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Chinese medicine to regulate intestinal bacteria in the treatment of atherosclerosis: A review

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Abstract: In recent years, several studies have shown that the stability of intestinal bacteria, which acts as a natural barrier, plays a key role fighting against cardiovascular disease. Dysbiosis of the gut microbiome can trigger inflammatory responses and oxidative stress damage, cause abnormal cholesterol metabolism and disrupt the levels of short-chain fatty acids, oxidized trimethylamine and bile acids, thus influencing the occurrence and development of atherosclerosis. Chinese medicine believes that the formation of atherosclerosis is related to the malfunction of the spleen in transportation and transformation, with the interplay of phlegm and blood stagnation being the primary pathogenesis. In addition, the gut micro biome is inextricably linked to the spleen. Ancient medical books recorded "the spleen is the source of phlegm production" and "the heart and small intestine are in close proximity to each other", exploring its TCM pathogenesis from the perspective of phlegm and stasis. Therefore, this review explores the mechanisms by which intestinal micro biota affects atherosclerosis and summarizes the role of Chinese herbal medicine in modulating intestinal microbes in the treatment of AS, providing new ideas for the prevention and treatment of cardiovascular disease.

Keyword: Intestinal bacteria, atherosclerosis, inflammation, Chinese medicine.

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INTRODUCTION

Cardiovascular disease is still one of the greatest threats to human health and one of the major causes of death (Zhao *et al*., 2019). Atherosclerosis (AS) is an important pathological basis of cardiovascular disease in middle-aged and elderly people, which begins with endothelial lesions, followed by lipid accumulation, fibrous tissue proliferation and calcium deposition and ultimately evolves into intra-plaque haemorrhage, rupture and thrombosis, etc (Jiang *et al*., 2022). The etiological mechanisms of AS include the theory of lipid infiltration, thrombosis, immuno-inflammation and smooth muscle cloning, etc (Wang *et al*., 2021). At present, many scholars believe that the endothelial damage response theory is related to inflammation and bacteria is an important factor leading to inflammation (Lotti *et al*., 2023). At present, in addition to surgical or interventional treatment, the main preventive and curative measures of conventional western medicine drugs are lipid-lowering, antiplatelet, anticoagulation and thrombolytic therapy (Lin *et al*., 2020). The treatment principles are as follows: First, for patients with LDL-C >1.8, lipid-lowering therapy should be performed to prevent plaque rupture and slow down the rate of plaque progression (Jukema *et al*., 2019). Statins such as atorvastatin can be used and if the effect is not good, cholesterol inhibitors such as ezetimibe tablets can be used to bring the lipid level to the desired level; second, antiplatelet therapy can inhibit platelet aggregation and prevent vascular embolism, such as aspirin, clopidogrel, etc. (Pęczek *et al*., 2021); third, vasodilator therapy can improve the endothelial proliferation in the atherosclerotic blood vessels effectively and relieve the vasospasm; finally, anti-inflammatory therapy is also essential for the treatment of AS (Su *et al*., 2020). Therapy is also an essential and important means of treating AS, which can reduce the causative factors of AS triggered by inflammatory factors, such as statins, aspirin and other drugs (Su *et al*., 2020). Although these drugs have obvious efficacy in stabilising plaques, their side effects cannot be ignored, especially they can cause some damage to liver and kidney functions and regular review is needed during the period of administration. In recent years, with the continuous deepening of the research on AS, we found that traditional Chinese medicine can play an anti-atherosclerotic role by regulating the intestinal flora (Xu *et al*., 2021). Traditional Chinese medicine can effectively control the occurrence and development of AS, improve lipid metabolism, inflammatory response and stabilise plaque and achieve the goal of treating cardiovascular disease from the root (Verhaar *et al*., 2020). At the same time, compared with conventional Western medicine treatment, the side effects of traditional Chinese medicine are significantly reduced (Ma *et al*., 2024). In this paper, we will explore the relationship between traditional Chinese medicine, intestinal flora and AS, and provide an overview of the research progress of Chinese medicine in treating AS.

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Mechanisms of action of intestinal flora in the treatment of atherosclerosis: Influence on immune cells

AS is an autoimmune and chronic inflammatory disease whose greatest danger is plaque rupture. During this process, cholesterol, inflammatory factors and immune cells (monocytes-macrophages, T-lymphocytes, dendritic cells, etc.) accumulate, leading to thickening of the blood vessel wall, which promotes the onset and progression of AS. Experimental studies have shown (Chan *et al*., 2016) that dysregulation of intestinal flora accelerates the progression of AS and that probiotics (e.g.: Lactobacillus, Bifidobacterium, Bacteroidetes and Bacteroidetes, etc.) in the intestinal flora can slow down the development of atherosclerosis by regulating the levels of immune cells and inflammatory factors.

Structural changes in the intestinal flora can lead to the development of an inflammatory response. Lipopolysaccharide (LPS) is a pro-inflammatory mediator usually derived from Gram-negative bacteria (Yang *et al*., 2015). It has been shown (O'Morain *et al*., 2021) that reduced intestinal barrier function as well as increased permeability may lead to the entry of endotoxins into the bloodstream, which may cause vascular endothelial damage and promote plaque formation. Victoria L. *et al*. demonstrated (Kim *et al*., 2013) that under *in vitro* Lactobacillus culture conditions, there was a reduction in the proliferation of vascular smooth muscle cells, as well as a reduction in the formation of monocytes, macrophages and foam cells; it was further demonstrated that Lactobacillus lowered plasma cholesterol and LDL levels thereby reducing lipid aggregation in plaques, allowing inflammation to be reduced and plaques to be stabilised. More recently, Kim (El-Daly *et al*., 2018) *et al*. found that lipophosphatidic acid (LTA) in Lactobacillus could reduce the inflammatory response of plaques and inhibit the development of atherosclerosis by down-regulating vascular adhesion factor, inhibiting lipopolysaccharide (LPS) production, attenuating the adhesion of THP-1 cells to HUVEC and inhibiting the production of pro-inflammatory factors and CO. All of these studies suggest that probiotics, as a type of intestinal flora, can be effective in treating atherosclerosis by improving the accumulation of macrophages and foam cells, etc., in the vascular wall and attenuating the inflammatory response of plaques.

