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Short Communication

Gut Flora Metabolite Trimethylamine n-Oxide, Predict Cardiac Events in Patients with Acute Coronary Syndromes

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Introduction

Recent studies indicate that microbes in the gut can predispose the development of atherosclerosis, chronic kidney disease and the heart failure as well as diabetes. The increase in the concentrations of trimethylamine N-oxide (TMAO) is associated with risks for both prevalent atherosclerotic heart disease and incident major adverse cardiac events in multiple independent cohorts [1-3]. There is evidence that foods such as meat, egg yolks and high fat dairy products appear to be dietary precursors TAMO generation, which is a metabolite that accelerate atherosclerosis and cardio-metabolic diseases (CMDs). These foods are rich sources of choline, phosphatidylcholine and carnitine and hence trimethylamine (TMA).

Further studies also revealed an increased risk of CMDs with changes in the composition of the gut microbiota and links between the host environment and microbiota [3,4]. These studies have shown that patients with CMDs frequently exhibit enrichment or depletion of certain bacterial groups in their resident microbiota compared to healthy individuals. Transfer of resident gut microbiota from mice or humans into germ-free mouse models, or between human patients, has enabled us to characterize the causative role of the gut microbiota in CMDs [3,4]. It is now possible to identify that dietary intake of choline, which is metabolized by the gut microbiota, is associated with CMDs. TMAO concentration which is a metabolite derived from the gut microbiota may also be associated with poor cardiovascular outcomes in patients with CVDs including acute coronary syndrome (ACS) and is also elevated in patients with chronic kidney disease (CKD). This view point summarizes data suggesting a link between the gut microbiota and derived metabolites with risk of chronic CMDs as well as with ACS.

Trimethylamine n-Oxide (TMAO)

TMAO, a gut-microbiota-dependent metabolite, enhances both; atherosclerosis in animal models and cardiovascular risks in clinical studies [3]. There is a need to study the impact of targeted inhibition of the first step in TMAO generation, commensal microbial TMA production, on diet-induced atherosclerosis. A structural analog of choline, 3,3-dimethyl-1-butanol (DMB), is shown to non-lethally inhibit TMA formation from cultured microbes, to inhibit distinct microbial TMA lyases, and to both inhibit TMA production from physiologic polymicrobial cultures (e.g. intestinal contents, human feces) and reduce TMAO levels in mice fed a high-choline or L-carnitine diet [3]. Further evidence showed that DMB inhibited choline diet-enhanced endogenous macrophage foam cell formation and atherosclerotic lesion development without alterations in circulating cholesterol levels [3]. Therefore, targeting gut microbial production of TMA specifically and non-lethal microbial inhibitors in general may serve as a potential therapeutic approach for the treatment of CMDs.

TMAO and cardiometabolic diseases

The phenotypes of the host are under strong influence of gut microbiome which is a complex and metabolically active community [5]. The Metabolic Syndrome In Men (METSIM) study involving 531 Finnish men revealed novel associations between gut microbiota and fasting serum levels of a number of metabolites; fatty acids, amino acids, lipids, and glucose [5]. There were significant associations with fasting plasma TMAO levels, with coronary artery disease (CAD) and stroke. It is possible that **t**he composition of the gut microbiota and circulating metabolites may be a resource for future studies to understand host–gut microbiota relationships.

It is known that TMAO, a metabolite derived from gut microbes and dietary phosphatidyl- choline and L-carnitine, may be associated with pathogenesis of CAD and risk of CVDs [6]. There is no study to demonstrate that plasma TMAO can predict mortality risk in patients with stable CAD. The relationship between fasting plasma TMAO and all-cause mortality was examined among 2235 patients with stable CAD during a follow up of 5-year [6]. The results revealed that higher plasma TMAO levels were associated with a 4-fold increased mortality risk, after adjustments for traditional risk factors, high-sensitivity C-reactive protein, and estimated glomerular filtration rate. TMAO remained predictive of incident mortality risk following cardiorenal and inflammatory biomarker adjustments to the model (adjusted hazard ratio 1.71, 95% CI 1.11 - 2.61; P = 0.0138) and provided significant incremental prognostic value for all-cause mortality. It is possible that higher plasma TMAO levels portended higher long-term mortality risk among patients with stable coronary artery disease managed with optimal medical treatment [6].

