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Research Progress on the Correlation between Hyperhomocysteinemia and Arteriosclerosis Obliteran

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ABSTRACT

Arteriosclerosis Obliteran (ASO) is a local manifestation of systemic arteriosclerosis in the lower extremity arteries. ASO is a chronic progressive disease caused by arteriosclerosis, such as intimal thickening, lumen stenosis or occlusion of the blood supply arteries of the lower limbs, insufficient blood supply of the diseased limbs, and clinical manifestations such as intermittent claudication, decreased skin temperature, pain, and even ulceration or necrosis of the lower limbs. Its pathogenesis is complex, the treatment is difficult and has gradually attracted extensive attention in clinic. In recent years, more and more studies have shown that hyperhomocysteinemia is an independent risk factor of atherosclerosis, but there is no clinical report on hyperhomocysteinemia and ASO. Therefore, this paper reviews the correlation between hyperhomocysteinemia and ASO, in order to provide theoretical basis and support for the follow-up study of ASO.

INTRODUCTION

Arteriosclerosis Obliteran (ASO) refers to a chronic progressive disease,
arteriosclerosis causes intimal thickening, lumen stenosis or occlusion of blood supply arteries of lower limbs, and insufficient blood supply of diseased limbs, clinical manifestations such as intermittent claudication, decreased skin temperature, pain, and even ulceration or necrosis of the lower limbs. ASO is often the local manifestation of systemic arteriosclerosis vascular disease in the lower limbs, and its pathogenesis is complex, the treatment is difficult and has gradually attracted extensive attention in clinic. At present, there are many studies on the relevant risk factors of ASO in the clinic, including smoking [1-3], diabetes [4,5], hypertension [6], inflammatory response [7,8], etc. The recent guidelines clearly pointed out another independent risk factor of ASO - hyperhomocysteinemia, and explained that the combined probability of hyperhomocysteinemia in ASO patients is significantly higher than that in the general population [9]. Since McCully [10] first proposed that hyperhomocysteinemia may lead to atherosclerotic vascular disease in 1969, more and more studies have shown that hyperhomocysteinemia is an independent risk factor of peripheral vascular atherosclerotic disease, but there is no clinical report on hyperhomocysteinemia and ASO. Therefore, this paper reviews the correlation between hyperhomocysteinemia and ASO, in order to provide theoretical basis and support for the follow-up research of ASO.

Clinical Manifestations of ASO

ASO is a local manifestation of systemic arteriosclerosis in the lower limbs, and its lesion location mainly occurs in the large and medium arteries [11]. According to the lesion scope of ASO, it can be divided into three types; Type I: Main iliac artery type. The lesion site mainly involves the bifurcation of abdominal aorta and common iliac artery. In clinic, it is characterized by intermittent claudication and sexual dysfunction; Type II: Main iliac femoral artery type, involving the bifurcation of aorta, proximal femoral artery, common iliac artery and external iliac artery. The main symptom is intermittent claudication of lower limbs; Type III: multi segment occlusion, which is the most common type in clinic. The lesions mostly occur in a wide range from aortic bifurcation to tibial peroneal artery, and are mainly manifested by plane obstruction or stenosis [12-14].

The disease often occurs in the middle-aged and elderly people over 40 years old. In the early stage of the disease, there is no obvious manifestation of limb ischemia, or only sometimes feel mild limb numbness, and most elderly patients feel slow, they often mistake the early symptoms or signs as the manifestation of aging or other diseases, and then miss the best opportunity for intervention [15]. With the gradual aggravation of the degree of ischemia, intermittent claudication will gradually appear, followed by ischemic resting pain, ulcer, gangrene, Acute Limb Ischemia (ALI), and even amputation [9].

Source and Metabolism of Homocysteine (Hcy)

Source

The scientific name of Hcy is called 2-amino-4-mercaptobutyric acid, Hcy does not exist naturally, it is an important intermediate product produced in the metabolism of methionine and cysteine and a non-essential amino acid for human body, methionine is an essential amino acid of human body. Methionine obtained from food reacts with Adenosine Triphosphate (ATP) under the catalysis of adenosine transferase to produce S-adenosyl methionine (SAM), and then SAM removes methyl under the action of methytransferase to form S-adenosyl homocysteine (SAH), Further, SAH removes adenosine and becomes Hcy under the action of hydrolase [16].

