutamine helps to regulate the utilization of the other amino acids. Zhao-Lai Dai et al. investigated glutamine's regulation of amino acid

utilization in the small intestine (3). In their experiment, bacterial strains from pigs were obtained under anaerobic conditions which include a tube with amino acid mixture, glutamine, and bacteria and a control tube that included the amino acid mixture, glutamine, but no bacteria. The bacterial utilization was dose dependent which means more glutamine decreased the net utilization of bacteria in the gut. Glutamine affected each amino acid differently. More glutamine increased arginine and proline utilization while citrulline lowered. The researchers concluded the amount and activity of bacteria affected the utilization and metabolism of amino acids in the small intestine. Also, the study showed the importance of glutamine and why it is crucial for maintaining gut health because the amount of bacteria impacts glutamine's effect on metabolism in the gut.

Glutamine Supplementation under Stress Conditions

Maintaining Autophagy and Inhibition of Apoptosis

A decrease in production of serum glutamine occurs under stress conditions which leads to intestinal permeability. Sakiyama et al. investigated if glutamine affected autophagy and if so, how (8). Western blotting and confocal microscopy was used to analyze rat intestines. Under stressed conditions, glutamine maintained autophagy and inhibited apoptosis. Under heat stress of 42°C for 30 min., LC3-11 decreased then returned to normal during recovery. In cells incubated with 0.7 or 0 mmol/L glutamine for 24 hours, the autophagy response decreased to heat stress. They concluded that serum glutamine decreases under stress conditions which leaves the gastrointestinal tract more susceptible. Low serum glutamine impairs the gut's ability to signal an autophagy response which increases the mucosal permeability and can lead to infections. Therefore, glutamine helps maintain autophagy and inhibits apoptosis in the gut, so

atrophy does not occur. If endogenous glutamine is insufficient then supplementation is beneficial.

In a more recent animal study, glutamine was shown to also inhibit apoptosis. Swaid et al. evaluated the preventative effects of glutamine supplementation on injury induced rats by acetic acid (9). The study used male Sprague-Dawley rats and lasted 5 days. The researchers created 4 groups in the experiment: a control group, control-glutamine group, an acetic acid group, and an acetic acid –glutamine group. Laparotomy was conducted on the rats and Western blotting was used to analyze the proteins in the intestines. The results showed that the body weight of the acetic acid rats decreased ($p > 0.5$), injury in the acetic acid intestinal damaged rat decreased a mean of 1/3-fold and 6-fold ($p < 0.5$), and in the intestinal mucosa, acetic acid damaged rat's bowel weight decreased. For enterocyte proliferations, enterocytes in the control group, the acetic-acid glutamine group, and the control-glutamine group increased. The acetic-acid injured rats decreased in enterocyte proliferations and decreased in cell proliferation. In conclusion, glutamine helped injury to the mucosa and increased recovery in rats injured by acetic acid. Glutamine increased enterocyte proliferation while decreased cell apoptosis. When injury occurs to the small intestine, glutamine supplementation provides a protective effect for intestinal barrier function.

The same effect of glutamine occurs in injury induced by drugs. Basivereddy et al. investigated glutamine's effect on indomethacin-induced alterations of the mucosa in a 7 day rat study (10). The researchers created two groups: a control and indomethacin-induced rat group. Glutamine was given in both diets. The intestines were removed from the rats and they measured their oxidative stress and BMM function. The results showed that the number of enterocytes in the indomethacin-induced rats lowered as well as their oxidative stress. The researchers

concluded that glutamine supplementation can help prevent drug-induced damage in the mucosa. Therefore, an adequate amount of glutamine is important to prevent damage in the intestinal mucosa.

Enteral and Parental Nutrition

Glutamine was also shown to inhibit apoptosis in enteral and parenteral supplementation. Han et al., discovered that glutamine impacts apoptosis of the mucosa (5). The laboratory and animal study used 80 SD rats. For 11 days, glutamine supplementation was given to rats through enteral and parenteral nutrition. The rats were split up into 5 clusters: (1) Sham-operated, (2) SAP + PN, (3) SAP + EN, (4) SAP + EN + Gln, and (5) SAP + PN + Gln. The results showed that in the SAP cluster, A1 decreased more than the shame-operated cluster. The SAP + EN + Gln cluster A1 was less than the SAP + EN or SAP + PN cluster after 7 days. Also, the SAP cluster A1 decreased. The researchers concluded that glutamine in enteral or parenteral nutrition inhibited cells from apoptosis which helps to maintain the integrity of the mucosal barrier. This study provides evidence that supplementation of glutamine benefits enteral and parenteral nutrition support.