TMAO, a gut microbial-dependent metabolite may be also elevated in chronic kidney diseases (CKD), apart from CVDs and may be associated with pathogenesis of CAD in patients [7-9]. The relationship between fasting plasma TMAO and all-cause mortality over 5-year follow-up in 521 stable subjects with CKD (estimated glomerular filtration rate [eGFR] < 60 ml/min/1.73m^2) was examined [7]. Median TMAO level among CKD subjects was 7.9 μM (interquartile range 5.2 - 12.4μM), which was markedly higher (P < 0.001) than in non-CKD subjects (n = 3,166) [7]. A higher concentration of TMAO level was associated with a 2.8-fold increased mortality risk, after adjustments for traditional risk factors, hsCRP and eGFR, elevated TMAO levels remained predictive of 5-year mortality risk (HR 1.93 [95%CI 1.13 - 3.29], p < 0.05). TMAO provided significant incremental prognostic value. It seems that plasma TMAO levels are both elevated in patients with CKD and portend poorer long-term survival and increased dietary intake of foods that increase TMAO appear to directly contribute to progressive renal fibrosis and dysfunction [7]. Microflora-dependent trimethylamine-N-oxide (TMAO) formation, which results from intake of choline and L-carnitine-rich food, shows promise as a predictor of CVD risk, but these associations have not been examined in ethnically diverse populations. In a multiethnic population-based study of adults involving 1285 adults, the stability of TMAO and L-carnitine in stored serum samples and their association with intimal medial thickness, prevalent risk factors, and clinical events were examined [8]. In 292 consecutive individuals (99 CVD cases and 193 unmatched control subjects), L-carnitine and TMAO concentrations were assessed. The mean (± SD) TMAO level was 1.998 ± 3.13 μM and L-carnitine was 42.29 ± 11.35 μM. TMAO levels showed a significant, graded association with prevalent CVD (odds ratio, 3.17; 95% confidence interval, 1.05-9.51; *P* trend = 0.02), whereas no significant association of l carnitine was observed. The findings revealed an association between TMAO with prevalent CVD in a multiethnic population.

TMAO levels and risk of acute coronary syndromes

The prognostic value of the gut-derived metabolite, TMAO was confirmed for the first time, using 1-year major adverse cardiovascular events (MACE) risk, in a large Swiss cohort of ACS undergoing coronary angiography [1]. High plasma levels of TMAO in patients with ACS can independently predict major adverse cardiac events (MACE) at 30 days and 6 months and mortality at 7 years, also in United States [1]. In two independent cohorts of ACS, the relationship of TMAO levels with incident cardiovascular risks among sequential patients was examined [1]. The Cleveland Cohort, comprised of 530 patients with chest pain of suspected cardiac origin, an elevated plasma TMAO level at presentation was independently associated with risk of MACE, including myocardial infarction, stroke, need for revascularization, or death during the follow up ensuing 30-day (P < 0.01) and 6-month (P < 0.01) intervals. TMAO levels were also a significant predictor of the Long term (7-year) mortality (P < 0.05) Those subjects with negative troponin T (< 0.1 ng/ml), TMAO level at initial presentation

predicted risk of incident MACE over the near-term (30 days and 6 months, $p < 0.01$) The prognostic value of TMAO was also assessed An independent multi-center Swiss Cohort of ACS patients (n = 1683) who underwent coronary angiography, TMAO levels again predicted enhanced MACE risk (1-year, $P < 0.05$). It is clear that plasma levels of TMAO among patients with chest pain can predict both near- and long-term risks of incident cardiovascular events. TMAO levels may provide clinical utility in risk stratification among subjects presenting with suspected ACS.