Attenuates oxidative stress damage

Oxidative stress injury results from an imbalance in the ratio of the body's own antioxidant capacity to the production of reactive oxygen species (ROS), which leads to dysfunction of the vascular endothelium and the production of pro-inflammatory mediators such as leukotrienes and thromboxanes, which activate leukocytes and increase adhesion to endothelial cells (El-Daly *et al*., 2018). Hyperuricaemia is one of the risk factors for the formation of atherosclerosis, uric acid has antioxidant capacity and intestinal metabolism as the main pathway of metabolism in the human body, if uric acid is elevated and reactive oxygen species in the body can not be removed in a timely manner, the antioxidant capacity is reduced, which will be directly involved in the development of atherosclerosis and cardiovascular disease (Singh *et al*., 2022). It has been found (Jitapunkul *et al*., 1991) that intestinal flora dysbiosis correlates with hyperuricaemia and atherosclerotic cardiovascular disease. Uric acid is mainly excreted through the kidneys, but a small portion is also degraded by intestinal bacteria, and thus disturbances in the intestinal flora correlate with blood levels of uric acid (Yin *et al*., 2022). By measuring uric acid levels in the faeces of patients with hyperuricemic coronary artery disease and normal control subjects, the researchers found that the patients with coronary artery disease had significantly higher numbers of gut bacteria, including *Escherichia coli, Helicobacter pylori* and Streptococcus, while there were no significant differences in the numbers of *Staphylococcus, Pseudomonas aeruginosa* and Weilonella. In contrast, uric acid levels were significantly higher in bacteria (Yin *et al*., 2022). This suggests that high uric acid is one of the factors contributing to oxidative stress damage, and also provides direct evidence that high uric acid has a significant impact on cardiovascular disease (Wang *et al*., 2023). In addition, probiotic interventions can reduce oxidative stress, inflammatory responses and improve gut flora imbalance, as well as enhance the resistance of the natural immune system to hyperuricaemic damage (Zhao *et al*., 2022). These studies suggest that oxidative stress plays a key role in the development of atherosclerotic cardiovascular disease and is also closely associated with inflammatory responses, which together influence disease progression.

Metabolites of intestinal flora affect AS

Intestinal flora can not only delay the progression of AS by affecting the immune system, oxidative stress and inflammatory response, but also its metabolites have a certain impact on the formation and rupture of atherosclerotic plaques, which include metabolites such as short-chain fatty acids (SCFAs), trimethylamine oxidation (TMAO), bile acids (BAs) and so on (Yoo *et al*., 2022).

Short-chain fatty acid

Short-chain fatty acids (SCFAs) are a class of organic fatty acids with a carbon chain length of less than 6, which are mainly produced by intestinal flora involved in the metabolic activities of dietary fibre and carbohydrates, including acetic acid, butyric acid and propionic acid. Bai Hongbo *et al* (Li *et al*., 2011) conducted a clinical randomised controlled trial and found that short-chain fatty acids have an anti-AS effect, which can regulate the expression of intestinal compact proteins mediated by GPR41, resulting in a significant reduction in intestinal permeability and LPS levels, which reduces the area of AS plaques and inhibits the development of

atherosclerosis. Studies have shown (Bartolomaeus *et al*., 2019; Yoo *et al*., 2020) that SCFAs can prevent and treat atherosclerosis by regulating Treg cell production and inhibiting histone deacetylase (HDAC) to enhance protection of vascular endothelial function and reduce inflammatory expression. Arash *et al*. explored the role of SCFAs in their study (Haghikia *et al*., 2022) and found that propionic acid lowered total cholesterol levels in high adiponectin mice total cholesterol levels and significantly ameliorated lesion size in diet-induced atherosclerosis in ApoE-/- mice. Propionic acid also down-regulated major intestinal cholesterol transporter proteins by increasing IL-10 levels in the intestinal environment, thereby stabilising the intestinal cholesterol internal environment and enhancing anti-atherosclerotic effects (Haghikia *et al*., 2022). Overall, as one of the metabolites of intestinal flora, SCFAs can inhibit the production of inflammatory factors by lowering LDL and play an important role in lipid metabolism in AS, which is a new direction to study the treatment of atherosclerosis.

Trimethylamine oxide

Recent studies have shown that trimethylamine oxide (TMAO) is also one of the risk factors for the development of cardiovascular disease, which has become a new hot spot in the study of atherosclerosis (Swanepoel *et al*., 2022). Trimethylamine (TMA) is absorbed into the blood and enters the liver via the portal circulation, where it is oxidised by flavin monooxygenase (FMO) to TMAO, which is produced by phosphatidylcholine metabolised by intestinal flora (Lv *et al*., 2022). Studies have shown (Wang *et al*., 2011) (Zhong *et al*., 2024) that elevated levels of TMAO *in vivo* can upregulate the number of scavenger receptors on the surface of macrophages, increase foam cell production and macrophage cholesterol accumulation, thereby accelerating atherosclerotic plaque formation. A study by Tang Jinghui *et al* (Zhong *et al.,* 2024) found that the levels of cholesterol reverse transcription factors, such as Sr-b1, Abcg5, Cyp7a1, etc., in the livers of ApoE-/- mice were significantly increased after administration of choline chloride or Lactobacillus, while the in vivo levels of TMAO were effectively reduced in ApoE-/- mice. In addition, the detection of local AS plaques revealed a significant decrease in the levels of macrophages and lipid metabolism, suggesting that Lactobacillus was able to promote the efflux of lipids metabolised by the liver by decreasing the levels of TMAO, increasing cholesterol reverse transcription and ultimately preventing atherosclerosis due to the elevation of TMAO in the serum (Tan *et al*., 2019). In addition, monocyte adhesion and endothelial dysfunction are also key links in the process of AS development (Ma *et al*., 2017; Ma *et al*., 2015). Studies have shown that TMAO can lead to endothelial cell injury through activation of protein kinase C (PKC) and NF-kB, as well as enhance monocyte adhesion and up-regulate the expression of adhesion molecules in vascular endothelial cells, and these results suggest that TMAO may participate in the prevention of atherosclerosis by affecting the activity of monocytes and the endothelial cell function, which is involved in the pathophysiological process of AS (Ma *et al*., 2017). In summary, the production of TMAO is related to the metabolism of intestinal flora. In addition, TMAO is closely related to macrophage and foam cell production, inhibition of reverse cholesterol transport and enhancement of monocyte adhesion to the arterial wall. All three of these aspects are important factors that trigger the development of AS, as well as cardiovascular disease (Luo *et al*., 2022).

