Microbiota and Coronary Artery Disease

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1 Introduction

Atherosclerosis and derived CAD has been the first leading cause of death around the world (GBD 2013 Mortality and Causes of Death Collaborators, 2015). Inflammation, both systemic and peripheral, plays a key role in CAD progression (Hansson, 2005; Rein et al., 2015). The rising recognition of the microbiota as a forgotten organ has been a major topic of research interest in systematic and peripheral inflammation (Purchiaroni et al., 2013). Recent studies suggest that microbiota with various metabolic products are closely associated with CAD, but the detailed processes and molecular mechanisms involved remain not fully understood (Caesar et al., 2010). This review will highlight the major findings and recent advances in the study of microbiota-dependent mechanism involved in the development of CAD and discuss important roles of probiotic bacteria and plant compounds in prevention and treatment of CAD.

2 CAD Featured Microbiota

In the process of atherosclerosis, it is hypothesized that CAD involved a bacterial infection and chronic inflammation of the arterial wall (Libby et al., 2002). But the mechanisms of infection and related immune responses remain unexplained. In the past, the isolation of infected bacterial strain was limited to the bacterial culturing condition. By using DNA-based molecular biological techniques, bacterial and fungal DNA were

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found in the atherosclerotic lesions of cardiovascular disease patients (Lehtiniemi et al., 2005; Ott et al., 2006; Ott et al., 2007; Pessi et al., 2013). A vast amount of certain microorganisms (> 50 different species), including Chlamydia, Staphylococcus, Lactobacillus, Klebsiella pneumoniae, and Streptococcus species, may infect the arterial wall and are associated with the development of coronary diseases (Table 1). Besides, Oral cavity derived antibiotic-resistant Enterococcus faecalis induced infective endocarditis may have an influence on CAD development and related to high mortality (Okui et al., 2015). The source of these microbes aroused great interest and were probably from oral and gut microbiota (Leishman et al., 2010; Org et al., 2015).

In a clinic study, CAD patients have different oral microbial flora from healthy control especially a periodontal pathogen called Prevotella intermedia (Nonnenmacher et al., 2007). It is demonstrated that oral infective microorganisms may increase the risk of occurrence of CAD (Suzuki et al., 2010). Another large-scale clinic finding revealed that periodontal microbiota was related to subclinical atherosclerosis in patients via the inflammatory C-reactive protein values (Desvarieux et al., 2005). Recently, high-throughput sequencing technology may facilitate the overview of microbiota composition involved in various diseases, as well as explore the causal relationships between microbes and host. The composition of all bacteria can be more accurately evaluated by sequencing the hyper variable regions of the 16S ribosomal RNA (rRNA) bacterial gene in total DNA with next generation sequencing technologies such as Roche 454-pyrosequencing or Illumina MiSeq platform (Mardis et al., 2008). The high-throughput sequencing not only explore the unculturing microbes but also providing insights into low abundances of bacteria. By using 454 pyrosequencing, the bacterial diversity in atherosclerotic plaque, oral, and gut were evaluated in patients to underscore the clinical importance of the association between microbiota dysbiosis and atherosclerotic lesions (Koren et al., 2011). In fact, the various bacteria from oral and gut affects atherosclerosis far beyond our expectation. More detailed, Abundances of Veillonella and Streptococcus in the atherosclerotic plaques are consistent with that in oral samples and some OTUs (operational taxonomic units) in plaques were considered gut-derived (Koren et al., 2011).

Besides, blood microbiota dysbiosis was suspected to induce CAD onset in a large-scale longitudinal study by 16S rDNA sequencing. Proteobacteria was found to be the dominated bacteria in blood which correlated with the onset of cardiovascular complications (Amar et al., 2013).

