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Review Article

Trimethylamine N-oxide, The Important Therapeutic Target for Heart Failure

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Abstract

Heart Failure (HF) is a common cause of morbidity and mortality and is characterized by high morbidity and mortality. The pathophysiology of HF is complex, and it is important to elucidate the molecular mechanism of HF, identify key agents, and conduct further research to discover new therapeutic targets to decrease the incidence and economic burden associated with HF. In 2011, a metabolomic-based study identified that Trimethylamine N-Oxide (TMAO) could predict Cardiovascular Diseases (CVDs) risk. In subsequent studies, TMAO has been found to influence the pathological processes of various CVDs significantly. This review clarifies the effect of TMAO on heart failure from three aspects: the production of Trimethylamine (TMA) and TMAO, TMAO in the pathological process of HF and TMAO can be the therapeutic target for HF, hoping to provide reference for clinical treatment of HF and research on HF.

Keywords: TMAO; Heart failure; cardiovascular diseases

Abbreviations: HF: Heart Failure; TMAO: Trimethylamine N-Oxide; CVDs: Cardiovascular Diseases; TMA: Trimethylamine; FMOs: Flavin-Containing Monooxygenases; FMO3: FMO Isoform 3; DMB: 3,3- Dimethyl-1-Butanol; NF- κB: Nuclear Factor-k-Gene Binding; TGF-β1: Transforming Growth Factor beta-1; VECs: Vascular Endothelial Cells; NLRP3: Leucine-Rich Repeat Protein 3; FMD: Fasting-Mimicking Diet; CKD: Chronic Kidney Disease; TCM: Traditional Chinese Medicine; BBR: Berberine; B - GB: Ginkgolide B

Introduction

As the end stage of various Cardiovascular Ddiseases (CVDs), Heart Failure (HF) is a group of clinical syndromes caused by abnormal cardiac structural and functional changes due to various factors, leading to ventricular systolic and diastolic dysfunctions [1]. It is a common cause of morbidity and mortality. Although an increasing number of medical scientists and researchers have focused on therapeutic targets for HF and great progress has been made in the treatment strategy and new drug research, the incidence of HF is still very high, and the number of patients with HF is increasing. According to statistics, there are 5.7 million people over the age of 20 years with HF in the United States, and the number of adults with HF is expected to rise to 8 million by 2030 [2]. Overall, the prognosis of patients with HF remains poor, and readmission and mortality rates remain high. In a study, 83% of the patients were admitted to the hospital at least once a year, and 43% were admitted at least four times a year [3]. All-cause mortality ranged from 21.6% to 36.5% and 6.9% to 15.6% in patients with acute HF and chronic HF, Respectively [4]. The pathophysiology of HF is complex, including hemodynamic abnormalities, neuroendocrine system activation, cardiac remodeling, and inflammatory response [5]. The investigation of the mechanisms underlying the pathological progression of HF is still ongoing. It is important to elucidate the molecular mechanism of HF, identify key agents, and conduct further research to discover new therapeutic targets to decrease the incidence and economic burden associated with HF.

Recent studies have identified the importance of gut microbiota in the pathophysiology of HF [6-13]. Reduced cardiac output and altered systemic circulation due to HF can cause intestinal hypoperfusion, intestinal mucosal ischemia, and edema

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[12]. This increases the likelihood of intestinal barrier damage and permeability, promotes microbial translocation, leads to microbial metabolites entering the blood circulation, increases proinflammatory mediators, and finally leads to chronic inflammation in patients with HF [7,13]. Increased intestinal permeability leads to bacterial translocation across the intestinal barrier, increasing endotoxin and other bacterial wall compounds, which may exacerbate the pathophysiological progression of HF [9,14]. Higher levels of enteropathogenic candida, including Campylobacter spp, Shigella spp, and Yersinia enterocolitica, have been observed in patients with CHF [15].

