The Role of Gut-Microbiota in Atherosclerosis and Cardiovascular Disease

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Abstract

A variety of microorganisms colonize the human body and are important for human health. They play a role in the digestion and absorption of key nutrients from the diet, but have also been implicated in disease. Gut-microbiota can metabolize various quaternary amines, such as phosphatidylcholine, choline, and L-carnitine to trimethylamine (TMA). TMA is then hepatically oxidized to trimethylamine-N-oxide (TMAO). TMAO is known to promote atherosclerosis by reducing reverse cholesterol transport via proposed mechanisms including the up-regulation of macrophage scavenger receptors and/or the bile acid synthetic pathway. Additionally, elevated levels of this metabolite have been linked to an increased risk for cardiovascular disease. Removal of choline, carnitine, and other TMAO precursors from the diet is not recommended since these essential nutrients are required for physiological processes. Instead, treatment should be targeted at reducing the conversion of precursors to TMA or TMAO. A promising therapeutic approach includes the use of meldonium to inhibit the carnitine biosynthetic pathway and reduce levels of TMA/TMAO. Further investigations into the biochemical mechanisms involved in trimethylamine formation can accelerate the development of more feasible treatment strategies.

Introduction

A variety of microorganisms colonize the human body and play an important role in maintaining health.[1] An emerging topic in research is the link between these microorganisms and disease. Microbes found on the skin, nasal/oral cavity, urogenital tract, and gastrointestinal tract have been linked to diseases such as inflammatory bowel disease, colorectal cancer, obesity, diabetes, non-alcoholic fatty liver disease, and atherosclerosis.[2] Of specific interest for this article are the implications of microbiota living in the gastrointestinal tract.

It is reported that the gastrointestinal tract harbours 70% of microbes in the body,[3] predominantly from the *Bacteroidetes* and *Firmicutes* phyla.[4, 5] This gut flora consists of non-pathogenic bacteria involved in the digestion and absorption of nutrients from the diet as well as pathogenic bacteria that can cause infection.[6] Specifically, certain microorganisms play an indirect role in atherosclerosis and cardiovascular disease via the formation of key trimethylamine intermediates.[7, 8] The microbiota-dependent metabolic pathway, the mechanism of trimethylamine intermediates in atherosclerosis, and implications to cardiovascular disease will be discussed below.

Metabolism of Quaternary Amines to TMAO

Quaternary amines, including phosphatidylcholine, choline, and L-carnitine, are metabolized by gut microbiota to the gas trimethylamine (TMA). TMA is then hepatically oxidized by flavin monooxygenases to form trimethylamine-N-oxide (TMAO), which has been implicated in atherosclerosis[9] (Figure 1). It is hypothesized that the production of TMAO from such precursors is dependent on the presence of gut microbiota. [7, 8] Specifically, using an "L-carnitine challenge", antibiotic suppression of intestinal microbiota before the consumption of L-carnitine by humans almost completely inhibited the production of TMAO in comparison to the same challenge conducted in the absence of antibiotics.[7] Similar studies were conducted for phosphatidylcholine and choline.[8] These results verify the role of intestinal microbiota in the production of TMAO.

Diet has been shown to influence the composition of gut flora as a result of unique interactions between dietary antigens, microbes, and intestinal epithelium. These antigens can ultimately allow certain microbes to thrive and dominate over others.[10] For example, regular red meat consumption favored the growth of *Prevotella* sp. while vegetarian diets favored the growth of *Bacteroide* sp.[11] Thus, it is suggested that vegan/vegetarians do not possess the same ability to convert dietary L-carnitine, a nutrient rich in red meat, into TMAO. Significantly lower TMAO levels were observed when the "L-carnitine challenge" was repeated on vegan/vegetarian subjects compared to omnivores.[7] Therefore, although the formation of TMAO is dependent upon gut microbiota, the natural flora can vary in individuals as a result of differences in dietary habits. Studies have shown that individuals following a Mediterranean diet, featuring little red meat intake, had reduced incidences of major cardiovascular events.[12] This indicates the possibility that vegan/vegetarian or Mediterranean diets may be beneficial due to lower ingested levels of carnitine and choline as well as a decreased capacity to produce TMAO from these precursors.[7]