### 3 The Role of Microbiota in Cardiovascular Health

Previous data demonstrated that atherosclerosis-prone mice were shown no enhancement in atherosclerosis with normal diet while suppression of intestinal flora completely by antibiotic treatment (Wang et al., 2011). But antibiotics treatment failed to benefit CAD in humans (Andraws et al., 2005). More importantly, Stepankova et al., (2010) found that germ-free ApoE/-/ mice could develop atherosclerotic plaques in the aorta whereas their conventional controls had no plaques with the same diet. It is sug-
<table>
<thead>
<tr>
<th>Species</th>
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<tr>
<td>Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia</td>
<td>Infected plaques</td>
<td>Periodontal disease related pathogens (Gaetti-Jardim et al., 2009)</td>
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<tr>
<td>Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Tannerella forsythensis, Eikenella corrodens, Prevotella intermedia, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans, Treponema denticola, C. pneumoniae.</td>
<td>Atherom-atous plaques</td>
<td>Periodontal disease related pathogens; P.gingivalis invade human oral endothelial cells (Kozarov et al., 2006)</td>
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<td>Chlamydia pneumoniae</td>
<td>Infected plaques</td>
<td>Enter the bloodstream via monocytes and infect the vessel wall by leukocyte infiltration (Berger et al., 2000)</td>
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<td>Streptococcus gordonii</td>
<td>Oral cavities</td>
<td>Platelet adhesion and subsequent aggregation (Petersen et al., 2010)</td>
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<td>Neisseria sp., Streptococcus mitis, Enterococcus sp., Lactococcus lactis sp., Haemophilus parahaemolyticus, Streptococcus pyogenes, Streptococcus salivarius, Streptococcus mitis, Prevotella sp., Lactobacillus fermentum, Lactobacillus delbrueckii</td>
<td>Fibroath-eroma</td>
<td>Enter the bloodstream during toothbrushing or by leaking through mucosal surfaces (Lehtiniemi et al., 2005)</td>
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<td>Helicobacter pylori</td>
<td>Atherosc-lerotic plaque</td>
<td>Gastric mucosal damage (Kowalski et al., 2001)</td>
</tr>
<tr>
<td>Enterococcus facalis</td>
<td>Oral cavity</td>
<td>Infective endocarditis (Okui et al., 2015)</td>
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**Table 1:** The identified CAD-associated species.
gested that gut commensal microbiota are presumed to have contributed to the prevention of atherosclerosis development. The effects of the gut microbiota on the cardiovascular health may not be limited to the presence of intestinal bacteria, but may involve systemic immune responses driven by these bacteria. Gut microbiota appear to influence host inflammatory responses in large part by producing LPS (Lipopolysaccharides) that enter the host circulation through impaired intestine (Org et al., 2015).

Though some microbes from the oral or gut may be associated with that in atherosclerotic plaques, there no single study has directly demonstrated how the way of microbe get from their external environment to the heart to cause CAD. In my opinion, transplantation of single risk microbe to oral or gut of germ-free ApoE-/- mice provide a useful model to trace the way of microorganisms causing atherosclerosis. Atherosclerotic bacteria would be shown to activate inflammatory pathways, altering lipid metabolism or producing risk substances. Moreover, mice may be protected from atherosclerosis via microbiota improvement by dietary intervention.

4 TMAO and Serotonin-novel Microbiota related Factors for CAD

An earlier previous metabolomic study compared the differences in serum metabolites between 36 severe CAD patients and 30 healthy controls. The result showed that choline-containing metabolites have considerable difference in these two groups and may used as a diagnostic biomarker (Brindle et al., 2002). In recent years, trimethylamine N-oxide (TMAO), exhibiting a strong association with CAD , has been considered as a novel risk factor for cardiovascular disease (Tang et al., 2013). Accumulating evidence indicates that the gut microbiota can modulate the metabolism of choline to trimethylamine (TMA) and TMAO is generated from TMA via oxidization (Wang et al., 2011; Shah et al., 2012). However, the core gut microbiota has not been identified to contribute to the TMA formation.Further investigation in animals showed that dietary egg yolk rich in phosphatidylcholine increased the TMAO levels and atherosclerosis risks, while broad spectrum antibiotics treatment could suppress intestinal flora and prevent the TMAO formation in mice (Tang et al., 2013). It is thought that the presence of microbiota play an obligate role in TMAO formation which is contributed to atherosclerosis risks. Besides, a minor human study showed that daily consumption of above2 eggs that rich in choline could increase TMAO concentrations linked with altered intestinal microbiota composition (Miller et al., 2014). It is suggest that overconsumption of eggs may increase the risk of atherosclerosis and CAD.