In 2011, a metabolomic-based study identified metabolites of the dietary lipid phosphatidylcholine, including choline, Trimethylamine N-Oxide (TMAO), and betaine, in an independent large clinical cohort. This research also demonstrated that they could predict CVD risk. The germ-free mice in these studies confirmed that dietary choline and gut microbiota significantly affect TMAO production, macrophage cholesterol accumulation, and foam cell formation. This discovery that gut microbiota-dependent metabolism of dietary phosphatidylcholine contributes to the pathogenesis of CVD opened another way for diagnosing and treating CVDs [16]. In subsequent studies, researchers found that TMAO is associated with a variety of CVDs, such as atherosclerosis [17], hypertension [18], type 2 diabetes [19], myocardial infarction [20], and HF [21]. TMAO has been found to influence the pathological processes of various CVDs significantly. This review aims to discuss the generation of Trimethylamine (TMA) and TMAO, the effect of TMAO in the course of HF, and the potential of TMAO as an important therapeutic target for HF, to have significant references for clinical work.

Production of TMA and TMAO

TMA nutritional precursors, including choline, betaine, croton betaine, trimethyl lysine, G-butyl betaine, phosphatidylcholine, glycerol phosphate choline, and TMAO [22], can be transformed to TMA by particular gut microbial enzymes. Red meat, fish, eggs and products made from cow's milk are rich in these components. Most TMA enter the circulatory system and are subsequently oxidized to TMAO by flavin-containing monooxygenases (FMOs) [23-25]. In the five functional enzymes in the host, FMO isoform 3 (FMO_3) is the crux in this process, with the highest conversion efficiency [26]. In most cases, the kidneys clear TMAO, and the remaining TMAO is reduced to TMA. This process is finished by TMAO reductase in the intestines [27].

It has been shown that changes in the gut microbiota caused by HF can result in changes in TMAO. Nine human gut strains, including Firmicutes and Proteobacteria, were able to produce TMA [28]. Corresponding to these findings, in patients with HF, the increased proportion of gut strains is consistent with the nine human gut strains capable of producing TMA, suggesting that, changes in the gut microbiota can affect TMAO levels. This process is achieved by adjusting the intestinal TMA synthesis.

However, the inherent causes may also significantly affect TMAO levels in humans. Human genes directly influence gut microbiota and can affect immune pathways and metabolic phenotypes [29,30]. In some chronic diseases, such as type 2 diabetes, disease susceptibility can be partly because of genetics that changes the gut microbiome [31]. In an overall analysis of genome-wide gut microbial hosts, microbiota and genetic factors accounted for about 10% of the variation in gut microbiota [32]. On the basis of the significant role of genetics in the gut microbiota, genetics could be thought to play a role in TMAO production.

