

Research Progress on the Anti-Atherosclerotic Effect and Mechanism of Tetramethylpyrazine

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Abstract: Atherosclerosis is a chronic vascular disease and the most common pathological change of cardiovascular disease. Its pathogenesis is closely related to inflammation, oxidative stress, lipid accumulation, and calcinosis. Tetramethylpyrazine plays an anti-atherosclerotic role by regulating lipid metabolism, inhibiting foam cell formation, alleviating inflammation, inhibiting vascular calcification and abnormal platelet activation, and has a cardiovascular protective effect. Therefore, this paper summarized the research progress of the anti-atherosclerosis effect and mechanism of tetramethylpyrazine.

Keywords: Atherosclerosis; Chinese medicine; Tetramethylpyrazine; Mechanism of action; Research progress

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1. Introduction

Atherosclerosis (AS) is an important cause of death in most cardiovascular and cerebrovascular diseases. According to the newly released data report “China Cardiovascular Health and Disease Report 2022,” China currently has a staggering 330 million cardiovascular patients, and two out of every five deaths are due to cardiovascular diseases. Atherosclerosis is an important cause of death in most cardiovascular and cerebrovascular diseases^[1]. Atherosclerosis is characterized by damage to the inner lining of the affected artery, followed by lipid accumulation, hyperplasia of fibrous tissue, and calcium precipitation; the middle layer of the artery gradually undergoes degeneration and calcification at the same time, eventually leading to plaque formation, plaque rupture, internal bleeding, and thrombosis. This situation can lead to arterial wall thickening and hardening, causing vascular lumen stenosis^[2,3].

Tetramethylpyrazine (2,3,5,6-tetramethylpyrazine, TMP) is one of the main effective components of *Ligusticum striatum* and an active alkaloid extracted from the rhizome of *Ligusticum striatum*. Research shows that TMP has various pharmacological actions including delaying cell apoptosis, promoting angiogenesis, anti-inflammatory, antioxidant, and so on^[4]. In order to further study the mechanism of TMP against vascular endothelial damage, this paper summarizes the mechanism of action of TMP against atherosclerosis and its research progress and provides a scientific basis for rational drug use in clinical settings.

2. Anti-atherosclerosis effect of tetramethylpyrazine and its related mechanisms

2.1. Effects on lipid metabolism

AS is one of the risk factors and lesions of lipid metabolic disorders, peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors, including PPAR-alpha, PPAR-beta, and PPAR-gamma. Both PPAR- α and PPAR- γ are expressed in macrophages and play an anti-inflammatory role. PPAR- γ promotes lipid uptake and outflow from macrophages, so PPARs and their ligands may also be therapeutic targets for reducing inflammation and slowing the progression of AS [5]. We discovered a digital gene expression system that participates in regulating gene expression in lipid and energy metabolism pathways with the help of TMP. The occurrence of AS affects mitochondrial function by regulating the expression of genes involved in oxidative phosphorylation. TMP affects the function of mitochondrial function by down-regulating the oxidative phosphorylation pathway. PPAR signaling is exploited by TMP to suppress atherosclerosis. Therefore, the mechanisms of metabolic pathways involved in the treatment of AS with TMP are oxidative phosphorylation and PPAR signaling pathways [6]. Other studies have shown that TMP can effectively promote cholesterol metabolism in the aorta and liver, as well as the lipid content of the liver, and reduce aortic blood lipid levels while mitigating high-fat diet; it also plays a role in inhibiting liver function damage and oxidative stress caused by lipid peroxidation in mice and provides corresponding treatment. Liver X receptor (LXR) is an important transcription factor regulating cholesterol and lipid balance in the body. At present, the target genes directly regulated by LXRs include *ABCA1* and *ABCG1*, regulating intracellular cholesterol efflux protein and *CYP7A1* rate-limiting enzyme promoting bile acid metabolism. TMP can promote the reversal of systemic cholesterol transport by inhibiting oxidative stress and LXR α /ABCA1 signaling pathway. The potential mechanism is that TMP acts as a ligand agonist to up-regulate the expression of LXR and PPAR genes. In the process, TMP may enhance the PPAR-LXR α -ABCA1 pathway to improve lipid metabolism [7]. Sterol regulatory element binding proteins (SREBPs) on the endoplasmic reticulum membrane have an important relationship with lipid biosynthesis through complex regulatory mechanisms. Inhibiting the activation of SREBPs can reduce the biosynthesis of cholesterol, fatty acids, and other components. Thus, SCAP/SREBP signaling is a major pathway regulating lipid metabolism [8]. Zhang *et al.* showed that TMP helped to effectively reduce lipid levels in ApoE $^{-/-}$ mice fed a high-fat diet. Progesterin and adipoQ receptor 3 (PAQR3), as a novel anchor-protein of the SCAP/SREBP complex, also regulates cholesterol biosynthesis and SREBP activation. Lipid metabolism disorders and AS may be optimized through PAQR3 and inhibition of SCAP/SREBP-1c signaling pathways, thus inhibiting AS progression and improving lipid metabolism disorders [7].