Blood platelets are closely related to thrombus formation and coronary atherogenesis in the progression of CAD (Vikenes et al., 1999). Yet the crosstalk between the platelets and immune cells extends functionally far more than have been recognized before. Platelets was also contribute to bacterial clearance in blood and thus prevention for coronary arteries infection through the innate immune system with kupffer cells (Wong et al., 2013). Serotonin (5-hydroxytryptamine, 5-HT), secreted by platelets, are involved in a wide range of biological functions, including vascular wall development,
thrombogenesis regulation, proliferation of smooth muscle cells and even the alleviation of comorbid depression in CAD (Pizzi et al., 2011; Gershon, 2013). Over the past few years, it has become clear that gut microbiota play a role in bloodstream 5-HT levels and depression behavior by the comparison between germ-free and conventional animals (Diaz Heijtz et al., 2011). Most recently, it is found that germ-free mice exhibit significantly lower levels of colonic and blood 5-HT compared to SPF controls, suggesting that gut microbiota greatly affected the 5-HT biosynthesis (Yano et al., 2015). Furthermore, most of the body’s 5-HT is synthesized in the gut play a key role in blood platelet activation which acted as a potential regulator in CAD prevention and development (Yano et al., 2015). Another study showed that short-chain fatty acids produced by microbiota could influence the 5-HT biosynthesis by enterochromaffin cells (Reigstad et al., 2015). Serotonin therefore may be one of the intestinal microbiota-dependent factors involved in the development of CAD.

5 Cholesterol: Reducing Effect of Probiotics in the Prevention of CAD

The high incidence of hypercholesterolemia imposes an enormous burden on healthcare systems and contributes to the development of atherosclerosis and related heart diseases. CAD is characterized by an imbalanced lipid metabolism and the early onset of coronary lesion is mostly independent of high circulating cholesterol level and followed by an accumulation of low-density lipoprotein (LDL) (Weber & Noels, 2011). Recent studies reveal a connection between hypercholesterolemia and microbiota (Martínez et al., 2009). As a crucial player in regulating gut microbiota, some probiotics also have lipid-lowering effect (Chen et al., 2013).

Mechanistically, one of main mechanisms in cholesterol-reducing is to enhance the conversion to bile acids through liver CYP7A1 activity. Another important cholesterol-reducing effect of probiotic is the direct adherence to cholesterol by bacterial cells. Lactobacillus acidophilus ATCC 4356, a probiotic with favorable cholesterol-reducing effect, was found to attenuate the development of atherosclerotic lesions in ApoE(-/-) mice (Chen et al., 2013; Huang et al., 2014). Furthermore, a comparison of intestinal microbiota between ApoE(-/-) mice with L. acidophilus and non-L. acidophilus showed significant differences in the composition of fecal lactobacillus and bifidobacterium, and these differences exhibited a correlation with inhibition of intestinal cholesterol absorption and decreased plasma cholesterol levels as well as reducing oxidative stress and inflammatory responses (Chen et al., 2013). In addition, a recombinant β-glucan-producing Lactobacillus paracasei NFBC 338 showed a significant promotion of fecal cholesterol excretion in ApoE(-/-) mice compared to wild type strain (London et al., 2014). However, obesity-preventing probiotic L. reuteri ATCC PTA 4659 exhibited no effects on inflammatory markers, blood cholesterol or atherosclerosis in ApoE(-/-) mice (Fåk & Bäckhed, 2012). These results indicated that, for some reason, the intestinal microbiota changes by probiotic may affect the intestinal cholesterol absorption which contribute to suppress atherosclerotic progression. However, there is controversial evidence to support the use of
probiotics in patients of CAD. In one study, the findings suggest that Lactobacillus plantarum was effective for the improvement of intestinal isovaleric acid and valeric acid levels but not in blood markers in arteriosclerosis (Karlsson et al., 2010). More recently, an open-label, randomized study showed Lactobacillus casei Shirota have limited efficacy in terms of decreasing CAD related TMAO levels in patients with metabolic syndrome (Tripolt et al., 2015). Of course, more high-quality and large-scale randomized controlled trials are necessary to examine the benefits of probiotics on CAD.