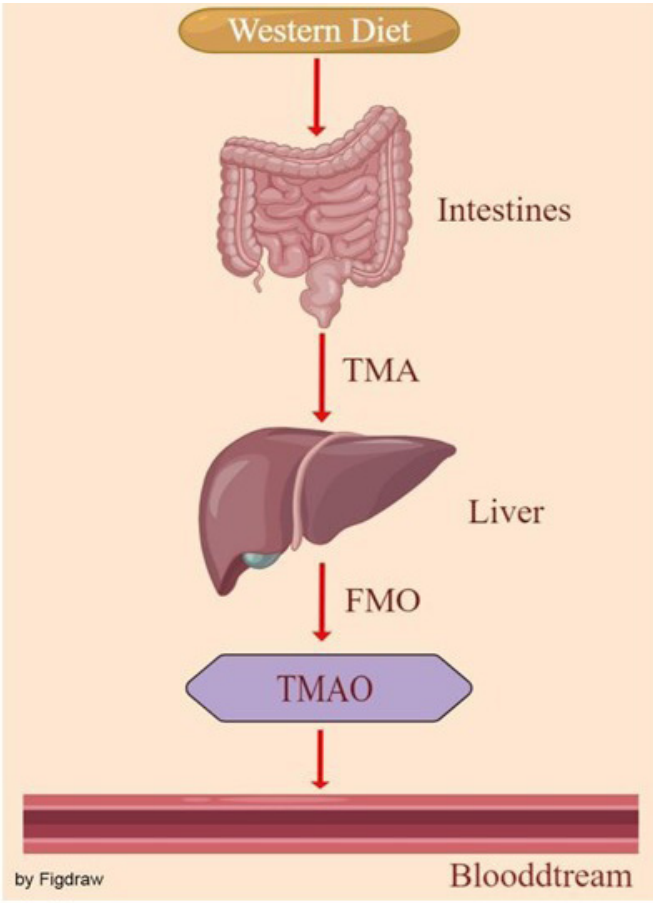

Figure 1: Western diets are abundant in precursors of TMA, which are converted into TMA by specific intestinal microbial enzymes. Most TMA is oxidized to TMAO via FMO. Among the FMOs, FMO3 is key in this process, with the highest conversion efficiency.

TMAO in the Pathological Process of HF

Plasma TMAO level is significantly higher in patients with HF with a preserved ejection fraction [33]. Rats induced by cross-sectional aortic coarctation are excellent animal models of HF. In this model, circulating TMAO levels were significantly higher than in the sham-operated group [34]. In an animal study, mice fed with TMAO or choline exhibited more severe left ventricular dilatation, pulmonary edema, and myocardial fibrosis; the circulating brain natriuretic peptide levels were higher than those in control mice. In patients with HF, higher plasma TMAO levels are associated with poorer prognosis [35]. The effect of TMAO on HF progression is significant [36].

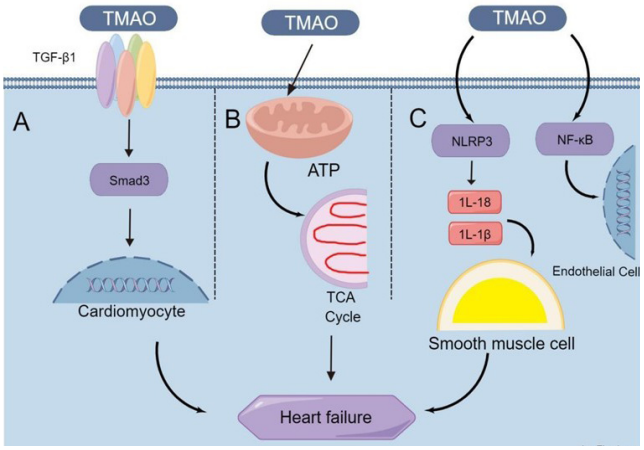

Figure 2: TMAO mainly induces HF by (a) aggravating myocardial hypertrophy and fibrosis, (b) aggravating mitochondrial dysfunction, and (c) inducing inflammatory responses.

TMAO Exacerbates Myocardial Hypertrophy and Fibrosis

In an animal study, TMAO induced cardiac hypertrophy and fibrosis via Smad3 signaling, and a specific inhibitor of Smad3 blocked this effect [37]. 3,3-Dimethyl-1-Butanol (DMB), an inhibitor of TMAO biosynthesis, can prevent myocardial hypertrophy, fibrosis, and inflammation by negatively influencing the p65 Nuclear Factor-k-Gene Binding (NF-κB) and Transforming Growth Factor Beta-1 (TGF- β1)/Smad3 signaling pathways [38]. TMAO can also reprogram skin fibroblasts, adipocyte progenitors, and Vascular Endothelial Cells (VECs) into myofibroblasts via TMAO receptor protein R-like endoplasmic reticulum kinase. Under TGF-β1 stimulation, FMO3 expression can be observed in skin fibroblasts [39].

TMAO Exacerbated Mitochondrial Dysfunction

Plasma TMAO increased after feeding mice with 120 mg/kg of TMAO for 8 weeks. Impaired pyruvate and fatty acid oxidation disrupted cardiac energy metabolism and mitochondrial dysfunction, leading to ventricular remodeling and HF [40]. Elevated TMAO levels hurt cardiomyocyte mechanics and intracellular calcium handling, and the cardiomyocytes exhibited glycogen accumulation, a higher number of mitochondria, and lipofuscin-like pigment deposition after TMAO exposure. Urolithin B- glucuronide caused cellular contractility and calcium dynamics to recover completely and reduced glycogen accumulation morphologically [41]. There is a potential relationship between TMA metabolism and mitochondrial dynamics, indicating a new direction for future research [42].

