Essential Role of Nonessential Amino Acid Glutamine in Atherosclerotic Cardiovascular Disease

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Atherosclerosis is a major disease that seriously harms human health and is known as the “number one killer” in developed countries and the leading cause of death worldwide. Glutamine is the most abundant nonessential amino acid in the human blood that has multifaceted effects on the body. Recent studies showed that glutamine is negatively correlated with the progression of atherosclerotic lesions. In this review, we focused on the relationship of glutamine with macrophage polarization, nitrification stress, oxidative stress injury, myocardial ischemia–reperfusion injury, and therapeutic angiogenesis to review its roles in atherosclerotic cardiovascular disease.

Keywords: glutamine, atherosclerosis, macrophages polarization, ischemia–reperfusion, angiogenesis

Introduction

CARDIOVASCULAR DISEASE (CVD) is the disorder of the heart and blood vessels including the coronary artery, kidney, and cerebral circulation, which is characterized by acute and chronic ischemic syndromes, such as acute coronary syndrome (ACS), angina pectoris, claudication, ischemic stroke, congestive heart failure, and sudden and nonsudden cardiac death (Libby *et al.*, 2018; Partida *et al.*, 2018). CVD is currently the number one killer in the society (Joseph *et al.*, 2017). In the past decades, identifying and reversing the pathogenesis of coronary heart disease (CAD) and preventing its complications remain a major challenge for CAD clinical management (Koton *et al.*, 2014).

Atherosclerosis (As) is the major cause of CAD (Rizzacasa *et al.*, 2019). It refers to the loss of elasticity of arteries and the accumulation of lipid and fibrous material in the arterial wall, narrowing of the arteries for the formation of atherosclerotic plaques (Wolf and Ley, 2019). With the progression of atherosclerotic lesions, plaques in the coronary arteries may block blood flow to the heart. The complete blockage of coronary artery and thrombus induced by plaque rupture can cause a heart attack and lead to massive cell death in the heart (Rigatelli *et al.*, 2019).

The pathophysiology of As is complicated. Recent studies revealed that amino acids are involved in the development of atherosclerotic lesions (Rom *et al.*, 2017). Glutamine is

the most abundant free amino acid in the human body (Huang *et al.*, 2017; Kim and Kim, 2017a). Li *et al.* (2018) showed that the knockout of P-selectin glycoprotein ligand-1 inhibits the initiation and progression of atherosclerotic lesions in low-density lipoprotein receptor (*LDLR*^{−/−}) mice accompanied by the changes in amino acid metabolism, which is characterized by increased glycine, valine, alanine, and glutamine. These results showed that glutamine is involved in the initiation and progression of atherosclerotic lesions. In this review, we present an overview of current evidence and functional roles of glutamine in atherosclerotic CVD and discuss the molecular pathways involved in atherosclerotic cardiovascular system.

The Resource and Function of Glutamine

Glutamine is considered as a conditional essential amino acid, which is synthesized by glutamic acid and ammonia through glutamine synthetase under normal physiological conditions (Wise and Thompson, 2010; Wu *et al.*, 2011). This molecule is the most abundant free amino acid and accounts for ~50% of the total free amino acids in the human body (Spodenkiewicz *et al.*, 2016). Diet is the most important source of glutamine (Labow and Souba, 2000). The normal fasting plasma glutamine concentration is 500–750 μM (Altman *et al.*, 2016).

Glutamine has multifaceted effects on the body. (1) It provides the body with necessary amount of nitrogen. The

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biosynthesis of other amino acids is heavily dependent on glutamine (Timmerman *et al.*, 2013). (2) Glutamine improves exercise endurance and can produce alkali, which prevents the decline of exercise capacity or fatigue caused by acidic substances to a certain extent (Coqueiro *et al.*, 2019). (3) Glutamine has important immunomodulatory effects that are required for lymphocyte secretion, proliferation, and maintenance of its function (Dos Santos *et al.*, 2017). (4) This molecule is involved in the synthesis of glutathione (GSH), which exerts strong antioxidative effect against stress damage (Hartmann *et al.*, 2017). (5) Glutamine is an essential nutrient for the metabolism of intestinal mucosal cells and maintains the integrity of the intestinal mucosal epithelial structure (Kim and Kim, 2017a). Under severe stress, such as trauma, infection, and fatigue, glutamine is rapidly depleted in the intestinal mucosal epithelial cells (Wang *et al.*, 2017). (6) This molecule is the substrate of the synthesis of proteins, lipids, and other macromolecular organisms and hence plays important roles in cells growth and proliferation (Fig. 1) (Showalter *et al.*, 2017; Ward, 2010; Son *et al.*, 2013; Bhutia *et al.*, 2015; Altman *et al.*, 2016; Mills *et al.*, 2016; Spinelli *et al.*, 2017).