Metabolism

Hcy is metabolized in four ways:

1. Methyltransfer pathway: Under the action of Methionine synthase (MS), Hcy uses Vitamin B12 (VitB12) as coenzyme and N5-methyltetrahydrofolate as methyl donor to produce methionine. N5-methyltetrahydrofolate is changed into tetrahydrofolate (FH4), under the action of Methylene tetrahydrofolate Reductase (MTHFR), with VitB12 as coenzyme, N5-methyltetrahydrofolate is reformed and participates in the metabolism pathway of Hcy again. This process can be carried out in all somatic cells;

2. Betaine contains three unstable methyl groups, under the action of Betaine homocysteine methyltransferase (BHMT), Hcy is methylated to methionine, and betaine becomes Dimethylglycine (DMG). This process is mainly carried out in the liver and kidney, and about 50% of Hcy is metabolized through the above two pathways;

3. Sulfur transfer pathway: Under the action of Cystathionine β-synthase (CBS), Vitamin B6 (VitB6) is used as coenzyme, Hcy combines with serine to form cystathionine, cystathionine is hydrolyzed to form cysteine and α-Ketobutyric acid, which is irreversible, it can be carried out in all somatic cells, and about 50% of Hcy is metabolized through this process;

4. After a small amount of Hcy is synthesized in cells, it is directly released into the blood to participate in circulation [17].

Hyperhomocysteinemia (HHcy)

HHcy refers to the continuous higher than normal level of fasting plasma total Hcy due to various genetic or acquired factors. The causes of HHcy are as follows [18-21]:

Source and Metabolism of Homocysteine

Hcy can be metabolized through four ways: Methyltransfer pathway, Betaine pathway, Sulfur transfer pathway, and direct release into blood circulation. As a result, ASO patients are associated with hyperhomocysteinemia, but no clinical report has shown a direct association between hyperhomocysteinemia and ASO.
(1) Nutritional factors: folate, VitB6, VitB12 and other factors play an important role in Hcy metabolism. These factors can directly or indirectly interfere with renal function or affect enzyme activity and participate in Hcy metabolism as cofactors [22];

(2) Genetic factors: The decreasing of heat tolerance and activity of key enzymes in the metabolic process such as CBS and methionine synthase will affect the metabolism of Hcy and lead to HHcy;

(3) Drugs: 3-isobutylmethylxanthine, methotrexate, phenytoin, contraceptives, azauridine triacetate and carbamazepine can interfere with the metabolism of folic acid, VitB6 and VitB12, resulting in the increase of Hcy concentration;

(4) Chronic diseases: Renal insufficiency, hypertension, diabetes, hypothyroidism, liver disease, psoriasis, etc. can affect the normal metabolism of Hcy;

(5) Others: obesity, smoking, anemia and old age can also cause the increase of Hcy concentration in plasma.

The plasma Hcy concentration of normal adults during fasting is 5-15 μM/L, if the concentration of Hcy in plasma continues to be higher than 15 μM/L is HHcy. According to the HHcy level, it can be divided into mild, moderate and severe: the Hcy concentration is 15-30 μM/L is mild; 30-100 μM/L is moderate; Greater than 100 μM/L is severe [23].

Study on the Pathogenesis of HHcy on ASO

Hcy is a toxic amino acid containing sulfhydryl [24]. It is an amino acid that can reflect vascular damage. It has become another new independent risk factor for arteriosclerosis after traditional factors such as hypertension, diabetes and dyslipidemia [25]. In recent years, more and more studies have shown that the increase of Hcy is a risk factor for arteriosclerosis [26-28]. Among them, vascular endothelial dysfunction is the initiating factor of ASO formation [29]. Improving vascular endothelial function and reducing endothelial injury can delay the process of ASO to a certain extent [30]. The mechanism of Hcy leading to endothelial dysfunction may include the following aspects:

Mitochondrial dysfunction

Mitochondrial dysfunction plays an important role in the process of vascular endothelial cell injury and apoptosis caused by Hcy [31]. Zhang Z, et al. [32] found that HHcy level can cause mitochondrial function imbalance, activate endoplasmic reticulum stress response of endothelial cells and promote apoptosis. At the same time, Yang F, et al. [33] found that Hcy inhibited the activity of mitochondria to damage to vascular endothelial cells, inhibited the activity of Cytochrome c oxidase (COX), increased the level of reactive oxygen species in cells and enhanced the apoptosis of endothelial cells, and this process can be restored by folic acid. Hcy can also promote the production of peroxide, inhibit the gene expression of antioxidant enzyme bundle factor, improve the toxicity of peroxide and cause damage to vascular endothelial cells. At the same time, it induces the oxidative stress response in vivo, resulting in the decrease of the activity of Glutathione peroxidase (GSH–PX), the dysfunction of cell mitochondria, and then accelerates platelet aggregation [33,34].