Secondary bile acid

Bile acids (BAs) are synthesised from primary bile acids in hepatocytes by cholesterol via the action of enzymes, and then primary bile acids are combined with taurine and glycine to form bile acid complexes, which enter the intestine and are synthesised into secondary bile acids by hydrolytic enzymes and 7α/β-dehydroxylase(Rani *et al*., 2024). Secondary bile acids have the following three main roles: Firstly, as signalling molecules involved in the regulation of substance metabolism, secondly, involved in lipid metabolism and absorption and thirdly, when bile acids accumulate in excess in the body, they can have toxic effects on the organism (Ma and Patti, 2014). Abnormal metabolism of bile acids promotes the development of AS, in which the bile acid receptor (FXR) and the G-protein-coupled receptor TGR5 play important roles (Branchereau *et al*., 2019). As a nuclear receptor for bile acids, FXR is mainly expressed in the small intestine and liver and induces fibroblast growth factor 19 (FGF19) to activate fibroblast growth factor receptor 4 (FGFR4), which inhibits CYP7A1 and reduces bile salt synthesis (Fu *et al*., 2016). A study by Jan *et al*. Revealed (de Boer *et al*., 2017) that FXR, upon activation, can induce blood cholesterol to enter the intestinal epithelium directly through the low-density lipoprotein receptor (LDL-R) and stimulate cholesterol influx into the intestinal lumen, i.e., increase cholesterol efflux from the TICE pathway, thereby reducing cholesterol levels in the body, improving lipid metabolism disorders, and delaying the onset of atherosclerosis. In addition, the study of Takeshi *et al*. also showed (Inagaki *et al*., 2006) that FXR agonists can effectively inhibit the damage caused by intestinal inflammation. Experimental results showed that mice lacking FXR were susceptible to bacterial attack, causing damage to the intestinal epithelial barrier and elevated bacterial levels in the ileum (Nijmeijer *et al*., 2014). This suggests that activation of FXR can prevent systemic infections and enhance protection of the intestinal tract by reducing its exposure to inflammatory damage, making it a potential therapeutic target for AS. As an important component of the bile acid signalling pathway, TGR5 is mainly expressed in the liver, gallbladder, stomach and intestine and regulates the anabolism of bile acids as well as influencing the expression level of bile acid synthase

(Reich *et al*., 2021). The findings of Thijs *et al*. Showed (Pols *et al*., 2011) that TGR deficiency exacerbates the progression of AS plaques by the mechanism of inhibition of macrophage NF-kB activity through the cAMP signalling pathway, causing the inhibition of macrophage NF-kB activity and uptake of oxidised LDL in macrophages, thus radically reducing the inflammatory response in atherosclerotic plaques. Sui Guoyuan *et al* (Chen *et al*., 2008) divided the ApoE -/- mouse model into blank group, statin group, and chemoexpectorant group, and gave mice a high-fat diet, and the results of the study showed that, after the intervention of chemoexpectorant formula, the expression level of hepatic FMO3 protein in mice was significantly reduced, and the inflammation and lipid deposition in the AS plaques were significantly improved, which radically inhibited the formation of aortic plaques. In summary, it can be seen that bile acids, as important signalling molecules produced by intestinal flora and host metabolism, play an important regulatory role in lipid metabolism. In addition, impaired cholesterol metabolism and inflammation are the direct causes of AS development, which has become a hot topic in the study of AS mechanism (Dinu *et al*., 2024; Sheng *et al*., 2023).

Modulation of intestinal flora by traditional Chinese medicine for the treatment of atherosclerosis

Chinese medicine mechanism of atherosclerosis

In Chinese medicine, atherosclerosis is not classified as an independent disease, but according to its pathogenesis, clinical manifestations and other characteristics, it is classified as "headache", "chest paralysis and heart pain", "true heart pain", "vertigo", "syncope", "stroke" and other categories.", "vertigo", "syncope", "stroke" and other categories, and now often the atherosclerosis in modern medicine is categorised as Chinese medicine's "Pulse paralysis". The name of pulse paralysis began in the "Neijing", "Suwen - paralysis theory" talked about "wind, cold and dampness three qi mixed to, combined as paralysis also." "Paralysis is in the veins, then the blood is condensed and does not flow", that qi and blood running in the veins and feel the evil will be blocked blood dispersed to the body in all directions, this "veins" is equivalent to the "blood vessels" in Western medicine, blood vessels Due to lipid metabolism and other factors that cause blood flow is not smooth, so the veins are not accessible. Wang Chunye *et al*. (Wang *et al*., 2024). searched the literature on the pathogenesis of atherosclerosis in Chinese medicine in the past five years, and concluded that its essence is a type of deficiency and deficiency mixed with reality, and concluded that the main symptoms include nine pathological factors, of which the top three pathogenic factors are blood stasis, phlegm, and heat. This indirectly confirms that the main factors promoting the formation of atherosclerosis are blood stasis, phlegm and heat. The disease of pulse paralysis is located in the blood vessels, and its primary deficiency is closely related to the three organs of the liver, spleen and kidney. The spleen is the source of qi and blood biochemistry, and its physiological function is to transport water and grains and water-dampness. It is pointed out in the Compendium of Evidence-based Treatments that "a deficiency of the spleen does not differentiate between the clear and the turbid, and phlegm arises from the retention of fluids". And "Medical Zong Biyan" mentioned: "Spleen and soil are weak, it is difficult to raise the clear, turbid is difficult to descend, stay in the diaphragm, coagulation into phlegm." Most of the patients with atherosclerosis are old and weak, and the spleen and stomach are unable to transport and transform, making the heart and various tissues and organs unable to get the essence of the spleen to transport and transform the material nutrients, so that over time, the creams and fats will be accumulated in the veins, and the deficiency of the spleen will cause phlegm and stagnation, affecting the operation of the qi, blood and fluid, and then aggravating the atherosclerotic cardio-cerebral and cerebral vascular diseases. Liver is the master of excretion, regulating the whole body's qi, qi line is blood line, qi stagnation is blood stasis, affecting the triple jiao and water and fluid metabolism. If the fluid can not be transported and distributed, it is easy to be condensed into phlegm. Kidney is the main water and essence, the whole body's water needs to be transpiration and gasification through the kidney, the "clear one" will be transported all over the body to nourish the meridians, and the "turbid one" will be turned into urine and passed down to the bladder. Medical Guan - phlegm theory" cloud: "kidney deficiency can not control water, the water does not return to the source, such as water against the flow, flooding and phlegm, is no fire." One of the kidney Yang has a warming effect, the blood is warm is travelling, cold is condensed, stagnation for a long time, the pulse is not smooth is easy to lead to atherosclerosis formation.