TMP reduces the levels of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) in the plasma of atherosclerotic rabbits, while increasing the levels of high-density lipoprotein cholesterol (HDL-C) [9]. Wang *et al.* showed that TMP could significantly reduce the formation of arteriosclerotic plaque and intimal hyperplasia in rabbits, as well as reduce the intima/media thickness ratio and the number of monocytes after experimental verification [10].

2.2. Inhibition of the transformation of macrophages into foam cells

The clearance receptors (SRs) contained in the inner membrane of macrophages play a key role in oxidized LDL uptake, such as cluster of differentiation 36 (CD36) and class A clearance receptor (SR-A). Intracellular lipid efflux mainly occurs through reverse cholesterol transport (RCT). Some proteins, such as ATP-binding cassette transporters A1 (ABCA1) and ABCG1, have been reported to play a vital role in the process of RCT. In the aspect of human atherosclerotic lesions, many genetic cohort studies have pointed out the importance of p38 MAPK and PI3K/Akt signal pathways. Mediated by TMP, the PI3K/Akt and p38 MAPK pathways split

the original AMP-activated protein kinase signaling pathway through clearance receptors (CD36, SR-A). This prompts the upregulation of ABCA1 and ABCG1, which suppresses macrophage lipid accumulation ^[11].

2.3. Inhibition of chronic inflammation

AS is a chronic inflammatory disease on the vascular wall characterized by autoimmune disease. Oxidized phospholipids combined with Toll-like receptors (TLRs) trigger inflammation of the blood vessel walls, TLRs are a group of pattern recognition receptors (PRRs) and can cause proinflammatory signals. In the clinic, oxidized LDL is a plaque inflammation marker. Natural LDL may also be absorbed by macrophages through micropinocytosis or by phagocytosis in the form of complexes or cholesterol crystals ^[12,13]. Research results show that by blocking the extracellular signal-regulated kinase (ERK), p38 MPAK, and nuclear factor κ B (NF- κ B) signaling pathways, TMP reduced the expression of IL-8 in the lipopolysaccharide (LPS)-stimulated human umbilical vein endothelial cells (HUVEC) ^[14]. In addition, Chen *et al.* suggested that TMP may interfere with the Rho/ROCK signaling pathway and help to maintain the balance of endothelial cells, thereby reducing the degree of LPS-induced inflammatory injury in HUVECs ^[15].

Oxidative stress is the main cause of vascular endothelial dysfunction. Oxidized LDL promotes atherosclerosis by accumulating in the blood vessel wall, causing early atherosclerotic vascular dysfunction, and significantly increasing the potential risk of cardiovascular disease. Therefore, the use of free radical scavengers may help to improve vascular disease induced by oxidative stress ^[16,17]. Injury to vascular endothelial cells (VECs) is an early event in the development of atherosclerosis, leading to VEC apoptosis. Subsequently, the accumulation of VECs or vascular smooth muscle cells (VSMCs) and macrophages/foam cells and the defective clearance of apoptotic cells gradually form atherosclerotic plaques. Jang *et al.* found that TMP acts as an antioxidant and can reduce oxidative stress. TMP can achieve these effects through two different ways: (1) TMP directly eliminates ROS; (2) TMP inhibits the activation of the nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NAD/NADP) oxidase system and reduces the production of malondialdehyde (MDA) in lipid peroxidation, thereby alleviating endothelial damage in rats. Therefore, TMP improves atherosclerosis in rats by inhibiting oxidative stress ^[9]. TMP can reduce oxidative damage by quenching ROS production, promoting the activity of endogenous antioxidant enzymes, blocking the S phase in the cell cycle, and apoptosis inhibition pathway ^[18]. Another mechanism is that TMP can regulate mitochondrial function, reduce oxidative stress, and prevent homocysteine-induced apoptosis in HUVECs. In addition, TMP prevented oxidative stress-induced endothelial dysfunction in isolated rat aortas without affecting endothelial nitric oxide synthase (eNOS) expression ^[19,20].