Nitric Oxide (NO) synthesis disorder

NO is an important cytokine with vasodilation produced by endothelial cells, arginine generates NO under the catalysis of Nitric Oxide Synthase (NOS). It is found that high concentration of Hcy will reduce the expression of endothelial Nitric Oxide Synthase (eNOS) and anti phosphorylated eNOS antibody (p–enos) [35], reduce endogenous NO and reduce or even disappear the vasodilation function of NO [36]. In addition, HHcy can induce oxidative stress response, aggravate the inflammation of vascular endothelial cells, further reduce the production and bioavailability of NO, and lead to endothelial dependent vasodilation dysfunction, which will further trigger vascular inflammatory response and stimulate the production of cytokines and vasoactive substances in vascular wall [37]. When vascular endothelial cells are damaged to a certain extent, it will in turn inhibit the production of NO, resulting in that NO cannot effectively inhibit the oxidation of Hcy, form a vicious circle and accelerate the development of ASO [38,39].

Imbalance of hydrogen sulfide signal pathway

Hydrogen sulfide is an endogenous signal molecule that participates in the fine regulation of endothelial cell homeostasis and plays a beneficial role in different signal transduction pathways [40]. Hydrogen sulfide can inhibit Nuclear factor kappa-B (NF-κB) pathway, activate potassium and calcium channels and inhibit vascular inflammation. At the same time, hydrogen sulfide can reduce the level of Reactive Oxygen Species (ROS) in endothelial cells, up regulate the expression of a variety of antioxidant enzymes (such as Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Glutathione S-transferase), mediate the relaxation of blood vessels, and can be used as an endogenous stimulator of angiogenesis, maintain the homeostasis of the vascular system. Therefore, it has been found that in mice with HHcy, after hydrogen sulfide donor treatment, the plaque area in the artery decreased, macrophage infiltration decreased and serum Hcy level decreased [41]. It is indirectly verified that the imbalance of hydrogen sulfide signal pathway is one of the pathogenesis of arteriosclerosis.

Oxidative stress response

6.4.1. Enhanced oxidative stress response: Hcy is mainly produced in the methionine metabolism process. It is a kind of chlorinated amino acid. After being oxidized, Hcy can
produce some substances that destroy vascular endothelial cells (such as peroxides, oxygen free radicals, etc.). The increase of these substances will produce oxidative stress reaction, damage the structure and function of vascular endothelial cells, destroy biofilm, and cause monocyte macrophages to turn into foam cells [42,43], which will lead to thickening of vascular lumen and reduction of vascular wall elasticity. Finally, it leads to stenosis and occlusion of vascular lumen [44]. At the same time, peroxide can react with NO to produce peroxynitrite [45]. Both peroxide and peroxynitrite can mediate tissue oxidation and modification, leading to the formation of lipid peroxide, thus inhibiting the normal vasodilation response [46].

Hcy can up regulate the expression of endoplasmic reticulum stress marker proteins BIP and chop in vascular endothelial cells, and induce the expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1) through endoplasmic reticulum stress, thereby damaging vascular endothelial function [47], leading to vascular endothelial dysfunction [48]. Hcy induced oxidative stress response, endoplasmic reticulum stress response, promoted the expression of death receptors and other mechanisms can destroy the protective barrier of endothelium, reduce the release of vasodilators and increase the generation of vasoconstrictors, resulting in increased vascular tension and decreased vascular compliance, induce platelet adhesion, lipid deposition, inflammation and other processes, and finally form atherosclerosis [49].

Regulation of ROS generation: Hcy has its own special structure, which contains highly active sulfhydryl groups. Its own oxidation can lead to inflammatory reaction, resulting in a large number of ROS in cells and a series of downstream stress reactions (such as endoplasmic reticulum stress, oxidative stress, methylation, etc.), damaging the integrity and connectivity between endothelial cells. At the same time, macrophages release a large number of inflammatory factors and platelet activation induces the formation of thrombomoduline, eventually lead to atherosclerosis [50].