TMAO Induced an Inflammatory Response

Inflammation is a vital factor in HF progression. TMAO can stimulate hepatocytes to produce exosomes widely distributed throughout the body and promote vascular inflammation. TMAO can also affect VECs by promoting the production of exosomes that carry important genetic information [43,44]. After a choline diet feeding, the aortas of low-density lipoprotein receptor-deficient mice showed increased inflammatory gene expression than those of controls. TMAO injection can induce the same inflammatory factors and activate mitogen-activated protein kinase, extracellular signal-related kinase, and NF-κB signaling cascades, leading to inflammation of vascular smooth muscle cells [45]. TMAO activated the Leucine-Rich Repeat Protein 3 (NLRP3) inflammasome to induce vascular inflammation, and NLRP3 inflammasome activation is mediated via the inhibition of the sirtuin 3-superoxide dismutase 2- mitochondrial reactive oxygen species signaling pathway. When NLRP3 inflammasome is activated, it releases inflammatory cytokines, including interleukin (IL)-1β and IL-18, ultimately leading to endothelial cell inflammation [46,47]. Meanwhile, TMAO levels decreased in NLRP3-deficient mice [48]. TMAO boosted the expression of vascular cell adhesion molecule-1, promoted monocyte adherence, and activated protein kinase C and p-NF-κB [49]. Molecular studies have shown that in sedentary mice fed a Western diet, plasma TMAO levels were elevated, myocardial inflammation and fibrosis were increased, and voluntary exercise could inhibit all of these [5].

TMAO can be the Therapeutic Target for HF

With increasing research on the important role of TMAO in the pathological process of HF, much progress has been made in reducing TMAO levels in various ways to slow down the symptoms of HF.

Changes in Lifestyle Habits

The TMAO levels are closely related to dietary habits [51]. Chronic exposure to a high-fat diet alters intestinal epithelial physiology and enhances E. coli cholinergic metabolism. In addition, cholinergic metabolism in E. coli increases the circulating TMAO [52]. Fasting-Mimicking Diet (FMD) is a type of intermittent fasting that restricts calories and limits protein sources of animal origin, and it is applied in daily cycles for 5 days. In one study, FMD decreased plasma TMAO levels by 2-fold, but it was not evident in volunteers eating a vegetarian diet. This suggests that FMD is a viable strategy for decreasing plasma TMAO levels by restricting the intake of calories and animal-derived proteins [53]. TMAO levels are reduced by either dietary TMAO or gut microbial blockade of TMAO generation. Furthermore, these therapeutic strategies alleviate the TMAO levels [54]. The metabolism of dietary L-carnitine and TMA in gut microbiota produces TMAO and aggravates atherosclerosis. Omnivorous volunteers consuming L-carnitine via microbiota-dependent mechanisms produced significantly higher TMAO levels than vegetarians [55]. Eating eggs and fish in bulk also causes markedly higher levels of plasma TMAO [56-60], and the impact of saltwater fish on TMAO is even greater [61]. A vegan diet can significantly reduce plasma TMAO levels [62]. The Mediterranean diet, mainly with fruits and vegetables, nuts, and whole grains, supplemented with small amounts of meat, eggs, and sugar, can optimize the gut microbiota, thus markedly reducing the incidence of HF [63]. Urinary TMAO levels were lower in participants on the Mediterranean diet than in those on the omnivorous diet [64]. Concentrations of TMAO are lower in the presence of a Mediterranean diet, even with a certain intake of red meat [65]. Regular consumption of cocoa and coffee may be an effective strategy for decreasing TMAO levels [66]. Ligustrum robustum is rich in flavonoids. It can reduce TMAO levels by modulating gut microbiota to attenuate diet-induced atherosclerosis [67]. In addition, consuming a vitamin D-rich diet can also reduce TMAO levels. A clinical study showed that leptin and TMAO levels were significantly reduced in vitamin D-deficient patients supplemented with 25-hydroxyvitamin D3 [68]. Vitamin D supplementation can modulate intestinal flora in mice fed with high-choline diets to reduce plasma TMAO levels [69]. Physical activity is strongly associated with cardiovascular health. Moderate to vigorous physical activity is associated with lower TMAO levels [70].