2.4. Inhibition of vascular calcification

Vascular calcification (VC) is widely used as an independent risk factor for cardiovascular events. TMP plays a protective role in β -GP-induced coronary artery calcification by activating the PPAR- γ pathway. Vascular smooth muscle cell (VSMC) proliferation is primarily associated with the expression of PPARs. Activation of PPARs functions to curb the proliferation and migration of VSMCs, thereby reducing vascular remodeling, and simultaneously exerting anti-inflammatory and antiproliferative effects. In addition, PPAR has been validated to possess several physiological functions, including anti-inflammatory and anti-atherogenic effects, and also aids in improving left ventricular remodeling. As an important regulator, PPAR plays an essential role in lipid and cardiovascular diseases. Under the action of alkaline phosphatase (ALP), β -glycerophosphate (β -GP) is decomposed to release inorganic phosphate, which leads to the increase of local phosphorus concentration and stimulates the high expression of ALP in VSMCs, thereby promoting the calcification process. Calcified vessels can adversely affect hemodynamics, thereby negatively affecting cardiac function and increasing the risk of cardiovascular events in patients ^[21].

During atherosclerotic plaque formation, oxidized LDL can promote angiogenesis, making the plaque more susceptible to damage and leading to the occurrence of intravascular thrombosis^[22,23]. Vascular endothelial growth factor (VEGF), as a highly specific endothelial growth factor, has the ability to promote vascular endothelial cell division, proliferation, and migration, promote extracellular matrix degeneration, and increase vascular permeability. It also has the effect of promoting inflammatory response and anti-apoptosis^[24]. Recent studies have indicated that TMP inhibits oxidized LDL-induced endothelial cell adhesion molecules, VEGF, VEGF receptor 2 (VEGFR2), Notch1, Jagged1, and Hes1 through the VEGF/VEGFR2 and Jagged1/Notch1 signaling pathways, which helps achieve anti-angiogenic effects^[25,26]. One study revealed that TMP inhibited angiogenesis by regulating the VEGF/Hippo/YAP pathway^[27].

2.5. Inhibition of abnormal platelet activation

Abnormally activated platelets play a crucial role in intravascular thrombotic events. TMP affects the inflammatory response of vascular endothelial cells by interfering with p38 MAPK and NF- κ B signaling pathways. TMP has an antiplatelet function at the same time^[28-30]. Li *et al.* observed that TMP inhibits adenosine diphosphate-induced platelet aggregation and platelet thromboxane A2 release by blocking Akt signaling pathways, contributing to reducing the development of atherosclerosis^[31]. Iron overload can lead to vascular endothelial injury^[32]. Recent studies have shown that TMP has been proven to be an effective antioxidant^[33]. In addition, the study also pointed out that the TMP can interfere with hyperlipemia mice signaling factor and activation of transcription activator 3 (STAT3) phosphorylation reaction, in order to achieve an anti-atherosclerotic effect^[34]. TMP has a significant positive effect on the prevention of coronary heart disease, mainly displayed in inhibiting platelet activation and aggregation.

3. Conclusion

Tetramethylpyrazine, an effective component of Chinese herbal medicine Chuanxiong, has been used in China for thousands of years, and its clinical application is extremely extensive, with high clinical efficacy. TMP is widely used and valuable in contemporary pharmacological research. The anti-atherosclerosis effects of TMP are mainly related to the regulation of lipid metabolism, the inhibition of foam cell formation, the reduction of inflammatory response, the inhibition of vascular calcification, and the inhibition of abnormal platelet activation. These effects can alleviate the occurrence and development of AS to a certain extent. Although there are many relatively in-depth studies on the modern pharmacological effects of TMP and reports on its derivatives and combination with other drugs, its mechanism of action is still unclear. It is necessary to strengthen the research work in this aspect, develop a preparation of TMP with better efficacy and higher stability, and further clarify the anti-atherosclerosis mechanism of TMP, so as to provide a more scientific and effective basis for the clinical treatment of cardiovascular and cerebrovascular diseases with TMP.