The most physiologically significant ROS are hydroxyl radical, superoxide anion and hydrogen peroxide. ROS and hydrogen peroxide are important activator of NF-κB signaling pathway, it can enhance the pro-inflammatory response related to Hcy. At the same time, Hcy can directly or indirectly increase the level of hydrogen peroxide and promote the oxidation of Low-Density Lipoproteins (LDL). This process is very important for the occurrence and development of arteriosclerosis. In addition, Hcy is an effective excitatory neurotransmitter, which can bind to N-methyl-D-aspartate receptor, accelerate the influx of cytoplasmic calcium and induce endothelial cell apoptosis [51]. High levels of Hcy can also lead to the production of hydroxyl free radicals, peroxides and other oxygen free radicals in vascular endothelium, reduce the ability of the body to scavenge oxygen free radicals, destroy elastic fibers and collagen fibers in vascular structure [52], and stimulate the proliferation of Vascular Smooth Muscle Cells (VSMCs). The above process is one of the independent risk factors leading to vascular sclerosis [53].

Weakening the antioxidant system: Many studies believe that the mechanism of Hcy weakening the antioxidant system is as follows: Hcy has cytotoxic effect on vascular endothelium itself, which will lead to the damage of vascular endothelial cells and the change of vascular cell function, and induce the apoptosis of vascular endothelial cells [54,55]. At the same time, HHcy will slow down the scavenging rate of oxygen free radicals and enhance the accumulation of peroxide, which will also have a toxic effect on endothelial cells. HHcy can also inhibit the expression and secretion of extracellular SOD, lead to the damage of antioxidant mechanism, and then trigger oxidative stress response [56].

There are two kinds of antioxidant systems in cells. One is enzymes that can neutralize ROS and reduce oxidative stress response, such as SOD, catalase, GPX, peroxide reductase, etc. The other is antioxidants, such as ROS scavengers such as glutathione and vitamins. Glutathione is an important cellular antioxidant. HHcy is related to the decrease of glutathione [57], and it is also related to the decrease of the expression of VittBl2, Vitamin E (VitE) [58], GPX (GPX-1, gpx-2) and SOD (SOD1, SOD2) [59]. The above processes show that the weakening of antioxidant system plays an important role in the pathogenesis of ASO induced by Hcy.

Dyslipidemia

Studies have confirmed that Hcy induced arteriosclerosis is also closely related to abnormal blood lipid metabolism, which is one of the important factors causing atherosclerosis. There is a close relationship between high Hcy level, abnormal blood lipid and atherosclerosis [60].

After the vascular wall is stimulated, endothelial cells are damaged, resulting in the release of inflammatory factors, promoting the adhesion and aggregation of inflammatory cells at the vascular endothelial injury, and inducing the occurrence of acute inflammation of vascular wall [61,62]. With the prolongation of the damaged time of the vascular wall, it gradually evolved into a chronic inflammatory reaction of the vascular wall. HHcy can induce a large number of LDL to gather at the lesion site. At the same time, monocytes in the blood gradually infiltrate into the lesion site. Monocytes will stretch out pseudopodia and differentiate into macrophages. Macrophages will become foam cells after swallowing a large amount of LDL at the lesion site [63,64]. At the same time, the degradation products of lipoprotein triacylglycerol, cholesterol and cholesterol esters will also be swallowed by monocytes to form foam cells and accelerate the process of atherosclerosis [65,66]. While foam cells are characteristic pathological cells in atherosclerotic plaques [67], a large number of foam cells accumulate under the vascular intima to form atherosclerotic plaques, which is a key link in the occurrence of atherosclerosis related diseases.
It is found that Hcy is closely related to the abnormal metabolism of LDL [68]. LDL, especially oxidized Low Density Lipoprotein (ox-LDL), plays an important role in the formation of ASO. After endothelial cells are damaged by Hcy, lipids (mainly LDL) in blood infiltrate into the vascular wall and are modified by ROS to generate ox-LDL. Monocytes phagocytize ox-LDL and turn into macrophages. Macrophages then phagocytize lipids to form foam cells and form the core of lipid plaque on the arterial wall. At the same time, Hcy can also change the lipid metabolism of the liver, increase the uptake of ox-LDL by macrophages, promote the accumulation of cholesterol and triglycerides in the vascular wall, and accelerate the occurrence and development of diseases [69].

