THROMBOSIS

Gut microbes modulate platelet function and thrombosis risk

“plasma TMAO levels independently predict the risk of incident thrombotic events such as stroke and myocardial infarction”

In a study that combines data from patients, *in vitro* studies with human platelets, and animal models, Weifei Zhu and colleagues show that gut microbes, through the generation of trimethylamine N-oxide (TMAO), directly modulate platelet hyperreactivity and thrombosis potential. Moreover, plasma TMAO levels independently predict the risk of incident thrombotic events such as stroke and myocardial infarction. Gut microbes, through TMAO production, have been previously linked to atherosclerosis development, adverse ventricular remodelling, and endothelial cell activation.

In a cohort of >4,000 stable patients undergoing elective diagnostic cardiac catheterization, plasma levels of TMAO were associated in a dose-dependent manner with increased incident thrombotic events (myocardial infarction or stroke). This association remained even after adjusting for multiple risk factors, cardiovascular status, renal function, and medication.

In vitro experiments with platelets isolated from healthy donors showed that physiological levels of TMAO enhanced platelet responsiveness to multiple agonists in a dose-dependent manner. TMAO enhanced platelet activation induced by ADP and thrombin (monitored by platelet aggregometry), and increased platelet adhesion to immobilized collagen (monitored by real-time microscopy). TMAO promoted platelet hyperresponsiveness by enhancing the release of intracellular cytoplasmic Ca^{2+} .

In animal models, the investigators demonstrated that gut microbes and dietary nutrients that increased TMAO generation modulated platelet hyperreactivity and thrombosis potential *in vivo*. For these studies, Zhu and colleagues used several mouse models, including germ-free mice, microbial transplantation, and dietary supplementation with choline — a nutrient that contains trimethylamine (TMA), the precursor for TMAO generation in the liver, and abundant in animal products such as eggs, liver, beef, and pork.

In mice, dietary supplementation with either TMAO or choline elevated the levels of TMAO in plasma, increased platelet aggregation induced by ADP, and shortened clot formation rate *in vivo* (monitored by time to blood flow cessation in the carotid artery following $FeCl_3$ injury).

The obligatory role of gut microbiota in the choline-dependent effects was demonstrated by suppressing gut microbes with a cocktail of broad-spectrum antibiotics. Oral antibiotics reduced plasma levels of TMAO and suppressed the prothrombotic phenotype only in the choline supplemented mice, but not in the TMAO supplemented group. The obligatory role of gut microbiota was further demonstrated in

germ-free mice, in which choline supplementation did not

produce TMA, or therefore TMAO, nor did it induce the prothrombotic phenotype observed in conventional mice.

Interestingly, choline supplementation altered the microbial composition, and specific microbial taxa

were associated with plasma TMAO levels and the thrombosis potential. Finally, in a mouse model of microbial transplantation, Zhu and colleagues showed that the thrombosis potential is a transmissible trait.

These findings “reveal a previously unrecognized mechanistic link between specific dietary nutrients, gut microbes, platelet function, and thrombosis risk,” conclude the investigators. These results suggest new potential therapeutic targets and nutritional interventions for preventing cardiovascular events.

The investigators suggest, for example, that TMAO levels could have clinical utility for identifying individuals who might benefit from antiplatelet prophylaxis therapies, and speculate that targeting this microbial pathway has the potential to reduce blood clot formation without the bleeding complications of other antiplatelet therapies.

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