Theory of the cardio-intestinal axis

With the deepening of the study of intestinal flora, the theory of intestinal-cardiac axis has been proposed, which not only better explains the close association between intestinal flora and cardiovascular diseases, but also proves the scientific nature of the theory of "the heart and the small intestine are mutually exclusive" in Chinese medicine. From the perspective of Chinese medicine, the heart and the small intestine are mutually exclusive. The Spiritual Pivot meridian 10 "recorded:" heart hand shaoyin vein, from the heart, out of the heart system, under the diaphragm, the small intestine; its branch, from the heart system on the hostage pharyngeal, the Department of the eye system; against the end of the palm of the back of the sharp bone, into the palm of the back of the Lian, by the little finger within the end of its(de Man *et al*., 2024)." The original description of the hand Shaoyin heart meridian and hand sun small intestine meridian in the small finger end of the articulation, constitute the viscera table relationship. In the "Suwen"

has long been recorded: "the heart, the official of the monarch, the gods out of it," "the small intestine, the officer who receives Sheng, changes out of it. From a physiological point of view, the heart of the blood, for the five viscera and six bowels of the Lord, can moisten the small intestine blood and help it to receive the fullness of the compounds; and Chinese medicine that the small intestine "urinary don't clear and turbid" function with modern medicine in the intestinal digestion, absorption of a variety of human body needs the function of the same material. The small intestine through the absorption of the stomach by the stomach of the essence of the grain and its red blood to moisten the heart pulse, while the dregs of the small intestine through the secretion of turbidity out of the body, and therefore each other for the relationship between the surface and the inner. Pathologically, "medical jinjian" cloud: "the heart and small intestine for the table, but also, but see the mouth and tongue sores, urine red and yellow, stem in the pain, hot drenching diarrhoea and so on, are heart to move the heat in the small intestine of the evidence," if the heart fire is hyperactivity, the heat is moved down to the small intestine affects the function of small intestinal urination of the other clear and turbid and small intestinal heat can be upward fumigation of the fire to the heart. Wang's medical deposit" said: "dirty, small intestine heat are upward, so can not sleep." The heart is the master of the mind, and solid heat from the small intestine can also affect the mind of the heart, thus making the heart and veins unsupported, aggravating the accumulation of pathological products such as phlegm, stasis of blood, and heat and toxins in the veins and blocking the veins. Intestinal flora as the small intestine to play a normal physiological function of the material basis, its research shows that once the intestinal microbiota metabolic disorders, will lead to a series of heart vein loss of nourishment of the disease occurs (Wu *et al*., 2020). Therefore, the "heart and small intestine" is the main factor to ensure the smooth flow of blood.

Chinese medicine regulates intestinal flora to treat atherosclerosis

The human gut contains a variety of microflora, and traditional Chinese medicine (TCM) is often used to treat AS by oral administration of herbal tonics. Studies on the association between intestinal flora and its metabolites and the heart have found that many Chinese medicines can change the structure and barrier of intestinal flora, thereby effectively controlling lipid metabolism disorders, oxidative stress damage, inflammation, etc., and thus achieving the effect of treating cardiovascular diseases such as atherosclerosis (Song *et al*., 2021). From the perspective of traditional Chinese medicine, phlegm and stasis, and stagnation of qi and blood are the pathomechanisms of atherosclerosis. Therefore, the use of blood activation, elimination of blood stasis and detoxification to improve the intestinal barrier function and to increase intestinal probiotics has become a new approach for the treatment of this type of disease.

Herbal monomers

Some herbal substances have been found to be able to prevent and control the development of atherosclerosis by modulating the intestinal flora and these herbal substances include aspalathin, berberine, curcumin, resveratrol and danshen (Shabalala *et al*., 2020). It has been found (Chen *et al*., 2016; Liu *et al*., 2021) that berberine, an active ingredient in aspalathin and curcumin, can increase the number of certain specific flora, as well as change the structure and function of the intestinal flora, thus increasing the content of probiotics and SCFAs in the intestinal tract, decreasing the level of the intestinal carnitine precursor substance TMAO and alleviating infiltration of inflammatory cells into the intestinal mucosa and damage and preventing the further deterioration of AS. Resveratrol, as a natural plant of anti-AS protein, can increase the content of Lactobacillus and Bifidobacterium by remodelling the intestinal microbial structure, and promote hepatic bile acid excretion to reduce the level of TMAO (Chen *et al*., 2016). Another study found that Salvia miltiorrhiza, when administered to rats after a high-fat diet induced obesity, reduced the number of harmful bacteria and increased the levels of Aspergillus and Actinobacillus, which significantly improved the integrity of the intestinal mucosa, as well as dramatically improving the metabolic build-up of lipids and decreasing the risk of diseases associated with AS (Ai *et al*., 2022).