Probiotic Flora

Probiotics are live microorganisms that benefit intestinal health [71]. They have a significant effect in adjusting the gut microbiota and maintaining the intestinal environment. A growing body of research suggests that probiotics may be associated with the modulation of myocardial recovery in patients with HF. For instance, in rats, Lactobacillus plantarum protects the heart during myocardial ischemia and attenuates reperfusion injury by reducing the left ventricular infarct size and improving cardiac recovery [72]. Both Enterobacter aerogenes ZDY01 and Lactobacillus plantarum ZDY04 can reduce TMAO levels, showing that the positive effects of them on the heart may be partially realized by reducing TMAO levels [73,74]. Enterobacter aerogenes ZDY01 utilized cecal TMA as a nutrient to reduce cecal TMA and serum TMAO levels, but not via altering hepatic FMO3 expression and gut microbiota composition [75]. In an animal study, the researchers chose three lactobacilli with strong adherence capability, and mice with high choline were fed the Multistrain Formula (MF). On days 7, 14, and 28, researchers

observed that, in the MF, L. amylovorus LAM1345, Lpb. plantarum LP1145, and Lim. fermentum LF33 significantly decreased serum TMAO levels [76]. A. muciniphila can reduce the level of TMAO by adjusting the microbial enzymes associated with TMA synthesis. In cold environments, these probes are destroyed [77].

Microbial TMA Inhibitors

In Lu'an GuaPian tea, the Kaempferol 3-O-rutinoside, quercetin 3-O-rhamnosylgalactoside, kaempferol 3-O-rhamnosylgalactoside, and myricetin 3-O-galactoside bind well with TMA-lyase [78]. Aiming at a major TMA-generating enzyme pair, CutC and CutD (CutC/D), researchers have developed potent, time-dependent, and irreversible inhibitors that do not affect commensal viability. In animal models, the Tookccccc CutC/D inhibitor significantly decreased plasma TMAO levels. This process lasted for 3 days [79]. DMB is a substance present in extra-virgin olive oil. It is a kind of the main components of the Mediterranean diet. DMB reduced circulating TMAO levels in choline-fed mice by inhibiting its formation [38]. Gallic acid and chlorogenic acid also exhibited strong TMA inhibitory activities. Up to 80–90% of TMA was inhibited, with a half-maximal inhibitory concentration of approximately 5 mM [80].

Natural Phytochemicals and Chinese Medicine

Some chemicals in plants can reduce plasma TMAO levels. In human volunteers with high TMAO levels, TMAO formation was reduced after a week of raw garlic juice intake [81]. In a rat model of impaired cardiac function established by intraperitoneal injection of TMAO for 50 days, after administering Ganoderma lucidum spores extract, a decrease in serum TMAO level was observed [82]. Resveratrol is a polyphenolic plant anti-toxin. It has anti-inflammatory effects; according to the report, it can alleviate atherosclerosis caused by TMAO by changing intestinal microbiota composition and accelerating the synthesis of hepatobiliary acid [83]. Perinatal resveratrol treatment protects adult male offspring from maternal Chronic Kidney Disease (CKD) and prenatal exposure to asymmetric dimethylarginine-related nitric oxide deficiency and TMAO-induced hypertension, mainly by regulating intestinal flora and its metabolites [84,85]. In resveratrol-fed tilapia, the TMAO level was significantly lower than that in the high-fat diet group at 3, 6, and 9 weeks [86]. Resveratrol and β-sitosterol can also inhibit the production of TMA [87]. The black raspberry extract decreased serum low-density lipoprotein cholesterol and TMAO in rats by affecting the gut bacterial community and microbial metabolites [88]. Traditional Chinese Medicine (TCM) and its extract could also effectively reduce TMAO levels [89]. Berberine (BBR), an extract of Coptis chinensis, effectively reduces plasma TMAO levels [90,91]. BBR inhibited FMO3 and generated plasma TMA/TMAO levels in hypertensive mice [92]. Ginkgolide B (GB) is a natural product extracted from Ginkgo leaves. GB could inhibit the mRNA and protein expression of FMO3 and then reduce TMA/TMAO concentrations [93]. In Xuefu Zhuyu decoction, naringin, paeoniflorin, β-ecdysterone, 18β-glycyrrhizic acid, amygdalin, albiflorin, and saikosaponin A reduced FMO3 activity, and TMAO synthesis [94]. Gypenosides can remodel the microbiota, reduce plasma TMAO levels, and affect TMA-lyase, which is required to convert choline to TMA in the gut microbiota. They can also negatively affect tricarboxylic acid and lipid metabolism enzymes, affecting TMAO and lipid metabolism [95]. Danlou tablet combined with Salvia miltiorrhiza ligustrazine injection decreased TMAO levels in patients with central sleep apnea [96]. Alisma orientalis is a TCM combined with various medicinal plants. In an animal study, it decreased the serum TMAO and hepatic FMO_3 levels [97]. In the treatment of acute ischemic stroke, Sanhuang Xiexin Decoction can also decrease the levels of TMAO [98].

