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Protective mechanisms of glutamine in intestinal diseases

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Abstract. Glutamine, the most abundant free amino acid in the human body, is a major molecule utilized by intestinal cells. It has been reported that glutamine is involved in intestinal physiology and management of multiple intestinal diseases. In gut physiology, glutamine promotes enterocyte proliferation, regulates tight junction proteins, suppresses pro-inflammatory signaling pathways, helps regain microbiota composition, and protects cells against apoptosis and cellular stresses during normal and pathologic conditions. The promising reported protective effects of glutamine supplementations on different experimental animal models of DSS-induced, TNBS-induced and NSAID-induced colitis, speculated a similar effect on clinical settings. As glutamine stores are depleted during severe metabolic stresses, including those associated with trauma, sepsis, and inflammatory bowel diseases, the effect of glutamine supplementation has been examined in patients to improve their clinical outcomes. In this review, we discuss the physiological roles of glutamine in intestinal health and its underlying mechanisms. In addition, we discuss recent evidence regarding the efficacy of glutamine supplementation in treating intestinal diseases.

Keywords: glutamine, intestinal function, tight junction, microbiota, animal models, inflammatory bowel disease, glutamine supplementation, clinical outcomes

Introduction

Glutamine is the most abundant amino acid in human blood, skeletal muscle, and free amino acid pool. It belongs to a family of amino acids known as glutamates (Calder 1994). It has several important physiological roles in multiple metabolic processes and energy metabolism. It is involved in the synthesis of many peptides and non-peptides, such as nucleotide bases, glutathione, and neurotransmitters (Albrecht et al. 2010; Amores-Sánchez, Medina 1999; Coster et al. 2004). In addition, it helps in the detoxification of ammonia and systemic control of acid-base balance (Patience 1990). Over the last few decades, the role of glutamine metabolism in immune system (Calder 1994; Newsholme 2001; Newsholme et al. 1999) and cancer cells (Cairns et al. 2011; DeBerardinis, Cheng 2010; Kim, Kim 2013) has been documented, which is an important finding in the field of clinical medicine. In intestinal and renal illnesses, immune cells were found to utilize large amounts of glutamine, exceeding its endogenous production. This finding gave rise to the theory that glutamine is "conditionally essential" because plasma and muscle glutamine levels were found to be markedly reduced in these conditions compared to those in healthy individuals (Askanazi et al. 1980). Another glutamate member is the glutamic acid, which is best known

as a component of monosodium glutamate (MSG) that is responsible for the umami taste in food (Calder 1994).

Intestinal tissue utilizes about 30% of total glutamine (Wu 1998), reflecting the essential role of glutamine as a key nutrient. Approximately three quarters of enterally taken glutamine is absorbed into the splanchnic tissues, and most of it is metabolized within the intestine (Dechelotte et al. 1991; Newsholme, Carrie 1994). Glutamine functions in the intestine include maintaining nucleotide metabolism, intestinal barrier function, and modulation of inflammation, as well as regulating stress responses and apoptosis (Kim 2011; McCauley et al. 1998; Wang et al. 2015). Therefore, it is important to ensure sufficient glutamine intake during intestinal illnesses. This review aimed to highlight the role of glutamine in intestinal diseases by comparing the pathological and clinical consequence of either glutamine deficiency or abundance in previously reported experimental and clinical conditions. Some reports in literature regarding clinical outcomes after glutamine supplementations were confusing; therefore, further research should focus on the underlying protective mechanisms of glutamine, thus uncovering these conflicting results.

Physiological roles of glutamine in the intestine

Tissue integrity

Intestinal epithelial cells renew every four to five days because a continuously high level of cell proliferation is required for homeostasis (van der Flier, Clevers 2009). Generally, cell proliferation is controlled by a number of hormones and signaling pathways. When activated, crypt-residing intestinal stem cells start to differentiate into specialized epithelial cell types, including enterocytes, goblet cells, and Paneth cells, which help maintain intestinal tissue integrity (Bjerknes, Cheng 2005).

Effect of glutamine on signaling pathways

Glutamine has a profound effect on several signaling pathways. To begin with, glutamine is required for activating multiple mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated (ERK1/2) and c-Jun N-terminal kinases (JNK1/2). These protein kinases orchestrate cell proliferation and differentiation (Zhang, Liu 2002). Additionally, glutamine augments the effect of several growth factors, including epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), and transforming growth factor-α (TGF-α). Cell lines cultured on media deprived of glutamine impaired EGF-stimulation of DNA, RNA, protein synthesis, and cellular replication in IEC-6 cells (Ko et al. 1993). On the other hand, high glutamine intake significantly enhanced IGF-I-mediated DNA and protein synthesis in a rat model with short bowel syndrome (Ziegler et al. 1996). During experimental ischemia, treatment with glutamine enhanced TGF-α action on mucosal cell proliferation (Blikslager et al. 1999).