As a sulfur-containing amino acid, the sulphydryl group in the structure of Hcy can promote the production of oxygen free radicals and hydrated peroxides, promote the production of Low Density Lipoprotein (LDL) and inhibit its ability to reverse transport cholesterol, which increases the formation and expansion of lipid necrosis core and promotes the transformation of arterial plaque to an unstable state [71]. At the same time, it will consume a large amount of HDL in the process of metabolic treatment [72], resulting in arterial vascular blockage. At the same time, LDL accumulation caused by HHcy aggravates this blockage. Endothelial dysfunction caused by nutrient artery blockage will lead to vascular endothelial cell swelling, which further hinders LDL from passing through narrow nutrient vessels. The two factors interact Cause and effect each other, form malignant circulation and induce the formation of fragile plaque [73].

Hcy’s own endothelial cytotoxicity induces the aggregation of LDL-C and the formation of oxygen free radicals [74]. Oxygen free radicals can induce inflammatory response, damage vascular endothelial cells, promote the proliferation of vascular smooth muscle cells and the accumulation of apolipoproteins on vascular walls [75]. At the same time, the metabolites of Hcy will combine with LDL-C to form a dense lipoprotein, which accumulates in the smooth muscle cells, subintima, collagen fibers and elastic fibers of the arterial wall, and further promote the proliferation of VSMCs [76].

In contrast to LDL, the plasma level of HDL is negatively correlated with arteriosclerosis. Hcy can reduce the levels of Lecithin Cholesterol Acyl Transferase (LCAT) and Cholesterol Ester Transfer Protein (CETP), reduce the transport efficiency of HDL, reduce the activity of Paraoxonase 1 (PON1) in HDL particles, reduce the anti-inflammatory and antioxidant capacity of HDL, and promote the occurrence and development of arteriosclerosis [77]. At the same time, Qinfeng Z, et al. [78] found that Hcy may also weaken the antioxidant function of HDL by reducing the level of paraoxonase, thereby interfering with the anti-atherosclerotic effect of HDL.

**Dysfunction of Vascular Smooth Muscle Cells (VSMCs)**

The possible pathogenesis of ASO caused by VSMCs dysfunction induced by Hcy is as follows:

1. Enhance the expression of caveolin, and the amino acid residues in its structure form a complex with tyrosine phosphorylated eNOS [79], which can reduce the synthesis and release of NO and promote the migration and proliferation of VSMCs;

2. Enhance the expression of key proteins and molecules in phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signal pathway and induce the migration and proliferation of VSMCs [80];

3. Affect the activities of osteopontin, cardiac myosin polypeptide and calmodulin, make the morphology of VSMCs round and smaller, and then accelerate the proliferation of VSMCs, secrete a large amount of extracellular matrix, and promote the formation of vascular atherosclerosis [81];

4. Induce VSMCs to secrete Matrix metalloproteinase-2 (MMP-2) and affect the dynamic balance of intracellular and extracellular matrix [82];

5. High concentration of Hcy can reduce the mitochondrial membrane potential, reduce the activity of mitochondrial ATP synthase, open the mitochondrial pathway of cell apoptosis and induce the apoptosis of VSMCs [83];

6. Gene level: Ma SC, et al. [84] found that Hcy affects the methylation of atherosclerosis related genes, which may be the characteristic expression of Hcy induced VSMCs proliferation, which also provides a new perspective for Hcy induced VSMCs proliferation and atherosclerosis. Hcy can inhibit the activity of methyltransferase and cause DNA hypomethylation, affect gene expression and lead to the proliferation of smooth muscle cells [85]. In addition, the newly produced mRNA in vascular smooth muscle cells is also closely related to HHcy. They can make VSMCs in arterial wall apoptosis rapidly, and promote a large number of arterial endothelial cells to fall off, thus accelerating the process of atherosclerosis [86]. MiRNA is a component of epigenetics and is also involved in the proliferation of VSMCs induced by Hcy. For example, miR-143 plays an important role in the transformation of VSMCs from differentiation phenotype to proliferation phenotype. MIR-143 is mainly expressed in vascular wall smooth muscle cell layer and plays a key role in maintaining VSMCs contraction phenotype and regulating VSMCs differentiation. Xiaoling Y, et al. [87] found that when Hcy induced the proliferation of VSMCs, the expression of miR-143 increased significantly. When miR-143 inhibitor was added, it could significantly...
inhibit the proliferation of VSMCs, indicating that miR-143 is the key miRNA in the process of Hcy induced VSMCs proliferation;