Compound prescription of Chinese medicine

The Chinese medicine compound prescriptions mainly include Qingxin Xieyu Fang, Tongxinluo, Tongxinhuayu Tang and Resolving Phlegm and Resolving Stasis Fang, which are mainly for removing blood stasis, and Spleen Strengthening, Resolving Turbidity and Regulating Lipids Granules and Semixia Baijutsu Tianma Tang, which are mainly for strengthening the spleen and removing phlegm (Zhang *et al*., 2019). A study conducted serum metabolomics analysis after intervening in patients with stable coronary artery disease using Qingxin Xieyuquan, and further investigated lipid metabolism in the ApoE-/- mouse AS model (Chen *et al*., 2022), and found that the use of Qingxin Xieyuquan (Astragalus, Dangshen, Rhizoma Ligustici Chuanxiong, Huoxiang, Huanglian) increased the abundance of intestinal bacterial genera positively related to serum HDL-C, such as Microcystis warticuliformis (Akkermansia spp.) and SCFAs-producing bacteria, while reducing triglyceride (TG) levels, serum inflammatory factor (e.g., interleukin) levels, and AS plaque extent in the ApoE-/- mouse model of AS. Qi *et al* (Qi *et al*., 2022) induced AS in rabbits using a high-fat diet plus balloon injury, and intervened with the proprietary Chinese medicinal drug Tongxinluo. The results showed that Tongxinluo reduced the

inflammatory response by decreasing the NLRP3 signalling pathway, and increased the content of probiotics and gut bacteria metabolites, which stabilised vulnerable plaques and slowed the progression of AS. Some studies have shown that Chinese herbal formulas have a synergistic effect with Western medications (e.g., atorvastatin). The combination of the two can significantly reduce the inflammatory response of AS plaques (Qi *et al*., 2022). In addition, the efficacy of Tongxinhuoyu Tang has been confirmed, which can regulate the structural composition of intestinal flora, especially reduce the level of TMAO generated by the metabolism of dietary choline, and improve platelet aggregation, thus exerting anti-atherosclerotic effects (Peng *et al*., 2018). Strudy found in animal experiments that the use of the formula for resolving phlegm and expelling blood stasis could significantly reduce the content of LPS in the AS model of ApoE-/- mice, increase the excretion of cholesterol and enhance the function of the intestinal barrier and change the structure of the bacterial flora (Tu and Xia, 2024). LPS is one of the pro-inflammatory mediators released by bacteria and dysfunction of the intestinal bacterial flora will lead to the impairment of the intestinal mucosal barrier, promoting the entry of LPS into the bloodstream and causing inflammatory responses to be Aggravation. The basic composition of the formula includes Codonopsis pilosulae, Gynostemma gibbosum, Astragalus membranaceus, Poria, Salvia miltiorrhiza, Rhizoma ligustici chuanxiong, Fructus schisandrae, and Uljin, which has the efficacy of tonifying qi, strengthening the spleen, activating blood circulation and removing blood stasis and it can regulate the structure of the intestinal flora and its mucosal barrier to maintain the homeostatic state of the internal environment, and balance cholesterol metabolism (Zhao *et al*., 2020). Recent studies have shown that intravascular cellular senescence caused by AS is related to intravascular ROS levels. When ROS levels rise, they activate inflammation via oxidative modification of lipoproteins and promote macrophage release (Kim *et al*., 2023). Further activation of the NF-κB signalling pathway prompts endothelial cell adhesion molecules to form plaques on the arterial wall and accelerates macrophage production, ultimately leading to AS lesions (Pang *et al*., 2019). Spleen-enhancing and lipid-regulating granules can effectively slow down the proliferation of vascular endothelial cells and macrophages caused by oxidative stress injury, mainly by increasing the abundance of Bifidobacterium and Lactobacillus to improve the structure of the intestinal flora and down-regulate the ROS/NF-κB signalling pathway, thus interfering with the progression of AS inflammation and the formation of aortic plaques (Xie *et al*., 2023).

Summary and prospects

In summary, we propose that intestinal flora can intervene in the occurrence and development of AS by regulating collective immune cells and influencing oxidative stress injury. In addition, the metabolites of intestinal flora are also one of the important factors affecting AS. With the deepening research on Chinese medicine for cardiovascular diseases, we have confirmed the close association between the intestinal environment and cardiovascular health through Chinese medicine theories. A large number of studies have also shown that Chinese medicine monomers and combinations can have a therapeutic effect on AS by regulating the intestinal flora, and their side effects are less harmful than those of conventional Western medicine treatments, e.g., mistletoe and other medicines can indirectly have a regulatory effect on the flora in order to improve the effect of cardiovascular diseases. However, due to the lack of clinical practice, further research and practice are still needed in the future. In this paper, we summarise the mechanism of intestinal flora modulation in AS, and describe the current research status of Chinese medicine modulation of intestinal flora in the treatment of AS, aiming to provide new ideas and inspiration for the treatment of AS with Chinese medicine.

REFERENCES

Ai ZL, Zhang X, Ge W, Zhong YB, Wang HY, Zuo ZY and Liu DY (2022). *Salvia miltiorrhiza* extract may exert an anti-obesity effect in rats with high-fat diet-induced obesity by modulating gut microbiome and lipid metabolism. *World J. Gastroenterol.,* **28**(43): 6131-6156.

Bartolomaeus H, Balogh A, Yakoub M, Homann S, Markó L, Höges S, Tsvetkov D, Krannich A, Wundersitz S, Avery EG, Haase N, Kraker K, Hering L, Maase M, Kusche-Vihrog K, Grandoch M, Fielitz J, Kempa S, Gollasch M, Zhumadilov Z, Kozhakhmetov S, Kushugulova A, Eckardt KU, Dechend R, Rump LC, Forslund SK, Muller DN, Stegbauer J and Wilck N (2019). Short-chain fatty acid propionate protects from hypertensive cardiovascular damage. *Circulation,* **139**(11): 1407-1421.