Disclosure statement

The authors declare no conflict of interest.

References

- [1] The Writing Committee of the Report on Cardiovascular Health Diseases in China, 2023, Interpretation of Report on Cardiovascular Health and Diseases in China 2022. Chinese Journal of Cardiovascular Medicine, 28(04): 297–312.

- [2] Libby P, Buring JE, Badimon L, et al., 2019, Atherosclerosis. *Nat Rev Dis Primers*, 5(1): 56.
- [3] Libby P, 2021, The Changing Landscape of Atherosclerosis. *Nature*, 592(7855): 524–533.
- [4] Lin J, Wang Q, Zhou S, et al., 2022, Tetramethylpyrazine: A Review on Its Mechanisms and Functions. *Biomedicine & Pharmacotherapy*, (150): 113005.
- [5] Xu P, Zhai Y, Wang J, 2018, The Role of PPAR and Its Cross-Talk with CAR and LXR in Obesity and Atherosclerosis. *Int J Mol Sci*, 19(4): 1260.
- [6] Fu R, Zhang Y, Guo Y, et al., 2014, Digital Gene Expression Analysis of the Pathogenesis and Therapeutic Mechanisms of Ligustrazine and Puerarin in Rat Atherosclerosis. *Gene*, 552(1): 75–80.
- [7] Zhang Y, Ren P, Kang Q, et al., 2017, Effect of Tetramethylpyrazine on Atherosclerosis and SCAP/SREBP-1c Signaling Pathway in ApoE^{-/-} Mice Fed with a High-Fat Diet. *Evidence-Based Complementary and Alternative Medicine*, (2017): 1–8.
- [8] Brown MS, Radhakrishnan A, Goldstein JL, 2018, Retrospective on Cholesterol Homeostasis: The Central Role of Scap. *Annual Review of Biochemistry*, 87(1): 783–807.
- [9] Jiang F, Qian J, Chen S, et al., 2011, Ligustrazine Improves Atherosclerosis in Rat Via Attenuation of Oxidative Stress. *Pharmaceutical biology*, 49(8): 856–863.
- [10] Wang G, Shi C, Sun M, et al., 2013, Tetramethylpyrazine Attenuates Atherosclerosis Development and Protects Endothelial Cells from ox-LDL. *Cardiovascular Drugs and Therapy*, 27(3): 199–210.
- [11] Duan J, Xiang D, Luo H, et al., 2017, Tetramethylpyrazine Suppresses Lipid Accumulation in Macrophages Via Upregulation of the ATP-Binding Cassette Transporters and Downregulation of Scavenger Receptors. *Oncol Rep*, 38(4): 2267–2276.
- [12] Wolf D, Ley K, 2019, Immunity and Inflammation in Atherosclerosis. *Circulation Research*, 124(2): 315–327.
- [13] Falck-Hansen M, Kassiteridi C, Monaco C, 2013, Toll-Like Receptors in Atherosclerosis. *International Journal of Molecular Sciences*, 14(7): 14008–14023.
- [14] Li X, He J, Liu H, et al., 2009, Tetramethylpyrazine Suppresses Interleukin-8 Expression in LPS-Stimulated Human Umbilical Vein Endothelial Cell by Blocking ERK, p38 and Nuclear Factor- κ B Signaling Pathways. *Journal of Ethnopharmacology*, 125(1): 83–89.
- [15] Chen J, Wang H, Gao C, et al., 2019, Tetramethylpyrazine Alleviates LPS-Induced Inflammatory Injury in HUVECs by Inhibiting Rho/ROCK Pathway. *Biochemical and Biophysical Research Communications*, 514(1): 329–335.
- [16] Chrissobolis S, Miller AA, Drummond GR, et al., 2011, Oxidative Stress and Endothelial Dysfunction in Cerebrovascular Disease. *Front Biosci (Landmark Ed)*, 16(5): 1733–1745.
- [17] Förstermann U, Xia N, Li H, 2017, Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. *Circulation Research*, 120(4): 713–735.
- [18] Li WM, Liu HT, Li XY, et al., 2010, The Effect of Tetramethylpyrazine on Hydrogen Peroxide-Induced Oxidative Damage in Human Umbilical Vein Endothelial Cells. *Basic & Clinical Pharmacology & Toxicology*, 106(1): 45–52.
- [19] Fan X, Wang E, He J, et al., 2019, Ligustrazine Protects Homocysteine-Induced Apoptosis in Human Umbilical Vein Endothelial Cells by Modulating Mitochondrial Dysfunction. *J Cardiovasc Transl Res*, 12(6): 591–599.
- [20] Ni X, Wong SL, Wong CM, et al., 2014, Tetramethylpyrazine Protects against Hydrogen Peroxide-Provoked Endothelial Dysfunction in Isolated Rat Aortic Rings: Implications for Antioxidant Therapy of Vascular Diseases. *Evidence-Based Complementary and Alternative Medicine*, (2014): 1–10.
- [21] Li H, Yang M, 2022, Ligustrazine Activates the PPAR-Gamma Pathway and Plays a Protective Role in Vascular Calcification. *Vascular*, 30(6): 1224–1231.
- [22] Camare C, Pucelle M, Negre-Salvayre A, et al., 2017, Angiogenesis in the Atherosclerotic Plaque. *Redox Biol*, (12): 18–34.