6 Chloride Ion Channels in CAD Pathogenesis

Although the bacterial conversion of bile acids in the human gastrointestinal tract has been well documented, the pharmacological role of various bile acids remains poorly understood (Ridlon et al., 2014). It is thought that bile acid is a determinant of the gut microbiota with a high-fat diet since bile acid could induce a rapid shift in dominating Bacteroidetes into Firmicutes (Yokota et al., 2012). Because bile acids acted as a rapid response for altered gut microbiota and initiated other pathways. Bile acid sequestrants (BAS), a specific drug for eliminating bile acids in intestine, have been shown the potential role in reducing CAD progression and the risk biomarkers (Insull, 2006). In animals, the novel atherosclerotic biomarker for TMAO level is rely on liver FMOs (flavin-containing monoxygenase) expression which is mediated by the composition of bile acids (Wang et al., 2011).

One of major signaling pathway for bile acids is the G protein coupled receptor TGR5. TGR5 has recently appeared to function as a target in the metabolism and inflammation of CAD (Pols et al., 2011). TGR5 activation performed essential function in reducing plaque macrophage inflammation and improving atherosclerotic processes. More importantly, there exist a bile acids-TGR5-chloride ion axis where bile acids elimination and chloride ion influx were regulated by TGR5 activation (Zeng et al., 2014).

Previous epidemiological survey showed that low serum chloride level had been a risk factor for cardiovascular events (De Bacquer et al., 1998). Hypertension, which is more likely to develop CAD, is associated with a lower serum chloride ion concentration (McCallum, 2013). However, the underlying mechanism of low serum chloride level for risk in hypertension with CAD is unclear. It is thought that serum chloride level reflects the tissue chloride level and expression of chloride ion dependent proteins.

A series of proteins, including Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), Chloride Channel-2 (CIC-2), Chloride Channel-3 (CIC-3), Chloride Channel Accessory (CLCA), Bestrophin (BEST), and Transmembrane member 16A (TMEM16A), are influenced by tissue chloride ion concentration. Previous experimental evidence has indicated that chloride ion dependent proteins may be involved in the regulation of various cellular functions, including cellular excitability, cell volume homeostasis, cell migration, proliferation, differentiation and apoptosis (Duan, 2009). Lactobacillus casei Zhang, a probiotic to modify the gut dominated flora, has been reported to reduce intestinal total bile acids level and enhance cardiac chloride ion concentration (Zhang et al., 2014). In addition, expression of chloride ion-dependent genes CIC2, be-
bestrophin-3 and CFTR were also upregulated with short-term probiotic intervention in heart. Collectively, a chloride ion influx from dependence on microbiota related changes of bile acids, has been implicated in bestrophin-3 and chloride channel promotion, suggesting a potential role of probiotic in improving coronary vasomotion and preventing microvascular dysfunction during earlier CAD pathogenesis (Adkins et al., 2015). Besides, cardiac CFTR over expression could modulate cell apoptosis in the basilar artery smooth muscle by regulating caspase-3 and -9 protein expression and reducing oxidative stress (Duan, 2011).

In recent years, CIC-3 has developed as a novel therapeutic target for the treatment of various cardiac and vascular diseases (Duan, 2011). CIC-3, a chloride ion channel highly expressed in cardiac myocytes and vascular smooth muscle cells, is combined with Nox1 for some synergetic effect on reducing generation of endosomal ROS and subsequent surpress NF-κB activation by inflammatory cytokines in VSMCs. Though the relationship between CIC-3 and CAD are not clearly identified, CIC-3 may be necessary for treating inflammation and oxidative stress in CAD progression and supporting cardiac function. These considerations provided novel mechanistic insight into the beneficial effects of probiotic in the surpression of atherosclerotic process and CAD through a chloride ion influx.