In another study, TMAO independently predicted death/MI at 2 years after hospitalization for acute myocardial infarction (AMI) [2]. Suzuki and his colleagues examined TMAO levels in plasma among 1079 patients of AMI admitted to the University Hospitals of Leicester between August 2004 and April 2007.Multivariable analyses revealed TMAO was an independent predictor of death/MI at 2 years (HR 1.21, 95% CI 1.03 - 1.43; $P = 0.023$) but not at 6 months ($P = 0.119$). Plasma levels of TMAO was the only biomarker that significantly predicted outcome, despite inclusion of such contemporary markers as cardiac troponin, N-terminal probrain natriuretic peptide (NT-proBNP), and copeptin, during the follow up of two years. The Global Registry of Acute Coronary Events (GRACE) score for calculating death/MI at 6 months, also revealed that plasma TMAO was able to down-classify patients with low risk (NRI 29.0, 95% CI 19.8 - 38.3; $P < 0.0005$) but unable to reclassify those at high risk (NRI −9.1, 95% CI −30.0 to 11.8). These results are quite interesting and provide evidence base for TMAO beyond stable patients, including those with heart failure or peripheral artery disease, and indicate new prospect of TMAO as a potentially modifiable risk marker for cardiovascular events.

These findings also indicate new perspectives in terms of intervention that need additional research, to find out their influence on TMAO levels directly either by dietary intervention. It is possible that in the near future, new compounds inhibiting the enzyme in the bacteria that produces TMAO, may be used for therapy for prevention of ACS and CMDs [10]. It may be proposed that some of the dietary components; omega-3 fatty acids, resveratrol, curcurmin, dietary fiber, probiotics etc. that are known to influence CVDs, may be examined for their beneficial effects on TMAO and other enzymes. TMAO is produced after gut bacteria break down dietary nutrients such as phosphatidylcholine, choline, and L-carnitine, found in red meat, eggs, and high-fat dairy products, all common in the Western diet. Hence nutrients rich in Mediterranean style diets may be considered for future therapy because such diets have been proven to provide beneficial effects in patients after ACS. It has also been already demonstrated that targeting trimethylamine (TMA), the first step in TMAO formation, with 3,3-dimethyl-1-butanol inhibits foam-cell formation and aortic atherosclerotic plaque formation in mice, without altering normal gut flora [3].

I the United States cohort, patients with the highest TMAO levels faced a nearly twofold increased hazard of 7-year mortality when compared with those with the lowest level (OR 1.81, 95% CI 1.04 - 3.15; $P < 0.05$) [1]. It is important that in adjusted analyses of patients with initially negative cardiac troponin, elevated TMAO levels maintained prognostic significance for MACE at 30 days (OR 5.83, 95% CI 1.79 - 19.03; $P < 0.01$) and 6 months (OR 5.51, 95% CI 1.90 - 16.01; $P < 0.001$) beyond traditional risk factors, biomarkers, and ECG data, including C-reactive protein (CRP), hypertension, hyperlipidemia, and diagnosis of STEMI, non-STEMI, or unstable angina. It is possible that the ability to generate rapid and accurate TMAO results through point-of-care testing could significantly improve rapid triaging and risk stratification among subjects presenting with suspected ACS. It has been observed that there may be intra-individual variation in TMAO, spiking, for example, in patients after simply eating a steak dinner [2]. However, some of the variations in TMAO concentrations, similar to CRP, may be attributed to circadian dysfunction due to late night sleep and late night eating which are known to alter circadian metabolism in liver and pancreas resulting in to CMDs. In view of the variability in TMAO levels, it may not be so useful as a diagnostic marker but as a prognostic marker.

The Swiss diet among Swiss patients appear to be quite a different diet, and this is of interest because TMAO levels are influenced by diet, in particular meat, shellfish, and eggs. Since American diets are quite rich in these foods, TMAO levels were much higher in patients in the US in association with higher cardiovascular complications in these patients, showing a graded increase in incident MACE risk [1]. The

adjusted risk of MACE at 1 year was 1.5 times higher in patients with the highest TMAO levels (> 4.85 μM) than in those with the lowest levels of TMAO (0.08 - 1.93 μM) (hazard ratio [HR] 1.57, 95% CI 1.03 - 2.41; $P < 0.05$). Inclusion of TMAO levels in the Cleveland Clinic's model significantly improved risk estimation for MACE at 30 days and 6 months, with significant improvement in C statistics for both time points which provides a further proof that TMAO levels may be a risk factor of ACS [1].