(7) The proliferation of VSMCs will weaken the elasticity of vascular smooth muscle and accumulate a large number of coagulation factors and platelets on the surface, leading to the formation of thrombosis. High level Hcy can also up regulate the expression of Platelet-Derived Growth Factor (PDGF), strengthen the mitogenic effect of PDGF, and then enhance the effect of PDGF on stimulating the proliferation of VSMCs [88];

(8) Hcy can lead to VSMCs proliferation, migration and aggregation, arterial wall thickening, lumen stenosis and occlusion; Hcy induces endothelial cell injury and hypertrophy, and a large number of fibrous tissues gather, which leads to the rupture of vascular elastic membrane [89]. The collagen fibers of arterial wall are damaged, and the elasticity [90] and compliance of vascular wall are weakened, thus promoting the occurrence of arteriosclerosis [91];

(9) Others: the proliferation and migration of VSMCs are the key factors causing the thickening of vascular inner wall and the formation of arteriosclerosis. Hcy can not only cause cell oxidative stress response, damage vascular endothelial cells and affect the relaxation function of endothelial cells, but also reduce the repair ability of endothelial cells, stimulate the proliferation of VSMCs and cause lumen stenosis and vascular wall remodeling [92]. Chiang JK, et al. [93] found that Hcy can interfere with the expression of cyclins A and D1 in human umbilical cord vascular smooth muscle cells and induce the proliferation of VSMCs. After removing cyclins A and D1, the induction of Hcy will also be inhibited. It indirectly verified that Hcy is closely related to the occurrence and development of peripheral arteriosclerosis [94,95].

Coagulation and fibrinolysis system dysfunction

Hcy can affect the process of coagulation and fibrinolysis and accelerate the formation of thrombosis [96–98]. Enhance the tendency of thrombosis in the body, cause the degradation of arterial elastic fibers and collagen fibers, and then cause the decline of arterial elasticity and compliance, and promote the occurrence and development of arteriosclerosis [99,100]. The pathogenesis is as follows:

**Hcy and platelets**: Platelet aggregation is a key factor in the process of thrombosis. The mechanisms of Hcy promoting platelet aggregation may be as follows:

1. Hcy hinders platelet synthesis of nitric oxide and enhances platelet activity and aggregation [111];
2. Hcy produces substances with strong oxidation, enhances the release of Ca2+ from platelets and guides the aggregation of platelets;
3. Activate arachidonic acid and produce extremely unstable Thromboxane A2 (TXA2) under the action of thromboxane synthase. TXA2 is an important inhibitor of adenylyl cyclase, which can promote platelet aggregation and constrict blood vessels [112–114];
4. Increased platelet adhesion and aggregation induce increased thrombosis [115]. It greatly increases the probability of thrombosis [116,117].

**5,10-methylenetetrahydrofolate reductase (MTHFR)**

Hcy is an independent risk factor for atherosclerotic vascular disease and has an obvious quantitative relationship with atherosclerotic lesions. The study found that the level of Hcy is affected by MTHFR in vivo. MTHFR is a key enzyme in the folate methionine metabolic pathway, which can maintain the normal level of Hcy in the body. When MTHFR gene is mutated, it will lead to the decrease of MTHFR enzyme activity and thermal stability, which will lead to Hcy metabolic disorder and HHcy [118,119]. It was found that C677T mutation of MTHFR gene can increase plasma Hcy level by inhibiting enzyme activity [120]. At present, it is found that MTHFR gene has more than 30 mutation types, among which the most common mutation is MTHFR gene C677T. Its genotype is divided into CC, CT and TT. When TT mutation occurs, MTHFR enzyme activity will decrease by 60%, and CT heterozygous mutant enzyme activity is between TT and CC enzyme activities. At present, many studies have shown that MTHFR gene C677T polymorphism is related to plasma Hcy level [121]. For example, studies by Cheng Dan [122] and others have shown that CT and TT genotypes in MTHFR gene
are associated with arteriosclerosis. Li, et al. [123] found that the level of Hcy in patients with TT type of MTHFR gene was significantly higher than that of CC type and CT type. At the same time, the degree of arterial disease in patients with TT type was more serious than that in the other two groups. Ni J, et al. [124] observed the correlation between MTHFR gene C677T polymorphism and plasma Hcy level and found that the probability of high Hcy in TT genotype population was significantly higher than that in CT and CC genotype population.