Although TMAO has been shown to be linked to ath-

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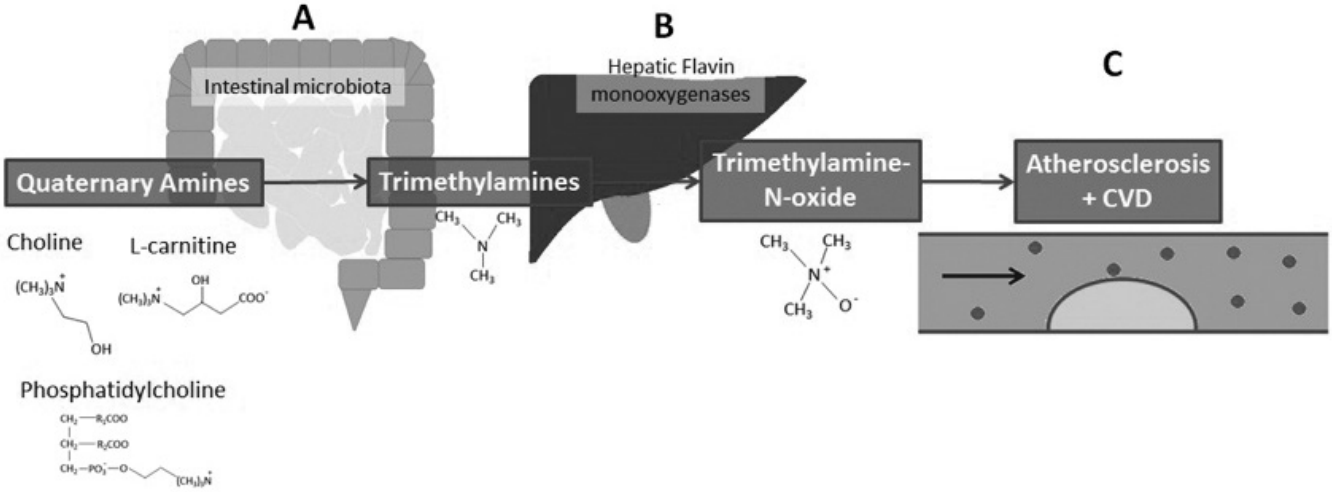

Figure 1. A) Quaternary amines such as choline, phosphatidylcholine, and L-carnitine are metabolized by intestinal microbiota to a trimethylamine gas (TMA). B) TMA is then oxidized by hepatic flavin monooxygenases to trimethylamine-N-oxide (TMAO). C) TMAO plays a role in the pathogenesis of atherosclerosis via the up-regulation of macrophage scavenger receptors and the bile acid synthetic pathway, resulting in an increased risk for cardiovascular disease (CVD).

erosclerosis, further investigations into the underlying biochemical and molecular mechanisms are needed to identify relevant structures or pathways that can be targeted to combat the formation of this metabolite. A recent study found that the *cntAB* gene in human microbiota, known to encode Rieske-type proteins, was essential for the conversion of L-carnitine to TMA.[13] This finding suggests the possible use of Rieske protein inhibitors as therapeutic agents to prevent TMA and thus TMAO formation after the ingestion of choline and carnitine-rich foods. Such inhibitors have already been developed and studied for other purposes, but investigation in animal and clinical trials is still warranted.[14] Targeting the conversion of quaternary to trimethylamines by intestinal flora is one method to prevent disease; however, a better understanding of the link between TMAO and atherosclerosis may provide alternative pathways.

