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Thrombosis Prevention: Let's Drug the Microbiome!

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The biologic relevance of the microbiome has been extensively investigated in the last decade and is an emerging topic in all aspects of healthcare. The gut is home to a vast number of commensal microorganisms, which influence physiology and metabolism throughout the body. Indeed, the microbiome has been implicated in a whole variety of health conditions, suggesting a critical role of our commensals in human health and disease. Disruption of the gut microbiome has been associated with a range of conditions, from infectious diseases to cardiovascular and metabolic disorders, gastrointestinal illnesses and neurologic diseases. The development of novel ad hoc interventions to both prevent and treat conditions linked to changes or disruptions in the gut microbiome is of increasing interest. Some of these drugs are meant to maintain the overall health of the microbiome, whereas others specifically target disease mechanisms by altering microbial functions and/or composition of the gut microbiome. Modulating the microbiome can be achieved in different ways, ranging from probiotics and prebiotics to fecal microbiome transplants, the use of bacteria as drugs and more traditional pharmaceutical approaches (e.g., small molecules). Treatments that try to regulate the microbial composition/activity for therapeutic purposes are potentially successful only when the disease is in a causative relationship to our symbionts.

Recent evidence has implicated the gut microbiota in the susceptibility to cardiovascular disease (CVD). Metabolomics studies identified choline and its derivative trimethylamine-N-oxide (TMAO) as metabolites that predispose to CVD and thrombosis.[1,2] The gut microbiota play an intermediate role in converting choline to trimethylamine (TMA). This metabolite then undergoes oxidation in the liver by flavin-monooxygenase enzymes (FMOs) to TMAO, which is finally released into the circulation.[3] In a mouse model susceptible to atherosclerosis, increased dietary choline resulted in elevated plasma levels of TMAO and accelerated plaque development.[4] Dietary exposure to TMAO elicited significant alterations in sterol/cholesterol metabolism, accounting for increased atherosclerosis. In contrast, mice deprived of the intestinal flora, either germ-free or treated with antibiotics, showed reduced circulating TMAO levels and atherosclerosis, even in association with a high-choline diet.[2,4] In addition, TMAO enhanced platelet responsiveness and thrombotic potential in animal models.[5,6] Platelet activation and aggregation and the subsequent generation of occlusive intraarterial thrombi are essential steps in atherothrombotic disease. Platelet exposure to TMAO enhanced stimulus-dependent platelet activation through increased Ca^{2+} release from intracellular stores.[5] Enhanced platelet reactivity is associated with both the extent of end-organ injury and adverse prognosis. Animal model studies employing dietary choline or TMAO, germ-free mice and microbial transplantation confirmed a role for gut microbiota-dependent TMAO production in modulating platelet hyperresponsiveness and thrombosis potential and identified microbial taxa associated with plasma TMAO and thrombotic potential.[5] Consistently, patients with the highest TMAO plasma levels had an increased risk of myocardial infarction or stroke, suggesting that elevated plasma TMAO concentration is predictive of thrombotic events and CVD.[7] Collectively, these studies demonstrated that the gut microbiota is an important player in atherogenesis and thrombosis and represents an environmental risk factor for CVD.

Thus, targeting gut microbiota-dependent TMAO formation is emerging as a novel potential therapeutic strategy to reduce thrombotic risk. A growing effort is currently made by scientists to "drug the microbiome" for clinical purposes, including the maintenance of cardiovascular health. In a recent *Nature Medicine* paper, Roberts et al[6] have developed and tested selective choline analogs capable to counteract TMAO formation. The aim of the authors was to select drugs that optimally target a gut microbial pathway with well-known relevance for a specific disease, in this case thrombosis. By applying a comprehensive screening strategy, 2 halomethylcholine-based inhibitors were identified. These compounds have the ability to interfere with the function of a