Effect of glutamine on tight junctions

Tight junctions form a continuous intercellular barrier between epithelial cells, which is required to separate tissue spaces and regulate selective movement of solutes across the epithelium. This barrier is built up of various proteins; to date, more than 40 types of tight junction proteins have been identified. They are dynamic rather than static structures, constantly remodeling their configuration with a relatively high turnover rate (Zihni et al. 2016). In the intestine, tight junction have an additional role maintaining intestinal integrity, which prevents pathogens and toxins from entering the intestinal lumen (Mitic, Anderson 1998). Tight junction protein components fall into four main families, namely claudins, occludin, tricellulin, and junctional adhesion molecules (Anderson, van Itallie 1995). As a response to different physiological stimuli and signal pathways, these proteins modulate the transport of luminal molecules into mucosal cells by adjusting their tightness (Harhaj. Antonetti 2004). Each signaling molecule interacts with a specific tight junction protein component. For example, activation of protein kinase C results in the upregulation of occludin, zonula occludens (ZO)-1, ZO-2, and claudin 1 in primary human epithelial cells, leading to enhanced transepithelial electrical resistance (TER) (Koizumi et al. 2008). MAPKs can directly interact with the C-terminal tail of occludin, which mediates the prevention of hydrogen peroxide-induced disruption of tight junction (Basuroy et al. 2006). Furthermore, myosin light-chain kinase-induced phosphorylation of myosin light chain was found to regulate tight junction permeability in Caco-2 cells (Rigor et al. 2013; Turner et al. 1997). As a demonstration of such effects, glutamine deprivation in the human colon carcinoma cell line Caco-2 was markedly reduced by the expression of several tight junction proteins, including claudin-1, occludin, and ZO-1 (Li et al. 2004). Moreover, it significantly increased epithelial cell permeability, as determined by TER. Mechanistically, deprivation of glutamine activated the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, which led to a reduction in claudin-1 expression and TER. On the contrary, addition of glutamine to cells previously deprived of glutamine rescued the impaired barrier functions

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and reduced the activation of the PI3K/Akt pathway, which reversed claudin-1 expression in the cells, suggesting that glutamine supplementation regulates the phosphorylation states of tight junction proteins. Therefore, glutamine-mediated tight junction maintenance is in part mediated by phosphorylation of tight junction proteins. Some reports showed that glutamine induced the expression of ZO-1, ZO-2, and ZO-3 and increased the abundance of claudin-1, claudin-4, and ZO-1 on plasma membranes (Wang et al. 2016). Additionally, maintaining intestinal permeability by tight junction proteins was beneficial for the treatment of multiple intestinal pathologic conditions, such as IBD and celiac disease (Lee 2015). These results suggested that glutamine supplementation could be beneficial for patients with impaired gut permeability.

Effect of glutamine on inflammatory pathways

Intestinal inflammation has shown to be the main underlying pathology in IBD, such as ulcerative colitis and Crohn's disease, as well as in colorectal cancer (Ullman, Itzkowitz 2011). Therefore, targeting intestinal inflammation is the key factor in treating such conditions. Several lines of evidence have indicated that glutamine has an anti-inflammatory property because of its influence on a number of inflammatory signaling pathways, including the nuclear factor κB (NF-κB) and activator of transcription (STAT) pathways (Rhoads, Wu 2009).