Branchereau M, Burcelin R and Heymes C (2019). The gut microbiome and heart failure: A better gut for a better heart. *Rev. Endocr Metab Disord.,* **20**(4): 407-414.

Chan YK, Brar MS, Kirjavainen PV, Chen Y, Peng J, Li D, Leung FC and El-Nezami H (2016). High fat diet induced atherosclerosis is accompanied with low colonic bacterial diversity and altered abundances that correlates with plaque size, plasma A-FABP and cholesterol: A pilot study of high fat diet and its intervention with *Lactobacillus rhamnosus* GG (LGG) or telmisartan in ApoE(-/-) mice. *BMC Microbiol.,* **16**(1): 264.

Chen ML, Yi L, Zhang Y, Zhou X, Ran L, Yang J, Zhu JD, Zhang QY and Mi MT (2016). Resveratrol

attenuates trimethylamine-N-oxide (TMAO)-induced Atherosclerosis by regulating TMAO synthesis and bile acid metabolism via remodeling of the gut microbiota. *mBio.,* **7**(2): e02210-02215.

Chen R, Chen T, Zhou Z, Song Z, Feng W, Yang X, Wang X, Li B, Ding X, Zhang H and Wang Y (2022). Integrated pyroptosis measurement and metabolomics to elucidate the effect and mechanism of tangzhiqing on atherosclerosis. *Front Physiol.,* **13**: 937737.

Chen RQ, Xiong P, Zhou XX, Liang ZQ and Wu LZ (2008). Effects of phlegm-dispelling and blood stasis-resolving traditional Chinese drugs on the blood pressure and cell cycle of left ventricular cardiac myocytes in hypertensive rats. *Nan Fang Yi Ke Da Xue Xue Bao.,* **28**(10): 1806-1809.

De Boer JF, Schonewille M, Boesjes M, Wolters H, Bloks VW, Bos T, van Dijk TH, Jurdzinski A, Boverhof R, Wolters JC, Kuivenhoven JA, van Deursen JM, Oude Elferink RPJ, Moschetta A, Kremoser C, Verkade HJ, Kuipers F and Groen AK (2017). Intestinal farnesoid x receptor controls transintestinal cholesterol excretion in mice. *Gastroenterology,* **152**(5): 1126-1138.e1126.

De Man AME, Amrein K, Casaer MP, Dizdar OS, van Zanten ARH, Gundogan K, Lepp L, Rezzi S, Shenkin A and Berger MM (2024). LLL 44-4: Micronutrients in acute disease and critical illness. *Clin Nutr ESPEN.,* **61**: 437-446.

Dinu LD, Gatea F, Matei F, Banciu C and Vamanu E (2024). Gut Microbiota Modulation by Selenium and Zinc Enrichment Postbiotic on Dysbiosis Associated with Hypertension. *Curr. Vasc Pharmacol.,* **22**(10): 365-374.

El-Daly M, Pulakazhi Venu VK, Saifeddine M, Mihara K, Kang S, Fedak PWM, Alston LA, Hirota SA, Ding H, Triggle CR and Hollenberg MD (2018). Hyperglycaemic impairment of PAR2-mediated vasodilation: Prevention by inhibition of aortic endothelial sodium-glucose-co-Transporter-2 and minimizing oxidative stress. *Vascul Pharmacol.,* **109**: 56-71.

Fu T, Kim YC, Byun S, Kim DH, Seok S, Suino-Powell K, Xu HE, Kemper B and Kemper JK (2016). FXR primes the Liver for intestinal FGF15 signaling by transient induction of β-Klotho. *Mol. Endocrinol.,* **30**(1): 92-103.

Haghikia A, Zimmermann F, Schumann P, Jasina A, Roessler J, Schmidt D, Heinze P, Kaisler J, Nageswaran V, Aigner A, Ceglarek U, Cineus R, Hegazy AN, van der Vorst EPC, Doring Y, Strauch CM, Nemet I, Tremaroli V, Dwibedi C, Kränkel N, Leistner DM, Heimesaat MM, Bereswill S, Rauch G, Seeland U, Soehnlein O, Müller DN, Gold R, Backhed F, Hazen SL, Haghikia A and Landmesser U (2022). Propionate attenuates atherosclerosis by immune-dependent regulation of intestinal cholesterol metabolism. *Eur Heart J.,* **43**(6): 518-533.

Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, Yu RT, Shelton JM, Richardson JA, Repa JJ, Mangelsdorf DJ and Kliewer SA (2006). Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci U S A.,* **103**(10): 3920-3925.

Jiang LP, Yu XH, Chen JZ, Hu M, Zhang YK, Lin HL, Tang WY, He PP and Ouyang XP (2022). Histone Deacetylase 3: A potential therapeutic target for atherosclerosis. *Aging Dis.,* **13**(3): 773-786.

Jitapunkul S, Chalaprawat M, Bunnag S, Bhuvapanich S, Kangkaya V, Pasatrat S and Vajanamarhutue C (1991). The relationship between glucose and uric acid metabolism: Influence of short term allopurinol on glucose metabolism. *J. Med. Assoc Thai.,* **74**(2): 80-86.

Jukema RA, Ahmed TAN and Tardif JC (2019). Does low-density lipoprotein cholesterol induce inflammation? If so, does it matter? Current insights and future perspectives for novel therapies. *BMC Med.,* **17**(1): 197.

Kim JY, Kim H, Jung BJ, Kim NR, Park JE and Chung DK (2013). Lipoteichoic acid isolated from *Lactobacillus plantarum* suppresses LPS-mediated atherosclerotic plaque inflammation. *Mol. Cells,* **35**(2): 115-124.

Kim SG, Sung JY, Kang YJ and Choi HC (2023). Fisetin alleviates cellular senescence through PTEN mediated inhibition of PKCδ-NOX1 pathway in vascular smooth muscle cells. *Arch Gerontol Geriatr.,* **108**: 104927.

Li N, Salter RC and Ramji DP (2011). Molecular mechanisms underlying the inhibition of IFN-γ-induced, STAT1-mediated gene transcription in human macrophages by simvastatin and agonists of PPARs and LXRs. *J. Cell Biochem.,* **112**(2): 675-683.