**Affecting gene methylation**

Hcy comes from methionine and has the function of synthesizing S-adenosylmethionine (SAM) and S-adenosine homocysteine (SAH). SAH, as the precursor of Hcy, can be transformed into Hcy under the action of SAH hydrolase, and the reaction is reversible. The increase of Hcy level can enhance the synthesis of SAH and lead to the increase of Hcy. Li Li, et al. [125] found that the plasma Hcy level and SAH level of patients with arteriosclerosis were significantly increased. Correlation analysis showed that Hcy was positively correlated with SAH. SAM is the only methyl donor composed of one carbon unit and participates in more than 100 different methyl transfer reactions in vivo, including DNA and RNA methylation. It is found that SAM and SAH play an important role in the process of genomic DNA methylation of atherosclerosis caused by Hcy, and they are expected to be used as new biological diagnostic indicators of vascular atherosclerosis [126].

DNA methylation refers to the under the action of DNA methyltransferase, with SAM as methyl donor, introduce activated methyl on the fifth carbon atom of cytosine, and modifies DNA on the basis of unchanged DNA base sequence, so as to regulate gene expression. Studies have shown that HHCy can cause DNA methylation modification and promote the formation of atherosclerosis [127]. High levels of Hcy in serum will weaken gene methylation, promote DNA damage, weaken cell development and differentiation, and reduce the transmethylation reaction in vivo. It is found that Hcy induced atherosclerosis is closely related to the induced hypomethylation state in blood vessels, and this process is partly mediated by the regulation of DNA methylation of oxidized Low Density Lipoprotein receptor-1 (LOX-1) gene [128,129]. Hcy induced VSMCs proliferation may also be caused by the methylation of atherosclerosis related genes and the methylation state of genes mediating cell proliferation, which may also be one of the mechanisms of Hcy induced methylation and then involved in the formation of atherosclerosis [130].

Scavenger Receptor Class-A (SR-A) is a glycoprotein mainly distributed on the surface of macrophages. SR-A can participate in the pathogen recognition and clearance activities of the immune system, and can also remove old red blood cells that lose sialic acid and some apoptotic cells in the circulation [131]. At the same time, macrophages can also phagocytize modified lipoproteins through SR-A, thereby promoting the formation of atherosclerotic plaque [132].

**Activation of inflammatory response**

Arteriosclerosis is a chronic inflammatory disease caused by a variety of inflammatory cytokines. The "inflammatory response theory" is one of the main theories of arteriosclerosis [133]. NF-κB signaling pathway is a key transcription factor regulating inflammation and cell proliferation [134]. Using immunofluorescence and immunohistochemical techniques, some scholars found that activated NF-κB could be detected in the intima-media and lesion areas of atherosclerosis and vascular fibrosis. But not found in healthy blood vessels [135]. In the early development of arteriosclerosis, NF-κB stimulated by inflammatory response, can regulate the expression of a variety of adhesion molecules and promote leukocyte adhesion and aggregation at the damaged vascular endothelium. As the same time. NF-κB is also a key molecule in regulating and chemotaxing monocytes, and stimulates inflammatory factors to participate in the transformation from monocytes to macrophages by regulating macrophage colony [136]. Therefore, NF-κB can promote atherosclerotic plaque growth and plaque instability, and inhibit NF-κB in macrophages activation can reduce the formation of foam cells and protect blood vessels.

High levels of Hcy activate NF-κB signaling pathway (existing in B lymphocytes, cardiomyocytes, VSMCs and vascular endothelial cells) up regulates the expression of monocyte chemoattractant protein and Interleukin-8 (IL-8) in inflammatory cells, promotes the secretion of downstream inflammatory factors, such as VCAM-1, MCP-1, ICAM-1[137], interleukin, interferon, leukocyte adhesion molecule, hematopoietic growth factor and histocompatibility antigen, and promotes the occurrence of inflammation [138]. Studies have shown that Hcy activates NF-κB by inducing miRNA-33 pathway, and then up regulate Tumor Necrosis Factor-α (TNF-α) and the expression of Interleukin-6 (IL-6), which increases inflammatory response and promotes atherosclerosis [139]. Cytoplasmic sol pattern recognition receptor - NOD like receptor family pyrin domin containing 3 (NLRP3) – mediated NLRP3 inflammatory body pathway is the central link of atherosclerotic inflammatory response [140]. Therefore, Wang R, et al. [131] found that high levels of Hcy can activate NLRP3 inflammatory bodies in reactive oxygen species dependent pathways in macrophages, leading to the aggravation of inflammation and atherosclerosis.