Mechanisms: The formation of TMAO in our body takes place following nutrient ingestion, via gut microbes which form TMA, and then host hepatic flavin monooxygenases (FMOs) catalyze the conversion of TMA into TMAO [3]. Recent studies highlight the importance of both TMAO and host hepatic FMO3, the primary FMO responsible for TMAO production, as important regulators in host lipid and sterol metabolism, as well as in development of atherosclerosis [3,9] (Figure 1). A structural analogue of choline, 3,3-demethyl-1-butanol (DMB) is an inhibitor of microbial TMA production (a choline TMA lyase inhibitor). It also inhibits choline diet dependent enhancement in TMAO, endogenous macrophage foam cell formation, and in development of atherosclerosis.

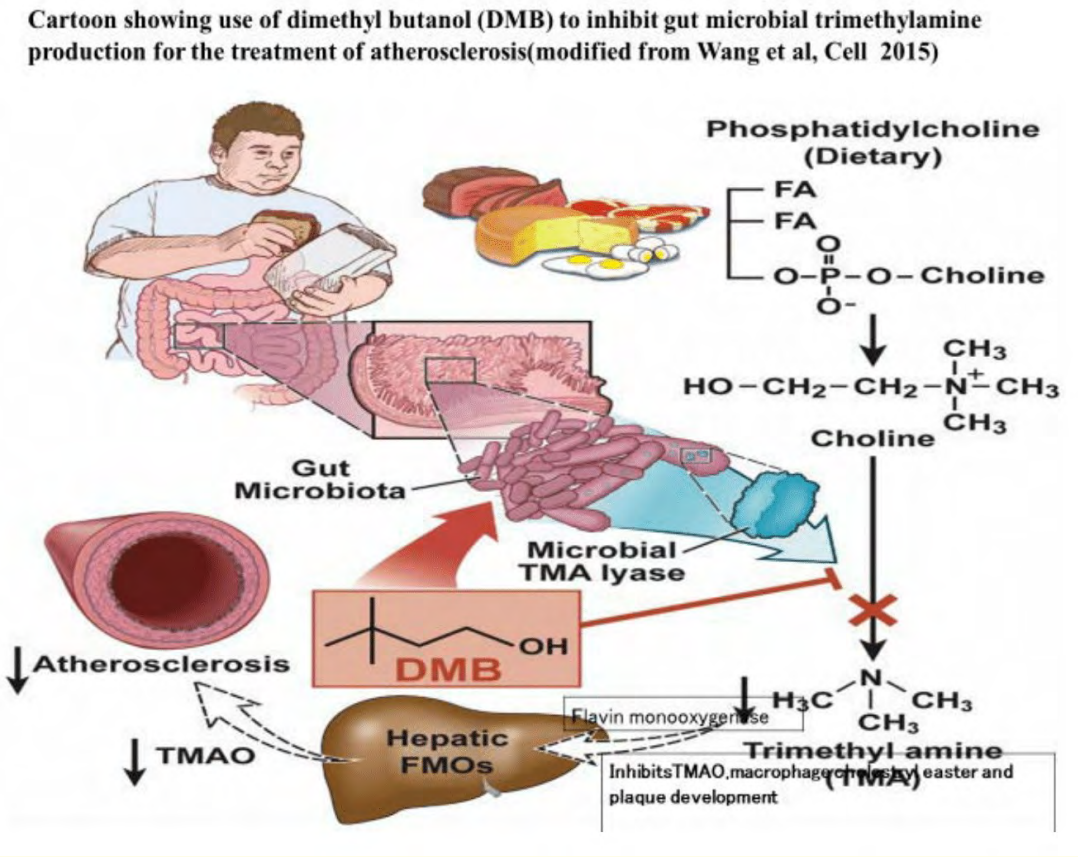

Figure 1: *Showing use of dimethyl butanol(DMB) to inhibit gut microbial trimethylamine production for the treatment of atherosclerosis (modified from reference 5).*