Antibiotic

After the use of antibiotics, the plasma levels of TMAO were significantly decreased. Nevertheless, a month after antibiotic withdrawal, the levels of TMAO increased again [99]. TMAO may lead to the failure of anti-infectious therapy. TMAO increased the minimum inhibitory concentration of quinolones, aminoglycosides, and β-lactams as the concentration of TMAO increased and increased the lethal dose of antibiotics against E. coli [100]. Nevertheless, it is controversial whether antibiotics have a protective effect in patients with HF. However, not all antibiotics are effective against TMAO. A randomized placebo-controlled trial showed that short-term rifaximin treatment could not reduce gut-derived cardiovascular toxins and inflammatory cytokines in patients with CKD [101]. In patients with HF, although polymyxin B can reduce proinflammatory cytokine production, its application is limited owing to its toxicity [102]. This suggests that, before prescribing antibiotics, doctors should carefully consider safety implications and the potential for antibiotic resistance, necessitating the cautious use of antibiotics.

Other Drugs

Statins have lipid-lowering and many CVD-reducing effects, providing new possibilities for clinicians to choose treatments [103]. Statins are also associated with TMAO. In clinical studies, the plasma TMAO level of patients treated with statins was significantly reduced, indicating that the decrease of TMAO mediated by statins can benefit the cardiovascular system besides improving lipid status and alleviating inflammation [104,105]. Metformin has a therapeutic effect by adjusting the intestinal flora structure and metabolic function. In choline-fed C57BL/6J mice, oral metformin significantly decreased the serum TMAO levels. Metformin can inhibit serum TMAO levels by remodeling the intestinal flora of choline-producing TMA [106,107]. A clinical trial has shown that the cardioprotective drug meldonium can increase the excretion of TMAO in urine and reduce the concentration of TMAO in plasma [108]. Enalapril also decreased the plasma concentration of TMAO in an animal experiment [109].

Conclusion

Although HF is still a serious disease threatening human health, research on its diagnosis and treatment is ongoing. Many studies have shown a direct association between CVD and TMAO. TMAO, a metabolite of the intestinal flora, provides an important reference for analyzing the pathological process of HF and optimizing its treatment. It has many effects on the pathological mechanisms of HF, such as aggravating myocardial hypertrophy, fibrosis, and mitochondrial dysfunction and inducing inflammatory reactions. There has also been much progress in the study of TMAO's impact on HF. It has been found that changing living habits, the use of probiotics, microbial TMA-lyase inhibitors, natural phytochemicals, TCM, and antibiotics can all affect HF by reducing TMAO levels. Despite many achievements, research on TMAO has only recently begun. With the advancement of TMAO research, it is believed that TMAO can profoundly affect the treatment of patients with HF.

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