Role of TMAO in Promoting Atherosclerosis

Atherosclerosis is characterized by the presence of raised intimal lesions that protrude into the lumen of blood vessels, also known as atheromatous plaques.[15] The key trimethylamine metabolite, TMAO, is believed to play a role in atherosclerosis and therefore, is associated with an increased risk for cardiovascular disease (CVD) (Figure 1). Specifically, an increase in aortic atherosclerotic plaque size was observed in mice consuming diets supplemented with choline and TMAO in comparison to diets lacking supplementation.[16] In order to gain a better understanding of the role of TMAO in the pathogenesis of atherosclerosis, it was necessary to explore the mechanism underlying this correlation.

To appreciate the role of TMAO in plaque formation, some background information is required to understand the key steps in the atherosclerotic pathway. First, damaging substances or conditions such as hypertension, hyperlipidemia, toxins, and infection can injure vascular endothelium to trigger the process.[15] The resulting inflammatory response is characterized by the influx of leukocytes, which adhere to and migrate into the intima of the endothelium.[17, 18] Circulating low-density lipoproteins (LDL) are recognized by scavenger receptors on macrophages and smooth muscle cells, leading to the uptake of LDL and formation of fatty streaks. The sequential intra and extra cellular lipid accumulation, proliferation of smooth muscle, and extracellular matrix deposition contributes to the growth of the plaque.[19]

With respect to the role of TMAO in atherosclerosis, a few possible mechanisms were explored including increased cholesterol synthesis versus reduced cholesterol clearance. It was found that mice consuming the dietary precursors choline and L-carnitine, demonstrated a 30% decrease in reverse cholesterol transport. This effect was only observed in the presence of intestinal microbiota. Furthermore, mice consuming dietary TMAO demonstrated a 35% decrease in reverse cholesterol transport, thus confirming the necessity for conversion of precursors to TMAO in cholesterol accumulation and atherosclerosis.[7]

On a cellular and molecular level, it was found that TMAO led to the up-regulation of macrophage scavenger receptors SRA and CD36, which are involved in the uptake of low-density lipoproteins to promote cholesterol accumulation within macrophages.[16] Activated macrophages containing LDL are referred to as "foam cells" due to unique characteristics of their cytoplasm[19] and it was found that a choline-rich diet led to an increase in foam cell formation.[16] However, further studies indicated that TMAO did not alter LDL receptor mRNA levels or cholesterol biosynthetic pathways in the macrophages.[7] More investigations are needed to determine whether a direct or indirect association exists between TMAO and macrophage scavenger receptors.

Also, recent experiments revealed contradictory findings suggesting that scavenger receptors might actually exert a protective role against atherosclerosis.[20] Therefore, if these receptors are shown to be influenced by TMAO, the extent to which this mechanism contributes to atherosclerosis must be evaluated.

More recent studies have suggested the involvement of the bile acid synthetic pathway in the mechanism of atherosclerosis.[7] Synthesis of bile acids in the liver is a chief mechanism for cholesterol excretion from the body. Several key enzymes are involved in the catabolism of cholesterol to bile acids, specifically members of the cytochrome P450 family of enzymes.[21] The expression of Cyp7a1 and Cyp27a1, both involved in the hydroxylation of cholesterol, were reduced in mice receiving a dietary source of TMAO. Several transport proteins, including the ATP binding cassette, responsible for exporting bile acids from the liver into the intestine, were also reduced in this study.[7] Both of these findings were accompanied by a corresponding decrease in the bile acid pool size in mice consuming TMAO,[7] suggesting that the synthesis and export of bile acids and thus the excretion of cholesterol from the body is reduced in the presence of TMAO. A role between decreased bile acid synthesis/excretion and atherosclerosis has been previously documented;[22, 23] however, more research is needed to ascertain the clinical significance of this mechanism.

It is clear that a link exists between TMAO levels and atherosclerosis. In the human body, atherosclerosis can lead to several complications, including cardiovascular disease. To understand the clinical applications of research revolving around the gut microbiome, in this case microbiota-dependent metabolism of quaternary amines, it is important to explore the relationship between TMAO and cardiovascular disease.