Despite no significant effects on circulating cholesterol, choline and other pro-atherogenic risk factors, DMB inhibits choline diet-dependent accumulation of both macrophage cholesteryl ester accumulation (foam cell formation) and development of aortic root

atherosclerotic plaque. Apart from inhibiting plasma levels of TMAO, DMB promotes reduction in proportions of some microbial taxa that are associated with plasma TMA and TMAO levels, and reduces extent of aortic plaque [3,9]. There is general agreement that changes in microbial composition induced by DMB indicate some degree of selective pressure, occurring with exposure to the agent, and thus raises the possibility of development of resistance.

It is possible that TMAO may be a toxic agent for the cardiovascular-renal system, but, it remains unclear exactly how TMAO exerts its effects. TMAO may also be a pro-thrombotic agent in the circulation *in vivo*, but whether or not it also stimulates macrophages and plaque formation is currently under investigation [3]. Since the molecular mechanisms in the athero-thrombosis and plaque in the vessel wall have not been fully elucidated, this poses the possibility that TAMO may have adverse effects on platelet aggregation and inflammation [10,11].

In brief, choline and L-carnitine are rich in the Western diets which can produce TMAO, a metabolite derived from gut microbes. TMAO may be important in the pathogenesis of CAD and in increasing the risk of CVDs, including ACS. The levels of TMAO could be considered in the prognosis of ACS as well as in the risk stratification of ACS.

Conflict of Interest

Conflict of interest has not been declared by the authors.

Bibliography

1. Li XS., *et al*. "Gut microbiota-dependent trimethylamine N-oxide in acute coronary syndromes: A prognostic marker for incident cardiovascular events beyond traditional risk factors". *European Heart Journal* 38.11 (2017): 814-824.

2. Suzuki T., *et al*. "Trimethylamine N-oxide and risk stratification after acute myocardial infarction". *Clinical Chemistry* 63.1 (2017): 420-428.

3. Wang Z., *et al*. "Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis". *Cell* 163.7 (2015): 1585-1595.

4. Aron-Wisnewsky J and Clément K. "The gut microbiome, diet, and links to cardiometabolic and chronic disorders". *Nature Reviews Nephrology* 12.3 (2016): 169-181.

5. Org E., *et al*. "Relationships between gut microbiota, plasma metabolites, and metabolic syndrome traits in the METSIM cohort". *Genome Biology* 18.1 (2017): 70.

6. Senthong V., *et al*. "Intestinal microbiota-generated metabolite trimethylamine-N-oxide and 5-year mortality risk in stable coronary artery disease: The Contributory Role of Intestinal Microbiota in a COURAGE-Like Patient Cohort". *Journal of the American Heart Association* 5.6 (2016): e002816.

7. Tang WHW., *et al*. "Gut Microbiota-Dependent Trimethylamine N-oxide (TMAO) Pathway Contributes to Both Development of Renal Insufficiency and Mortality Risk in Chronic Kidney Disease". *Circulation Research* 116.3 (2015): 448-455.

8. Mente A., *et al*. "The relationship between trimethylamine-N-oxide and prevalent cardiovascular disease in a multiethnic population living in Canada". *Canadian Journal of Cardiology* 31.9 (2015): 1189-1194.

9. Warrier M., *et al*. "The TMAO-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance". *Cell Reports* 10 (2015): 326-338.

10. Troseid M. "Gut microbiota and acute coronary syndromes: ready for use in the emergency room?" *European Heart Journal* 38.11 (2017): 825-827.

11. Fedacho J., *et al*. "Can gut flora metabolites predict cardiac events and cardiometabolic diseases?" *World Heart Journal* 9.2 (2017): 107-113.

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