Through activation of adenylate activated protein kinase and endoplasmic reticulum stress, Hcy can up regulate TNF-α and Matrix metalloproteinases-9 (MMP-9), which increase plaque instability [141]. At the same time, the percentage of immune related T lymphocytes in peripheral blood of patients with hyperhcy is significantly lower than that of patients with normal Hcy level [142], indicating that Hcy can promote the occurrence and development of atherosclerosis by affecting immunity [143].
Matrix metalloproteinases (MMPs)

MMPs are important endopeptidases for tissue remodeling and Extracellular Matrix (ECM) degradation. Studies have shown that the migration and proliferation of vascular smooth muscle cells need to be mediated by MMPs, and the catalytic activity of MMPs mainly depends on calcium and zinc ions. ECM is rich in elastin, collagen, proteoglycan and other substances, which are important components of vascular wall. Endothelial Cells (EC), VSMCs and inflammatory cells (including monocytes and macrophages) are the main sources of MMPs in vascular wall. The key role of MMPs in the pathogenesis of atherosclerosis has been supported by a series of evidence [144].

HHcy can enhance the activity of MMPs to induce elastin deterioration, resulting in the decrease of vascular elasticity. MMP-2 and MMP-9 in MMPs family are closely related to vascular endothelial cells and monocyte macrophages [145]. Activated MMP-2 can decompose collagen components in ECM and expose hidden functional sites. This process creates favorable conditions for basement membrane degradation, ECM remodeling and cell migration, further aggravates the pathological degree of vascular endothelium and increases the degree of arteriosclerosis [146]. Inflammatory cells in the adventitia and smooth muscle cells in the middle membrane of blood vessels can secrete MMP-2. MMP-2 can decompose elastin and cause calcium deposition in the blood vessel wall, leading to atherosclerosis. MMP-2 can also degrade type IV collagen, which is an important component of vascular basement membrane. At the same time, MMP-2 can also reshape ECM, activate cytokines, chemokines and growth factors, and then play a key role in the occurrence and development of atherosclerosis and its complications [147].

MMP-9 is the main enzyme that degrades vascular ECM. The excessive generation and activation of MMP-9 can degrade a large number of elastic fibers of vascular wall and affect the proliferation and migration of VSMCs, thus participating in intimal thickening and reactive remodeling of vascular wall to injury [148-150]. In vascular ECM, the pathological concentration of Hcy can change the expression of MMPs and its inhibitor mRNA in vascular ECM, increase the expression of MMP-9 in vascular ECM, but reduce the expression of its inhibitor, promote the degradation of ECM and reduce the stability of atherosclerotic plaque [151]. Studies have also shown that the higher the levels of MMP-2 and MMP-9, the greater the risk of arteriosclerosis [152]. In the process of atherosclerotic plaque formation, under the stimulation of a variety of inflammatory factors or ox-LDL, foam cells transformed from macrophages and VSMCs can continuously secrete MMP-9. MMP-9 has catalytic activity, which can degrade ECM, affect tissue structure and cell physiological activities, make fiber cap thinner, further weaken the stability of plaque, and eventually lead to plaque rupture and complications [153,154].

Summary

In conclusion, Hcy has become a new independent risk factor for ASO, and the level of serum Hcy is significantly correlated with the degree of ASO. There are many mechanisms for the occurrence and development of ASO caused by Hcy, including vascular endothelial cell dysfunction, mitochondrial dysfunction, oxidative stress response, coagulation dysfunction, VSMCs proliferation, abnormal lipid metabolism, body methylation and other mechanisms, which are interconnected and interact with each other, so as to cause and promote the occurrence and development of ASO. At present, the pathogenesis of ASO induced and promoted by Hcy remains to be further studied and explored, however, we believe that with the deepening of research and the continuous improvement of medical technology, the guiding role and clinical significance of Hcy detection for the diagnosis, treatment and prognosis evaluation of ASO will become more and more important, and will better be applied to the prevention and treatment of ASO, which has important clinical significance for improving people’s health level.

Fundamental Project

Construction project of the inheritance studio of the academic school of Chinese medicine of the Qilu School of Medicine (Lu Wei letter [2021] No. 45).

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