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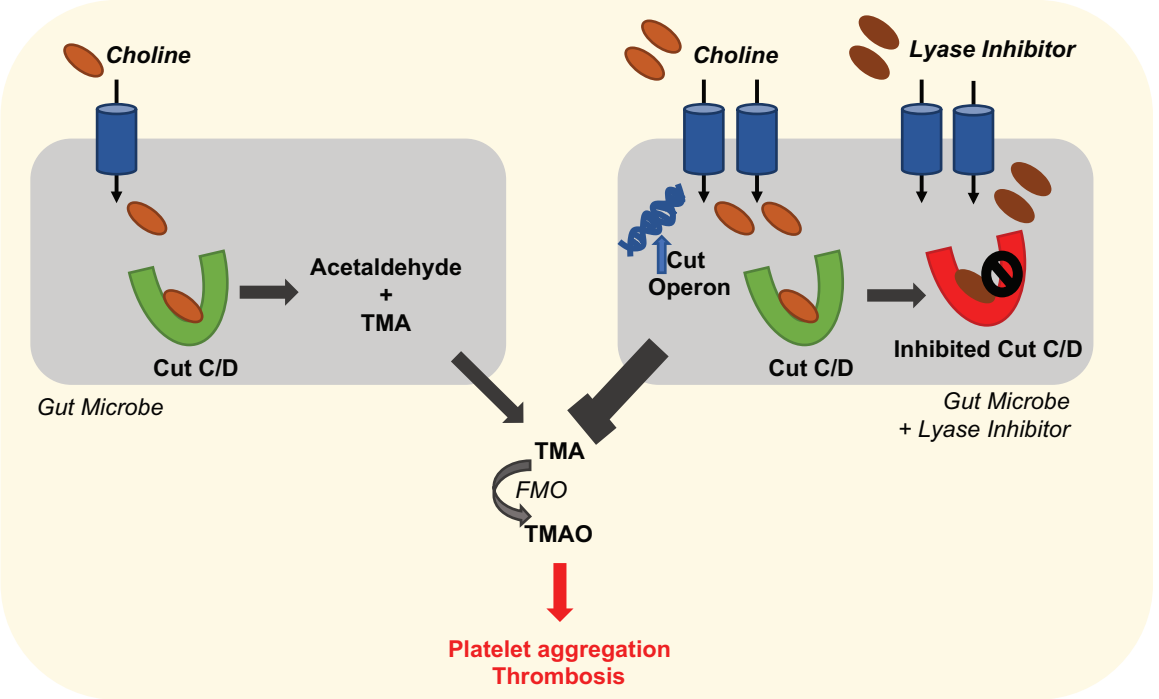

Figure 1. Novel choline analogs inhibit gut microbiota-dependent TMAO production and reduce atherothrombotic risk. Gut microbiota produce TMA from choline through the activity of choline TMA lyase CutC/D. Once released, TMA is converted in TMAO by hepatic FMO. TMAO, due to its proatherogenic and platelet-activating action, promotes atherosclerosis and thrombotic events. Upon CutC/D inhibition, microbial choline increases. Further accumulation of choline and the inhibitor occurs as a result of *cut* gene cluster upregulation. These mechanisms inhibit choline conversion to TMA and decrease choline availability to surrounding microbes, further contributing to TMA/TMAO reduction. As a result, platelet responsiveness and thrombotic potential are reduced.

major microbial TMA-generating enzyme, CutC/D, leading to its irreversible inactivation. Specifically, these drugs act on the CutC/D choline TMA lyase, which converts the substrate choline into TMA (Fig. 1). Importantly, these choline analog inhibitors are selectively transported into gut microbes, thus limiting systemic drug exposure in the host. The safety of these drugs is suggested by the lack of toxic side effects as well as microbe lethality. The administration of the compounds to mice fed a choline-enriched diet resulted in potent inhibition of plasma TMAO release. The selective accumulation of the inhibitors within the large intestine completely prevented TMA formation and led to a marked increase in intestinal microbial cytosolic choline levels. Choline accumulation is sensed as nutrient overload within gut microbes and promotes the induction of the *cut* gene cluster, encoding CutC/D itself as well as a choline transporter (Fig. 1). As a result, a positive feedback loop is established, whereby both the choline TMA lyase substrate (choline) and substrate analog (the drug inhibitor) are actively pumped and sequestered into the microbe. In turn, this event reduces choline availability to neighboring microbes, further contributing as a secondary mechanism to the reduction of TMA formation. The suppression of TMAO levels in mice treated with choline TMA lyase inhibitors significantly improved platelet responsiveness and reduced their aggregation. Taking advantage of the carotid artery $FeCl_3$-induced injury model, the authors elegantly show that clot formation was efficiently suppressed in those mice, suggesting a potent antithrombotic effect of these compounds. Importantly, bleeding was not observed upon administration of the drugs, which represents a key and uncommon advantage for their clinical development as antiplatelet therapies. Noteworthy, gut microbiota composition was partially altered by inhibitor treatment, which triggered a shift in the proportions of several microbial communities. The specific increase in the *Akkermansia* genus might be of further benefit in this setting due to its protective role in obesity and metabolic health. Therefore, the efficacy of these inhibitors is likely to be in part mediated by their ability to shift microbial composition to one that produces less TMAO and/or naturally counteracts thrombotic risk. Overall this study identifies promising mechanism-based drugs to apply in patients at risk of thrombotic complications and CVD[6] (Fig. 1) and represents a premise to the future development of several novel strategies to prevent/cure diseases through microbiome targeting.

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