Under steady-state conditions, NF-κB resides in the cytoplasm and is maintained inactive by a family of inhibitors, namely κB inhibitors (IκB). When phosphorylated and activated during the inflammatory state, IκB triggers the degradation and release of κB from NF-κB, thus activating it. The active NF-κB complex is then translocated into the nucleus, where it induces the expression of genes harboring NF-κB-binding elements, such as interleukin-6 (Il-6) and tumor necrosis factor-α (TNF-α). As a result, the production of IL-6 and TNF-α highly increases during inflammation, by activating multiple target cells, such as antigen-presenting cells and T cells, and by inducing acute-phase proteins (Ganeshan, Chawla 2014). Studies showed that glutamine suppressed NF-κB pathway activation, with intraperitoneal injection of glutamine or oral gavage suppressing NF-κB activation in rodent models of colitis (Hou et al. 2013; Xue et al. 2011). Additionally, glutamine influences IκB stability. In lipopolysaccharide (LPS)-treated Caco-2 cells, deprivation of glutamine reduced the expression of IκBα, triggering an increase in the number of NF-κB binding to DNA, as well as an increase in the expression of inflammatory cytokine interleukin-8 (IL-8) (Malhotra et al. 2002). An *in vivo* experiment in human ileocecal adenocarcinoma HCT-8 cells showed that glutamine pre-treatment reduced the level of IκBα degradation and production of IL-8 during TNF-α-induced inflammation (Liboni et al. 2005). In support of these data, glutamine supplementation for seven days was proven to significantly reduce IκBα degradation, leading to the suppression of NF-κB activation in a rat colitis model (Hubert-Buron et al. 2006). As glutamine status influences the production of IL-8, a cytokine that stimulates the migration of neutrophils to inflammatory sites, glutamine-mediated IL-8 regulation could be another important way for targeting intestinal inflammation (Kaplan 2013; Kretzmann et al. 2008).

STAT proteins have also been extensively studied for their roles in regulating inflammation. These proteins are transcription factors that modulate the immune system, cellular proliferation, and development (Kaplan 2013). In rat colitis models, glutamine administration via the rectal route reduced the phosphorylation of STAT1 and STAT5, indicating that glutamine influences STAT signaling activation (Kretzmann et al. 2008). Furthermore, in LPS-treated Caco-2 cells, glutamine depletion upregulated STAT4 expression, whereas glutamine supplementation downregulated STAT4 expression and IL-8 production (Liboni et al. 2005). Therefore, the anti-inflammatory effect of glutamine could additionally be achieved by inhibiting STAT activation and the expression of inflammatory cytokines, such as IL-6 and IL-8, in intestinal tissues.

Studies report that during inflammation NO may play a double role, with both beneficial and harmful effects. It is synthesized by multiple cells and modulates a variety of beneficial cellular signaling pathways, including inflammatory responses (Coleman 2001). Glutamine was found to be an important regulator of NO synthesis (Hecker et al. 1990; Swierkosz et al. 1990). The level of whole-body plasma nitrate, the stable end-product of NO production, was found to be reduced in rats fed a glutamine-enriched diet compared to that in control rats (Houdijk et al. 1998). Similarly, in rats with intestinal ischemia-reperfusion injury, a glutamine-enriched diet reduced the mucosal expression of inducible NO synthase, an inflammatory enzyme, and decreased the plasma NO concentration (Suh et al. 2003). Therefore, based on the *in vitro* and *in vivo* studies mentioned above, glutamine supplementation could be a promising method for treating intestinal inflammatory disorders by inhibiting the activation of NF-κB and STAT and suppressing the expression of inflammatory cytokines such as IL-6, TNF-α, and IL-8, as well as inflammatory enzyme inducible NO synthase.

Effect of glutamine on intestinal microbiota

Correction of dysbiosis in intestinal microbiota is another important factor in alleviating intestinal inflammation. It was reported that repeated administration of chemotherapeutic agents during anticancer therapy decreased the abundance of *Firmicutes*, which mostly comprise gram-positive bacteria, but increased that of *Bacteroidetes* and *Verrucomicrobia*, which mostly comprise gram-negative bacteria. The mechanisms underlying dysbiosis related to chemotherapy are still unclear, although the disruption of the intestinal epithelial barrier is a likely cause (Kato et al. 2017; Yasuda et al. 2012). Some studies reported that polyglutamate (PGA), a polymer of glutamic acid, had a remarkable effect on the composition of gut microbiota, enhancing the abundance of *Firmicutes*, specially of the genus *Lactobacillus*, compared with that of *Bacteroidetes*. High-molecular-weight γ-PGA with viscous characteristics can stabilize the gut microorganisms, while low-molecular-weight γ-PGA reaching the colon can increase the microbial diversity in the gut. The effect of γ-PGAs could be an additional mechanism by which glutamine can have a beneficial effect on gastrointestinal health (Kato et al. 2017).