Glutamine is heavily consumed and used for energy generation as carbon and nitrogen sources for biomass accumulation in rapidly dividing cells, such as lymphocytes, intestinal cells of the small intestine, and especially cancer cells (Zhang *et al.*, 2017). In this process, the glutamine requirement exceeds cellular production, and the cell must increase its intake of this molecule. Moreover, GSH con-

sumption is important in situations where catabolism is stressed, such as after surgery, injury, or sepsis (Singleton *et al.*, 2005; Lai *et al.*, 2014; Shu *et al.*, 2016).

Glutamine Promotes the Polarization of M2 Macrophages

Coronary plaque rupture and thrombosis are the most important pathological bases of ACS, which endangers patients' life with CAD (Bentzon *et al.*, 2014). Rupture of unstable plaque can lead to myocardial infarction (MI), which is the main cause of onset and death of ACS (Badimon and Vilahur, 2014).

The differentiation and activation of monocytes or macrophages are the key processes for the development and stability of As (Cochain and Zernecke, 2015). During this illness, monocytes migrate from the bloodstream into the subintima and differentiate into macrophages, which then undergo different polarization states after being stimulated by different signals (Kawano *et al.*, 2019; Liu *et al.*, 2019). The activation states of macrophages are divided into M1 (classical activation) and M2 (alternatively activation) polarizations (Murray *et al.*, 2014). *In vitro*, the activation of macrophages induced by lipopolysaccharide (LPS)+IFN- γ is the classical activation. However, the activation of macrophages induced by IL-4 is alternatively activation (Martinez and Gorgon, 2014; Orecchioni *et al.*, 2019). M1 macrophages induce a rapid proinflammatory response to infection and tissue damage by sensing the release of injury-related

FIG. 1. The important role of glutamine and glutamine-derived glutamate in biosynthesis. Gln, glutamine; SLC1A5, glutamine enters the mammalian cell through transporter (SLC1A5); GLS, glutaminase; GDH, glutamate dehydrogenase; GLDH1, glutamate dehydrogenase 1; α -KG, α -ketoglutaric acid; α -KG enters the TCA cycle to produce ATP; ACLY, ATP citrate lyase; Ac-CoA, acetyl-CoA, glutamine is reduced by carboxylation and reverse carboxylation produces acetyl-CoA to promote fatty acid production; Pro, proline; Asp, aspartic acid; BCAAs, leucine, isoleucine, valine, these amino acids contribute to protein together with nonessential amino acids (NEAAs: glycine, alanine, serine) produced by transaminase by glutamate synthesis. NEAAs, nonessential amino acids; TCA, tricarboxylic acid.

molecular patterns from microbial components, such as lipopolysaccharides (LPS) and damaged tissues. In contrast, M2 macrophages display anti-inflammatory and repair activities to resolve inflammation and promote tissue repair (Ivashkiv, 2013; Murray *et al.*, 2014). In general, M1 macrophages promote the progression of atherosclerotic plaque and its instability, whereas M2 macrophages inhibit the progression of atherosclerotic plaque and contribute to the stability of plaque (Jia *et al.*, 2017).