Lin Y, Zhu W and Chen X (2020). The involving progress of MSCs based therapy in atherosclerosis. *Stem Cell Res Ther.,* **11**(1): 216.

Liu FY, Wen J, Hou J, Zhang SQ, Sun CB, Zhou LC, Yin W, Pang WL, Wang C, Ying Y, Han SS, Yan JY, Li CX, Yuan JL, Xing HJ and Yang ZS (2021). Gastrodia remodels intestinal microflora to suppress inflammation in mice with early atherosclerosis. *Int. Immunopharmacol.,* **96**: 107758.

Lotti S, Dinu M, Colombini B, Amedei A and Sofi F (2023). Circadian rhythms, gut microbiota and diet: Possible implications for health. *Nutr Metab Cardiovasc Dis.,* **33**(8): 1490-1500.

Luo T, Liu D, Guo Z, Chen P, Guo Z, Ou C and Chen M (2022). Deficiency of proline/serine-rich coiled-coil protein 1 (PSRC1) accelerates trimethylamine N-oxide-induced atherosclerosis in ApoE(-/-) mice. *J Mol Cell Cardiol.,* **170**: 60-74.

Lv S, Wang Y, Zhang W and Shang H (2022). Trimethylamine oxide: A potential target for heart failure therapy. *Heart.,* **108**(12): 917-922.

Ma G, Pan B, Chen Y, Guo C, Zhao M, Zheng L and Chen B (2017). Trimethylamine N-oxide in atherogenesis: Impairing endothelial self-repair

capacity and enhancing monocyte adhesion. *Biosci. Rep.,* **37**(2): 244.

Ma H and Patti ME (2014). Bile acids, obesity, and the metabolic syndrome. *Best Pract. Res. Clin. Gastroenterol.,* **28**(4): 573-583.

Ma L, Guo X and Chen W (2015). Inhibitory effects of oleoylethanolamide (OEA) on H_2O_2-induced human umbilical vein endothelial cell (HUVEC) injury and apolipoprotein E knockout (ApoE-/-) atherosclerotic mice. *Int. J. Clin. Exp. Pathol.,* **8**(6): 6301-6311.

Ma ZM, Ouyang H, Ni J, Lyu Z and Li H (2024). Traditional Chinese medicine preparations in medical institutions: current situation and inspirations on research and development of new traditional Chinese medicine preparations. *Zhongguo Zhong Yao Za Zhi.,* **49**(5): 1415-1420.

Nijmeijer RM, Schaap FG, Smits AJ, Kremer AE, Akkermans LM, Kroese AB, Rijkers GT, Schipper ME, Verheem A, Wijmenga C, Gooszen HG and van Erpecum KJ (2014). Impact of global Fxr deficiency on experimental acute pancreatitis and genetic variation in the FXR locus in human acute pancreatitis. *PLoS One.,* **9**(12): e114393.

O'Morain VL, Chan YH, Williams JO, Alotibi R, Alahmadi A, Rodrigues NP, Plummer SF, Hughes TR, Michael DR and Ramji DP (2021). The Lab4P consortium of probiotics attenuates atherosclerosis in LDL receptor deficient mice fed a high fat diet and causes plaque stabilization by inhibiting inflammation and several pro-atherogenic processes. *Mol. Nutr. Food Res.,* **65**(17): e2100214.

Pang J, Hu P, Wang J, Jiang J and Lai J (2019). Vorapaxar stabilizes permeability of the endothelial barrier under cholesterol stimulation via the AKT/JNK and NF- κB signaling pathways. *Mol. Med. Rep.,* **19**(6): 5291-5300.

Pęczek P, Leśniewski M, Mazurek T, Szarpak L, Filipiak KJ and Gąsecka A (2021). Antiplatelet effects of PCSK9 inhibitors in primary hypercholesterolemia. *Life (Basel),* **11**(6): 466.

Peng S, Xu LW, Che XY, Xiao QQ, Pu J, Shao Q and He B (2018). Atorvastatin inhibits inflammatory response, attenuates lipid deposition and improves the stability of vulnerable atherosclerotic plaques by modulating autophagy. *Front Pharmacol.,* **9**: 438.

Pols TW, Nomura M, Harach T, Lo Sasso G, Oosterveer MH, Thomas C, Rizzo G, Gioiello A, Adorini L, Pellicciari R, Auwerx J and Schoonjans K (2011). TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell Metab.,* **14**(6): 747-757.

Qi Y, Liu W, Yan X, Zhang C, Zhang C, Liu L, Zheng X, Suo M, Ti Y, Ni M, Zhang M and Bu P (2022). Tongxinluo may alleviate inflammation and improve the stability of atherosclerotic plaques by changing the intestinal flora. *Front Pharmacol.,* **13**: 805266.

Rani D, Kaur S, Shahjahan, Dey JK and Dey SK (2024). Engineering immune response to regulate cardiovascular disease and cancer. *Adv. Protein Chem Struct. Biol.,* **140**: 381-417.

Reich M, Spomer L, Klindt C, Fuchs K, Stindt J, Deutschmann K, Hohne J, Liaskou E, Hov JR, Karlsen TH, Beuers U, Verheij J, Ferreira-Gonzalez S, Hirschfield G, Forbes SJ, Schramm C, Esposito I, Nierhoff D, Fickert P, Fuchs CD, Trauner M, García-Beccaria M, Gabernet G, Nahnsen S, Mallm JP, Vogel M, Schoonjans K, Lautwein T, Köhrer K, Häussinger D, Luedde T, Heikenwalder M and Keitel V (2021). Downregulation of TGR5 (GPBAR1) in biliary epithelial cells contributes to the pathogenesis of sclerosing cholangitis. *J Hepatol.,* **75**(3): 634-646.

Shabalala SC, Dludla PV, Mabasa L, Kappo AP, Basson AK, Pheiffer C and Johnson R (2020). The effect of adiponectin in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and the potential role of polyphenols in the modulation of adiponectin signaling. *Biomed Pharmacother.,* **131**: 110785.