Apoptosis and cellular stress

Because of the high level of cellular turnover in intestinal cells, it is critical to maintain a fine balance between proliferation and apoptosis to maintain normal intestinal function (Matés et al. 2002). Spontaneous apoptosis is essential for maintaining its normal architecture. However, a number of cellular stresses induced by exogenous agents or intracellular stimuli can disturb this balance. Dysregulated apoptosis could trigger several intestinal pathologic conditions (Demehri et al. 2013; Que, Gores 1996; Sánchez de Medina et al. 2014; Zatorski et al. 2016). Studies showed that glutamine displayed anti-apoptotic properties in the intestine. In rat intestinal epithelial (RIE-1) cells, glutamine deprivation resulted in apoptosis (Papaconstantinou et al. 1998). On the other hand, glutamine supplementation effectively reduced toxin-induced apoptosis in human intestinal epithelial T84 cells (Carneiro et al. 2006), suggesting that glutamine is critical for suppressing apoptosis. These interesting reports increased the enthusiasm towards uncovering the anti-apoptotic capacity of glutamine.

First, as a precursor for glutathione (GSH), glutamine maintains a normal cellular redox status because it is present in both reduced and oxidized forms (GSSG), which is crucial for preventing apoptosis. Along with other amino acids, glutamine generated from glutamate produces GSH, an important cellular antioxidant (Roth et al. 2002). Secondly, glutamine regulates the activation of caspases—a family of protease enzymes that play important roles in inducing apoptosis (Fan et al. 2005). They are normally present as proenzymes, and various stimuli can activate them through cleavage. In RIE-1 cells, glutamine-deprived cells showed significantly higher caspase-3 activity along with a higher level of apoptosis, while administration of glutamine reduced caspase-3 activity in neonatal piglet enterocytes, as well as caspase-8 activity in T84 cells (Carneiro et al. 2006). In addition, glutamine enhanced the expression of heat shock proteins (HSPs) (Wischmeyer 2002), which modulate apoptotic cell death, by acting as a molecular chaperone, allowing the cells to adapt to stressful conditions.

Another interesting effect of glutamine is related to its protective role over the endoplasmic reticulum (ER). A number of pathologic conditions, including IBD, disrupt ER function, resulting in ER stress (Kaser et al. 2008). As extensive ER stress triggers sustained apoptosis and further insults, attenuating ER stress could improve cell protection and survival. In *in vivo* experiments in rats with colitis, glutamine administration markedly reduced the activation of ER stress markers, such as glucose responsive protein 78 and caspase-12 (Crespo et al. 2012). Supporting these observations, *in vitro* glutamine treatment in Caco-2 cells reduced the activation of ER stress induced by pharmacological ER stress inducers. During the process of autophagy, cellular organelles and proteins are broken down to supply energy. Reports showed that autophagy provided a protective effect against intestinal pathologic conditions, as the autophagy-related 16-like 1 (Atg16L1) gene was implicated in Crohn's disease (Hampe et al. 2007; Rioux et al. 2007). Mice lacking Atg16L1 were more susceptible to induction of acute colitis. Furthermore, in the mouse intestinal epithelium, mutation of Atg5 and Atg7, which are autophagy-related genes, resulted in increased production of TNF-α and IL-1β following LPS administration. Glutamine increased autophagy in intestinal epithelial cells, namely Caco-2 and IEC-18 cells. As a result, glutamine suppressed intestinal apoptosis under stress conditions through promotion of autophagy and cellular survival (Fujishima et al. 2011).

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Clinical implications of glutamine supplementation in intestinal diseases

Glutamine supplementation has been considered and examined in many clinical settings, particularly in conditional glutamine-deficient statuses associated with acute critical illnesses. In these illnesses, muscle wastage is caused by a marked reduction in plasma glutamine concentration (Askanazi et al. 1980). Patients with intestinal diseases, especially Crohn's disease, displayed low plasma and cellular glutamine concentrations along with reduced mucosal glutaminase activity (Sido et al. 2006). These observations underlie our hypothesis that glutamine supplementation would improve the clinical outcomes of these diseases.

IBD induction in experimental animals highlighted that glutamine supplementation can protect the intestinal mucosa, supporting the possibility of its use in human patients. For example, in mice with dextran sulfate sodium (DSS)-induced colitis, oral glutamine supplementation (41.7 g/kg) for 10 days resulted in decreased severity of colonic inflammatory reactions (Hsiung et al. 2014), as well as increased expression of small-intestinal intraepithelial cells (Pai et al. 2014). In rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis that received dietary glutamine supplementation (20 g/kg or 40 g/kg) for two weeks, reduction in the production of pro-inflammatory cytokines, including TNF-α and IL-8, bacterial translocation, and inflamed lesions were evident (Ameho et al. 1997). In addition, oral glutamine supplementation (3%) in drinking water ameliorated abdominal radiation-induced mucosal injury and reduced the bacterial translocation in the gut mucosa of rats (Souba et al. 1990). Interesting results were reported about the effect of monosodium glutamate, which harbors glutamic acid, on the development and healing of the non-steroidal anti-inflammatory drug (NSAID) loxoprofen -induced small intestinal lesion in rats. Monosodium glutamate mixed with powdered food for 5 days before induction of intestinal lesions suppressed inducible nitric oxide synthetase expression and bacterial invasion. Moreover, the healing impairing effect of loxoprofen was depressed by 5% monosodium glutamate administration after induction of intestinal ulceration (Amagase et al. 2012; 2014).