Glutamine accumulation leads to macrophage polarization (Jha *et al.*, 2015). A high glutamine uptake by the macrophage indicates a high anti-inflammatory macrophage population (Tavakoli *et al.*, 2017). Glutamine can affect M2 macrophage polarization through uridine 5'-diphosphate-*N*-acetylglucosamine biosynthesis and *N*-glycosylation (Jha *et al.*, 2015). Deprivation or inhibition of *N*-glycosylation by glutamine reduces the polarization of M2 macrophages and the production of C-C motif chemokine ligand 22 (CCL22) (Jha *et al.*, 2015). One-third of the carbon source and >50% of the nitrogen in the tricarboxylic acid (TCA) metabolites of M2 macrophages are derived from glutamine (Jha *et al.*, 2015). After the removal of this molecule, the expression of the specific markers of M2 macrophages, including interferon regulatory factor 4 (IRF4), kinesin family member 4 (*KIF4*), *CCL2*, and interleukin 4-induced 1 (*IL4i1*), and the TCA cycling activity are substantially downregulated. However, this regulation has no remarkable effect on the polarization of M1 macrophages (Jha *et al.*, 2015). Liu *et al.* (2017b) revealed that during glutamine deprivation, the specific markers of M1 macrophages (including *IL-1b*, *TNF- α* , *IL-6*, and *IL-12*) are upregulated, whereas those of M2 macrophages (including *Arg1*, *Ym1* [*Chil3*], *Retnla*, and *Mrc1*) are downregulated through α KG-jmjd3 pathway (Fig. 2). Jha *et al.* (2015) showed that one-third of all the carbon in TCA metabolites in M2 cells came from glutamine, and more than half of the nitrogen in UDP-GlcNAc synthesis came from glutamine, which also confirmed that this molecule plays a key role in M2 polarization.

These results showed that glutamine plays an important regulatory role and promotes the polarization of M2 macrophages, thus inhibiting the progression of atherosclerotic lesions and benefitting the stability of atherosclerotic plaques.

Glutamine Inhibits Nitrification Stress Caused by Excessive Nitric Oxide

Nitric oxide (NO) is produced by NO synthase (NOS), a family composed of neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (Iwakiri and Kim, 2015). In this synthesis process, nicotinamide-adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin, Ca^{+2} /calmodulin, and flavin adenine dinucleotide act as cofactors (Hong *et al.*, 2019). The most important roles of NO is as an endodermal vasodilator that is critical for cardiovascular protection (Ramirez-Carracedo *et al.*, 2018). NO also inhibits platelet aggregation and adhesion, which contributes to the downregulation of chemotactic protein MCP-1 and surface adhesion molecules, such as *CD11/CD18*, P-selectin, intercellular cell adhesion molecule-1, and vascular cell adhesion molecule-1 (Saini *et al.*, 2012). However, under abnormal conditions such as hypercholesterolemic and portal hypertension, the excessive NO reacts with superoxide and leads to the formation of nitrous oxide or proximities (ONOO); this phenomenon is known as nitrification stress and has serious toxic effects on islet B cells and intermuscular plexus neuron cells (Halliwell, 2006; Terra *et al.*, 2012; Yuste *et al.*, 2015).

The excessive NO activates mitochondrial apoptosis through a variety of pathways, thereby inducing oxidative stress and nitrifying stress, which is closely related to the formation of ONOO by superoxide reactions during the late stages of As (Isshiki *et al.*, 2002). In addition, the combination of NO with the increased superoxide production leads to the formation of harmful proximities, which indirectly (through free radical-mediated reactions) or directly (through oxidation reactions) induces lipid peroxidation and affects cell components and accelerates the formation of atherosclerotic lesions (Rizzo *et al.*, 2003). Consistent with these findings, Shi *et al.* (2002) showed that high NO concentration aggravates late-stage atherosclerotic lesions by modifying low-density lipoprotein (LDL) oxidation (Shi *et al.*, 2002). In the intermuscular plexus of tumor-bearing rats, NO-mediated nitrification stress injury was alleviated by glutamine (Vicentini *et al.*, 2017).

Endothelial cells (ECs) can synthesize glucosamine from glutamine and glucose. Glucosamine reduces the cellular

FIG. 2. Glutamine affects macrophage polarization. Glutamine deprivation upregulated the expression of M1-type macrophage marker genes. Glutamine promotes the polarization of M2-type macrophages through the akg-jmjd3 pathway, and the expression of M2-type macrophages-related genes are downregulated after the removal of glutamine.

level of NOS cofactor NADPH, which mediates the inhibitory effect of glutamine on endothelial NO synthesis. Beyond this process, glutamine involves in the conversion to glucosamine in hexosamine biosynthetic pathway, thereby inhibiting the activity of oxidative pentose phosphate pathway and reducing the availability of NADPH to inhibit NO synthesis (Guoyao *et al.*, 2001). Besides, glutamine inhibits the accumulation of ammonia-induced striatum cyclic-guanosine monophosphate and limits the supply of arginine for NO synthesis (Hilgier *et al.*, 2009). In addition, Gong *et al.* (2017) showed that oral glutamine with probiotics remarkably reduces NO content by downregulating the expression of iNOS through increasing methylation of the iNOS gene, thereby reducing the expression of the inflammatory factors *TNF- α* , *IL-6*, and *IL-8*, which contributes to the inhibition of intestinal inflammation and oxidative stress in Wistar rat burn models. The deletion of iNOS promotes cholesterol efflux through increasing the expression of liver X receptor α , ATP-binding cassette transporter A1, and scavenger receptor class B type I, which retards the progression of As in *apoE*^{-/-} mice (Zhao *et al.*, 2014).