- [23] Wang L, Li G, Chen Q, et al., 2015, Octanoylated Ghrelin Attenuates Angiogenesis Induced by ox-LDL in Human Coronary Artery Endothelial Cells Via the GHSR1a-Mediated NF- κ B Pathway. *Metabolism*, 64(10): 1262–1271.
- [24] Apte RS, Chen DS, Ferrara N, 2019, VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell*, 176(6): 1248–1264.
- [25] Park JA, Kwon YG, 2018, Hippo-YAP/TAZ Signaling in Angiogenesis. *BMB Rep*, 51(3): 157–162.
- [26] Yuan R, Shi W, Xin Q, et al., 2018, Tetramethylpyrazine and Paeoniflorin Inhibit Oxidized LDL-Induced Angiogenesis in Human Umbilical Vein Endothelial Cells Via VEGF and Notch Pathways. *Evidence-Based Complementary and Alternative Medicine*, (2018): 1–12.
- [27] Zhu X, Shan Y, Yu M, et al., 2021, Tetramethylpyrazine Ameliorates Peritoneal Angiogenesis by Regulating VEGF/Hippo/YAP Signaling. *Frontiers in Pharmacology*, (12): 649581.
- [28] Sheu JR, Kan YC, Hung WC, et al., 1997, Mechanisms Involved in the Antiplatelet Activity of Tetramethylpyrazine in Human Platelets. *Thromb Res*, 88(3): 259–270.
- [29] Zhang Y, Ma XJ, Guo CY, et al., 2016, Pretreatment with a Combination of Ligustrazine and Berberine Improves Cardiac Function in Rats with Coronary Microembolization. *Acta Pharmacol Sin*, 37(4): 463–472.
- [30] Sheu JR, Kan YC, Hung WC, et al., 2000, The Antiplatelet Activity of Tetramethylpyrazine is Mediated Through Activation of NO Synthase. *Life Sci*, 67(8): 937–947.
- [31] Li L, Chen H, Shen A, et al., 2019, Ligustrazine Inhibits Platelet Activation Via Suppression of the Akt Pathway. *Int J Mol Med*, 43(1): 575–582.
- [32] Vinchi F, Porto G, Simmelbauer A, et al., 2020, Atherosclerosis is Aggravated by Iron Overload and Ameliorated by Dietary and Pharmacological Iron Restriction. *European Heart Journal*, 41(28): 2681–2695.
- [33] Zhou Q, Chen S, Li H, et al., 2020, Tetramethylpyrazine Alleviates Iron Overload Damage in Vascular Endothelium Via Upregulating DDAHII Expression. *Toxicology in Vitro*, (65): 104817.
- [34] Zhang M, Sun MY, Guo CY, et al., 2019, Effect of Tetramethylpyrazine and Hyperlipidemia on Hepcidin Homeostasis in Mice. *Int J Mol Med*, 43(1): 501–506.

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