Based on these positive findings in animal models, human studies have been conducted in an attempt to convey these results for improving disease status. Only a limited number of studies concluded that glutamine supplementation has a beneficial effect in intestinal diseases. However, a number of studies did not observe any improved outcomes from glutamine supplementation. Although some studies showed favorable effects, the clinical efficacy of glutamine supplementation in intestinal diseases remains a controversial issue. The "conditionally essential glutamine" theory is still a prediction and remains uncertain. Therefore, various experimental designs could help to assess the results of clinical studies. The first problem is that glutamine can be administered in two different ways: total parental nutrition and enteral nutrition. Generally, enteral nutrition is safer than parenteral nutrition for prolonged periods, whereas parenteral nutrition is often recognized as being better for achieving the targeted calorie requirement. Route of administration influences the contribution of glutamine (Boelens et al. 2006). In patients with acute ulcerative colitis, total enteral nutrition was shown to be nutritionally effective, as well as to produce fewer complications compared to parenteral nutrition (González-Huix et al. 1993). Given that total parenteral nutrition results in changes in intestinal morphology and function (Boelens et al. 2006), glutamine supplementation via parenteral nutrition might cause complications in the intestine. Second, a wide variety of doses, times, and modes of supplementation was used in previous studies. Doses of glutamine used in the studies varied up to 5-fold (Buchman et al. 1995), and treatment periods varied from two days (Noyer et al. 1998) to eight weeks (Beaugerie et al. 1997). Compared to the other periods, short-term glutamine administration during a flare-up phase could have a greater impact on outcomes (Noyer et al. 1998).

Conclusion

In this review, we described the roles of glutamine in the intestine, including the regulation of enterocyte proliferation, maintenance of tight-junction proteins, modulation of inflammatory pathways (e.g., NF-κB and STAT signaling), effect on intestinal microbiota, and protection against apoptosis and cellular stresses (Fig. 1). Even though previous *in vitro* and animal model studies showed

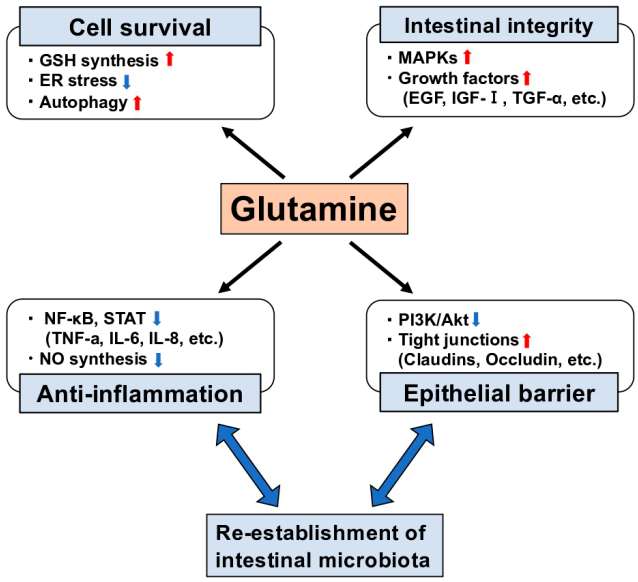

Fig. 1. The physiological effects of glutamine on the intestinal functions

significant beneficial effects and uncovered the mechanism by which glutamine alleviates intestinal inflammation, future research should focus on the use of glutamine supplementation in patients with intestinal diseases.

Conflict of Interest

The authors declare that there is no conflict of interest, either existing or potential.

Author Contributions

Nahla Hamouda performed the comprehensive literature search and wrote the paper. All authors participated in previously reported experimental testing of glutamine either *in vivo* or *in vitro*. Kikuko Amagase is the corresponding author of this paper and revised and edited the paper. All authors agree with the edited version.

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