Glutamine Blocks Oxidative Stress Injury Induced by Reactive Oxygen Species

Oxidative stress is characterized by an imbalance between the production and elimination of cellular oxidants, which damage the basic components of cells (including proteins, lipids, and DNA), interfere with homeostasis, and lead to various adverse consequences (Schieber and Chandel, 2014; Wu and Ni, 2015). Oxidative stress induces vascular endothelial cell (VEC) injury and is the main factor of As (Forstermann *et al.*, 2017). Atherosclerotic lesion is a high oxidative stress state characterized by the accumulation of oxidized lipids and proteins in blood vessel walls (Sun *et al.*, 2013). Reactive oxygen species (ROS), a marker of oxidative stress, is mainly produced in the mitochondrial electron transport chain during cell metabolism and plays important roles in cell biological behaviors, such as proliferation and apoptosis (Cameron *et al.*, 2019). Recent studies showed that ROS increases the pyroptosis of VEC that contributes to VEC dysfunction and As progression (Wu *et al.*, 2018; Zhaolin *et al.*, 2019). Lowering oxidative stress injury induced by ROS is considered as a promising therapy to inhibit the progression of As (Burtenshaw *et al.*, 2019).

GSH is a major endogenous cellular antioxidant that plays an important role in cellular responses to oxidative stress by neutralizing free radicals and ROS. Glutamine produces GSH and GSSG “buffers” of oxidative stress (Ballatori *et al.*, 2009). Decreasing glutaminase and consequently inhibiting glutamine production can downregulate this important antioxidant pathway, leading to high ROS levels and toxic effects on cells (Abu *et al.*, 2017). Glutamine starvation has been reported to deplete endogenous levels of the antioxidant GSH, promote oxidative stress in HuH-7 cells, and induce apoptosis (Yang *et al.*, 1998). ECs are highly susceptible to ROS-induced damage because of glutamine consumption (Huang *et al.*, 2017). In addition, glutamine-starved ECs are more sensitive to ROS-induced cell death (Huang *et al.*, 2017). Similarly, glutamine-depleted ECs show a tendency to decrease the overall level of GSH (Huang *et al.*, 2017). Thus, glutamine supplementation re-

duces mitochondrial ROS formation and apoptosis induced by high glucose and hypoxia reoxygenation by decreasing oxidative stress and inactivating intrinsic apoptosis pathways. This process is mainly dependent on the enhanced GSH synthesis (Li *et al.*, 2015).

Glutamine Improves Myocardial Ischemia-Reperfusion Injury with Antioxidative Stress

Ischemic heart disease is the leading cause of death worldwide (Mozaffarian *et al.*, 2015). Myocardial ischemia, known as MI, is mostly caused by As and usually causes a large number of cardiac cell death. At present, myocardial ischemia-reperfusion strategies, such as thrombolytic therapy or direct percutaneous coronary intervention, remain the standard and most effective treatment for acute MI because of the limited regenerative capacity of the heart (Laflamme and Murry, 2011). However, the process of myocardial reperfusion can itself induce further cardiomyocyte death, a phenomenon known as myocardial reperfusion injury (I/R) (Hausenloy and Yellon, 2013). I/R injury is mainly caused by excessive oxygen free radicals that are generated during reperfusion and trigger strong proinflammatory response, leading to further myocardial damage (Zhou *et al.*, 2015).