Implications to Cardiovascular Disease

Cardiovascular disease is the leading cause of death and morbidity worldwide.[24] The established relationship between TMAO levels and atherosclerosis[16] led to a proposed link between TMAO levels and the risk for CVD. Prior research has suggested a possible link between plasma choline levels and CVD, while other studies have not found evidence to support the use of plasma choline levels alone as a prognostic factor for CVD.[26] Given the conflicting studies, it was of interest to investigate the role of TMAO precursors vs. TMAO itself in the predicted risk for CVD.[25, 26]

Koeth et al investigated a relationship between fasting plasma L-carnitine levels with CVD, and found a relationship existed only in individuals with simultaneously elevated plasma TMAO levels.[7] Likewise, it was found that increased plasma choline levels were associated with enhanced risk for an individual to develop a major adverse coronary event. However, this correlation existed only in the presence of elevated TMAO levels.[27] These findings verify the essential role of TMAO in predicting the risk for CVD. The results also reinforce the conversion of quaternary amines to TMA/TMAO as a critical step in the pathogenesis of this disease.

Relevant quaternary amines for this microbiota-dependent pathway include choline, phosphatidylcholine and carnitine. Foods such as eggs, milk, liver, red meat, poultry, shell fish, and fish are major sources of dietary choline,[16] while L-carnitine is abundant in red meat. Diets rich in red meat have been linked to CVD due to their high amounts of cholesterol and saturated fats. However, research exploring the link between TMAO production and CVD highlights a role of gut-microbiota in pathogenesis.[16] Since TMAO is the primary metabolite responsible for the pathogenesis of CVD, simply removing choline and L-carnitine from one's diet is not expected to reduce the risk for CVD. Choline and carnitine are essential nutrients that may be digested into other metabolites required for many physiological processes, and therefore, the avoidance of such precursors through diet is not recommended.[27] Rather, the conversion of precursors to TMA and TMAO should be targeted to achieve benefit.

Given the increased risk for CVD in patients with elevated TMAO levels, exploration of possible preventative measures or treatments is warranted. One approach is to target conversion of TMA to TMAO by intestinal microbiota. Differences in intestinal flora in omnivores vs vegans/vegetarians led to an altered ability to produce TMAO from dietary trimethylamine precursors.[7] Thus, it has been suggested that a probiotic regimen designed to promote or favor the growth of certain microorganism species may alter the ability of an individual to form TMAO.[28, 16]

A different approach was taken to target bacterial production of TMA using the drug meldonium. Meldonium is a competitive inhibitor of gamma-butyrobetaine hydroxylase, a key enzyme in the carnitine biosynthetic pathway. The drug also inhibits the carnitine/organic cation transporter 2, responsible for the reabsorption of carnitine in the kidneys.[29] Meldonium is currently used as an anti-atherosclerotic agent, but its role in lowering TMAO levels has not been an area of interest, until recently. Administration of meldonium resulted in decreased gut-microbiota production of TMA and TMAO from L-carnitine. This suppression of TMAO was achieved by shifting the metabolic pathway of L-carnitine in favor of gamma-butyrobetaine (GBB), as noted by the elevated GBB levels.[30] Although results support the use of meldonium to reduce TMA and TMAO levels, the drug is only effective in the L-carnitine pathway. Therefore, in order to reduce the risk for CVD, more investigations are needed to target other quaternary amines (i.e. choline) as well.

Conclusion

Microorganisms colonizing the intestine play a role in human health and disease. Recent research suggests a role between quaternary amines (choline, phosphatidylcholine and L-carnitine) and the pathogenesis of atherosclerosis and CVD. This risk is dependent on the generation of TMAO via a microbiota-dependent pathway. While a change in diet to reduce ingested choline and L-carnitine levels can be considered, it should not be relied on too heavily since these nutrients are essential for health. Therapeutic measures to benefit patients should target the conversion of precursors

to TMA and TMAO instead. However, a better understanding of particular biochemical pathways is required to develop appropriate therapeutic agents. Nevertheless, this topic is only one great example of the unique interaction between microbiomes and human health vs. disease.

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