The benefits of enteral and parenteral nutrition has been observed in human studies. Supplementation of glutamine through enteral nutrition provides beneficial effects for burn patients. Peng et al. investigated protective effects on the mucosal barrier from supplementation of glutamine by enteral nutrition support in severe burn patients (7). In a 9 month study, 48 severe burn patients, 29 male and 19 female were identified between the ages of 18-60 years old. Two groups were created: one group was the burn control group of 23 patients and the other

group was the glutamine treated group of 25 patients. The groups were either given glutamine supplementation or a placebo with the same dose. The L/M ratio in urine was collected which was lower than the glutamine in the burn control group. Glutamine plasma concentration showed that glutamine increased in the control group and increased in the glutamine supplementation group. The researchers concluded that enteral glutamine helps patients to recover better and faster which would decrease hospital stay. Also, glutamine lessens the mucosal permeability. In conclusion, glutamine supplementation is necessary to protect the mucosa in burn patients.

Benefits of glutamine supplementation was also shown in abdominal surgery patients. Quan et al. investigated the impact of glutamine on post-operative patients (11). They evaluated the permeability in the intestine and its relation to the inflammatory response. The study was a randomized trial which was double blind, randomized, and controlled. The subjects were 20 patients, 13 men and 7 women between the ages of 18-65 years old undergoing abdominal surgery who had no severe diseases. The subjects were split into two groups: a glutamine and placebo group. After their abdominal surgeries, the patients took glutamine or a placebo for 4-7 days. They measured the patient's temperature, heart rate, blood, liver and kidney functions and urine samples. Temperature and heart rate lowered in the glutamine group and WBC counts lowered to normal faster. Glutamine serum raised in the glutamine group. The urine L/M sample showed that even though there was no difference initially, after 7 days the concentration in the placebo group was greater than the concentration in the glutamine group. Permeability of the intestinal mucosa increased due to damage and glutamine supplementation decreased intestinal permeability. Therefore, glutamine helps to maintain the intestinal barrier of the small intestine.

How Glutamine Decreases Intestinal Permeability

In the previous studies, glutamine maintained autophagy, decreased apoptosis, increased enterocyte proliferation, and decreased intestinal permeability, and prevented damage of the intestinal mucosa, but the mechanism of how glutamine has this effect was not found. Ban and Kozar discovered a mechanism of how glutamine inhibited apoptosis (4). The study explained glutamine's effects on gut protection and how it protected intestinal epithelial cells. The methods used were microarray analysis of small epithelial cells, Western blotting, and quantitative real time PCR. The results showed that glutamine had a protective effect because of its inhibition on the Sp3 protein. Sp3 is a marker for tumor cells which can lead to cancer and may signal apoptosis. Glutamine inhibits Sp3 which decreases apoptosis. The microarray gene analysis showed that Sp3 decreased 3.12-fold by glutamine and apoptosis decreased shown by the mRNA expression of Sp3 decreased 82.9%. These results show that glutamine decreased apoptosis under stress conditions. Glutamine has a protective effect on barrier function because it suppresses the Sp3 protein.

Glutamine was also shown to decrease intestinal permeability by specific signaling pathways. Niederlechner et al. examined the relationship of the pathways on the protection of glutamine after injury (6). The study included rats and Western blotting. In the experiment, the researchers treated epithelial-6-cells for 15 minutes with glutamine, with and without different inhibitors. The results showed that after heat stress, the SB203580 inhibitor increased cell survival. The researchers concluded that glutamine is protective after heat stress by the activation of certain signal pathways. Therefore, preservation of the gut barrier also occurs by activating signal pathways.

CONCLUSIONS

Glutamine is an important amino acid that is “conditionally” essential for gut health especially in patients undergoing stress because it protects the integrity of the mucosal barrier. As shown by Zhao-Lai Dai et al. glutamine protects the integrity of the gut by providing fuel for the small intestine and regulating amino acid utilization which is dependent on the amount and activity of bacteria. It maintains autophagy of the cells by preventing atrophy which could lead to infections according to Sakiyama et al., Swaid et al., and Basivereddy et al. Han et. al, Ban and Kozar, and Niederlechner et al. found that glutamine also protects the integrity of the gut by inhibiting apoptosis by the suppression of the Sp3 protein. As apoptosis decreases, cell proliferation increases which increases the integrity of the mucosa. By decreasing intestinal permeability which increases the integrity, glutamine prevents damage and infections to occur in the mucosa. Damage and infections can lead to IBS or gastrointestinal disorders. Preventing damage in the gut increases the recovery in stressed patients as shown by Peng et al. and Quan et al. which concludes that glutamine supplementation is beneficial. Therefore, glutamine is an amino acid that is “conditionally” essential to protect the mucosal barrier and maintain a healthy gut.

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