Oxidative stress is the main mechanism leading to myocardial I/R injury. Hence, antioxidative stress is an important strategy against such injury. GSH is an important endogenous antioxidant that has a prominent contribution against oxidative stress (Hartmann *et al.*, 2017). Lian *et al.* (2018) showed that glutamine improves the oxidative stress response induced by myocardial I/R by enhancing GSH synthesis. The study reported that the glutamine metabolism in rat heart is inhibited by I/R, and pretreatment with glutamine reduces I/R injury and MI area with I/R injury. MicroRNA-200c (miR-200c) aggravates cardiac I/R injury, whereas miR-200c directly targets glutamine to inhibit glutamine metabolism and promote intracellular ROS accumulation (Liu *et al.*, 2017a). Khogali *et al.* (1998) showed that glutamine enhances the recovery of acute ischemia in isolated rat hearts in a dose-dependent manner. Zhang *et al.* (2011) found that isolated rat hearts with I/R injury treated with glutamine resulted in the complete recovery of the cardiac output. Glutamine enhances heat shock protein 72 expression, which attenuates intracellular oxidant production, protects cardiomyocytes from I/R damage, and remarkably increases myocardial cell survival and recoveries of systolic function after I/R injury (Zhang *et al.*, 2011). These results showed that glutamine protects against I/R injury through the antioxidant pathway.

Glutamine Improves MI by Promoting Therapeutic Angiogenesis

Acute MI is a serious ischemic disease. With the introduction of new drugs and the improvement of treatment regimens, the short- and long-term mortality rates of acute MI have been remarkably reduced. The promotion of angiogenesis in ischemic regions has been recently used to treat ischemic diseases and may provide new treatment for CVDs (Kumagai *et al.*, 2018; Lee *et al.*, 2019).

EC metabolism is a regulator of angiogenesis (Huang *et al.*, 2017). Glutamine is the most consumed amino acid by

FIG. 3. Glutamine controls various metabolic functions in EC. ECs are a single layer of flat epithelial cells on the surface of blood vessels that come into direct contact with blood. Endothelial dysfunction was found to be the initiating factor of atherosclerosis. (1) Glutamine deprivation depletes the intracellular TCA metabolite pool but does not change the intracellular ATP level. Therefore, glutamine deprivation does not cause an energy crisis, possibly because EC produces most of the energy through glycolysis. (2) Glutamine starvation reduced the storage of glutamate (Glu), aspartic acid (Asp) and asparagine (Asn) in ECS, but did not affect the expression levels of alanine (Ala), serine (Ser) and glycine (Gly). (3) Glutamine deprivation reduces protein, nucleotide, and lipid synthesis. (4) Glutamine deprivation increased intracellular ROS levels and decreased glutathione expression. ECs, endothelial cells; ROS, reactive oxygen species.

vasodilation (Fig. 3) (Kim *et al.*, 2017b). Deprivation of glutamine in ECs or inhibition of glutaminase 1 (GLS1) leads to defects in vessel germination because of impaired proliferation and migration, indicating that glutamine metabolism is essential for EC to reach the tip of the vessel bud (Huang *et al.*, 2017; Kim *et al.*, 2017b). Asparagine is a downstream metabolite of glutamine; inhibition of asparagine GLS1 and deprivation of asparagine with glutamine are lethal for ECs (Li *et al.*, 2017). Supplementation with asparagine can promote the proliferation of ECs after glutamine deprivation. The combination of α -KG and nonessential amino acids (NEAAs) completely rescues the proliferation defects of glutamine-deficient EC. Asparagine is the only NEAA needed for complete recovery. Consistent with its proliferation-rescue effect, the α -KG plus asparagine combination restores intracellular dNTP and rescues all other glutamine-dependent phenotypes, including mTORC1 signaling, protein synthesis, ER stress response, and REDOX imbalances (Fig. 3). These data highlight the importance of asparagine and α -KG supplementation for the metabolism and cellular changes of glutamine-deprived ECs (Huang *et al.*, 2017).

Conclusions and Perspectives

Glutamine regulates macrophage polarization, oxidative stress, nitrification stress, and angiogenesis and might contribute to the delayed progression of atherosclerotic lesions and the prevention of MI injury. However, some adverse effects of glutamine on CAD are also reported. Rom *et al.*

(2017) showed that glutamine promotes the accumulation of macrophage triglyceride by enhancing the uptake of LDL and very LDL. Shah *et al.* (2010) showed that peripheral blood glutamine levels in patients with CAD are higher than that in healthy individuals. Therefore, deeper understanding of the relationship between glutamine and As is necessary to clarify the role of this molecule in the development of atherosclerotic lesions and plaque stability. Moreover, investigation of the underlying mechanism of glutamine in atherosclerotic CVDs will help develop novel strategies for the prevention and treatment of such diseases.

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