Sheng Y, Meng G, Zhou Z, Du R, Wang Y and Jiang M (2023). PARP-1 inhibitor alleviates liver lipid accumulation of atherosclerosis via modulating bile acid metabolism and gut microbes. *Mol. Omics.,* **19**(7): 560-573.

Singh HK, Biswas AK, Philip J, Kushwaha N, Mukherjee B and Baranwal AK (2022). A study to assess the relationship between donor uric acid levels and supernatant hemolysis in stored packed red blood cell units. *Asian J. Transfus Sci.,* **16**(2): 251-256.

Song L, Zhang J, Lai R, Li Q, Ju J and Xu H (2021). Chinese herbal medicines and active metabolites: Potential antioxidant treatments for atherosclerosis. *Front Pharmacol.,* **12**: 675999.

Su YS, Ali MS, Pukenas BA, Favilla CG, Zanaty M, Hasan DM and Kung DK (2020). Novel treatment of cerebral vasospasm using solitaire stent retriever-assisted angioplasty: Case series. *World Neurosurg.,* **135**: e657-e663.

Swanepoel I, Roberts A, Brauns C, Chaliha DR, Papa V, Palmer RD and Vaccarezza M (2022). Trimethylamine N-oxide (TMAO): A new attractive target to decrease cardiovascular risk. *Postgrad Med J.,* **98**(1163): 723-727.

Tan YL, Ou HX, Zhang M, Gong D, Zhao ZW, Chen LY, Xia XD, Mo ZC and Tang CK (2019). Tanshinone IIA promotes macrophage cholesterol efflux and attenuates atherosclerosis of apoE-/- mice by omentin-1/ABCA1 Pathway. *Curr. Pharm. Biotechnol.,* **20**(5): 422-432.

Tu R and Xia J (2024). Stroke and vascular cognitive impairment: The role of intestinal microbiota metabolite TMAO. *CNS Neurol Disord Drug Targets,* **23**(1): 102-121.

Verhaar BJH, Prodan A, Nieuwdorp M and Muller M (2020). Gut microbiota in hypertension and atherosclerosis: A review. *Nutrients,* **12**(10): 2982.

Wang F, Wang Z and Tang J (2023). The interactions of *Candida albicans* with gut bacteria: A new strategy to

prevent and treat invasive intestinal candidiasis. *Gut Pathog.,* **15**(1): 30.

Wang X, Luo D and Wu S (2021). Molecular dysfunctions of mitochondria-associated endoplasmic reticulum contacts in atherosclerosis. *Oxid. Med. Cell Longev.,* 2021: 2424509.

Wang Y, Qin C, Tian S, Meng Y, Chen Y, Fu S, Xu M, Wang B, Li Y, Ouyang Q, Ling H and Liu M (2024). Dataset on cardiac structural and functional parameters in TMAO-challenged mouse. *Data Brief.,* **54**: 110465.

Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ and Hazen SL (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature.,* **472**(7341): 57-63.

Wu M, Yang S, Wang S, Cao Y, Zhao R, Li X, Xing Y and Liu L (2020). Effect of berberine on atherosclerosis and gut microbiota modulation and their correlation in high-fat diet-fed ApoE-/- Mice. *Front Pharmacol.,* **11**: 223.

Xie L, Chi X, Wang H, Dai A, Dong J, Liu S and Zhang D (2023). Mechanism of action of buckwheat quercetin in regulating lipid metabolism and intestinal flora via Toll-like receptor 4 or nuclear factor κB pathway in rats on a high-fat diet. *Nutrition.,* **115**: 112148.

Xu L, Li X, Zhang Y, Ding M, Sun B, Su G and Zhao Y (2021). The effects of linalool acupoint application therapy on sleep regulation. *RSC Adv.,* **11**(11): 5896-5902.

Yang P, Li Z, Li H, Lu Y, Wu H and Li Z (2015). Pyruvate kinase M2 accelerates pro-inflammatory cytokine secretion and cell proliferation induced by lipopolysaccharide in colorectal cancer. *Cell Signal.,* **27**(7): 1525-1532.

Yin H, Liu N and Chen J (2022). The role of the intestine in the development of hyperuricemia. *Front, Immunol.,* **13**: 845684.

Yoo JY, Groer M, Dutra SVO, Sarkar A and Mc Skimming DI (2020). Gut microbiota and immune system interactions. *Microorganisms.,* **8**(10): 2046.

Yoo JY, Sniffen S, McGill Percy KC, Pallaval VB and Chidipi B (2022). Gut dysbiosis and immune system in atherosclerotic cardiovascular disease (ACVD). *Microorganisms,* **10**(1) : 108.

Zhang Y, Liu Y, Li Y, Zhao X, Zhuo L, Zhou A, Zhang L, Su Z, Chen C, Du S, Liu D and Ding X (2019). Hierarchical and complex system entropy clustering analysis based validation for traditional chinese medicine syndrome patterns of chronic atrophic gastritis. *J. Altern. Complement. Med.,* **25**(11): 1130-1139.

Zhao D, Liu J, Wang M, Zhang X and Zhou M (2019). Epidemiology of cardiovascular disease in China: Current features and implications. *Nat. Rev. Cardiol.,* **16**(4): 203-212.

Zhao H, Lu Z and Lu Y (2022). The potential of probiotics in the amelioration of hyperuricemia. *Food Funct.,* **13**(5): 2394-2414.

Zhao J, Li Y, Xin L, Sun M, Yu C, Shi G, Bao T, Liu J, Ni Y, Lu R, Wu Y and Fang Z (2020). Clinical features and rules of chinese herbal medicine in diabetic peripheral neuropathy patients. *Evid. Based Complement. Alternat. Med.,* **2020**: 5795264.

Zhong H, Wang L, Jia F, Yan Y, Xiong F, Li Y, Hidayat K and Guan R (2024). Effects of *Lactobacillus plantarum* supplementation on glucose and lipid metabolism in type 2 diabetes mellitus and prediabetes: A systematic review and meta-analysis of randomized controlled trials. *Clin. Nutr. ESPEN.,* **61**: 377-384.