Probiotics are generally defined as live microorganisms which confer health benefits when present in adequate amounts (FAO/WHO, 2001). The probiotics are considered to be strain-specific and previous research has mainly focused on the individuality of their function. In the previous studies, Lactobacillus acidophilus, Lactobacillus rhamnosus JB-1, Saccharomyces boulardii and Bifidobacterium breve C50 were found to enhance the chloride ion secretion or chloride ion related genes expression (Girard et al., 2005; Heuvelin et al., 2010; Raheja et al., 2010; Bravo et al., 2011). These experiments indicated that chloride ion-influx capacity might be a generality of probiotics. This characteristic will provide a new strategy for probiotic evaluation and consultation for probiotic related health claims.

The plant origin compounds are always considered important improvement to human cardiovascular health, and therefore identifying the mechanisms in reducing CAD is of significant interest. Plant origin compounds are always considered mostly interacted with colonic microbiota because of their relatively poor oral bioavailability and low plasma drug concentration (Cardona et al., 2013). Genistein, a soy isoflavone, have shown to lower cardiovascular risk markers and in postmenopausal women with a high risk of CAD (Atteritano et al., 2007). The previous report provided evidence that studies of genistein administration may exhibit increased Cl(-) secretion with activation of the CFTR chloride channel and thereby contribute to significant increases in basal I(sc) associated with intestinal epithelia function (Al-Nakkash et al., 2006; Tuo et al., 2009; Al-Nakkash et al., 2011). In addition, genistein have a direct influence on gut dominant communities and are amenable to further bacterial metabolism to yield equol and 5-hydroxy-equol via altered microbiota in postmenopausal women (Clavel et al., 2005; Matthies et al., 2012). These researches suggest that genistein might have a potential beneficial role in reducing CAD by regulation of microbiota and related intestinal chlo-
ride ion proteins. Curcumin, another plant compound from curry powder, was also shown to ameliorate the development of cardiovascular diseases and stimulate CFTR Cl(–) channels directly (Berger et al., 2005; Wongcharoen et al., 2009; Bernard et al., 2009).

Dihydromyricetin, an abundant ingredient in rattan tea, was reported to possess anti-inflammatory, antimicrobial activity, and can protect vein endothelial cells from oxidative stress damage, an effect that is potential to reduce the risk of CAD involving the mitochondrial pathways (Kou & Chen, 2012; Hou et al., 2015). More importantly, dihydromyricetin affected the expression of chloride ion-dependent GABA receptors protein in brain tissue to exert beneficial effect on alcohol intoxication and Alzheimer’s disease (Shen et al., 2012; Liang et al., 2014). These researches suggest that plant origin compounds may influence the whole body chloride ion movement not only be limited to intestine.

7 Conclusion

In this chapter, we summarized the CAD featured microbes, the oral or gut microbiota in cardiovascular health and described the potential role of bacterial metabolites such as TMAO and serotonin in the CAD pathogenicity. Though the underlying role and interaction of numerous CAD related microbes in CAD has been not fully understood, the causal relationship of bacterial metabolite TMAO and cardiovascular disease become more clearly. In addition, serotonin may act as a potential regulator in CAD development. Moreover, importance of probiotic as a cholesterol regulator in the control of CAD development and related mechanism was recalled. Cholesterol-reducing effect of probiotics is linked to CYP7A1 activity and bacterial adherence to cholesterol.

The identity and physiological roles of chloride ion channels and proteins has lagged behind that of many other drug targets (Verkman & Galietta, 2009). Bile acids and its receptor TGR5 affected by gut microbiota could influence the secretion of chloride ion and expression of chloride ion channels. The deficit of chloride ion channels function may be responsible for the altered microbiota and metabolic disturbance in response to cholesterol-rich diet, implicating a novel and important role of chloride ion channels in the development of CAD. Recently, targeted chloride ion related proteins regulated by probiotics or plant compounds seem have relevance to the suppression of CAD. In future, multiple omics view may provide a more complete understanding of the chloride ion-dependent gene function for CAD prevention in the context of microbiota changes.

References


