Mechanism and Targeting of Host-Microbial Trimethylamine Pathway to Reduce Neointimal Hyperplasia after Injury

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INTRODUCTION

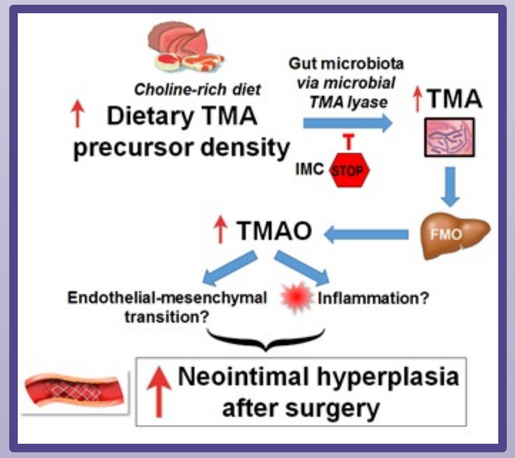
Each year, 7.4 million Americans with CVD undergo procedures. However, up to 50% of treated vessels exhibit restenosis. This is due to neointimal hyperplasia, a pathologic response of the blood vessel to vascular intervention. The pathologic neointima is composed of cells from multiple possible sources, including resident smooth muscle cells, adventitial fibroblasts, and endothelial cells that transform into myofibroblasts via endothelial-to-mesenchymal transition (endoMT). Major gaps exist in our understanding of the complex process of pathologic neointima formation after injury.

Gut microbiota exposed to a Western diet rich in compounds such as choline generate trimethylamine (TMA) via the enzyme TMA lyase, which is converted to trimethylamine N-oxide (TMAO). Elevated TMAO is associated with endothelial injury and the promotion of fibrotic cellular phenotypes.

We propose a novel pathway for neointimal hyperplasia that links gut microbiota, a TMAO produced by gut microbiota from dietary nutrients, and neointimal hyperplasia after vascular surgery.

OBJECTIVES

- **Determine if a choline-rich diet, via TMA generation by the gut microbiome, will increase the severity of neointimal hyperplasia after arterial surgery**
- **Define the role of endoMT as the mechanistic link between elevated TMAO, arterial injury, and neointimal hyperplasia**



METHODS

In vivo experiments: 15-week-old C57BL/6 mice were given control drinking water with 1% choline for 3 weeks prior to left carotid ligation. Morphometric analysis of arteries 4 weeks after injury to quantify the extent of injury was performed. In a subset of mice microbial TMA lyase activity was blocked using iodomethylcholine (IMC) and levels of TMA and TMAO were measured.

In vitro experiments: Human umbilical vein endothelial cells (HUVEC) were treated with TMAO and measurements of endoMT including cell morphology and mesenchymal-specific cell marker expression were examined.

RESULTS

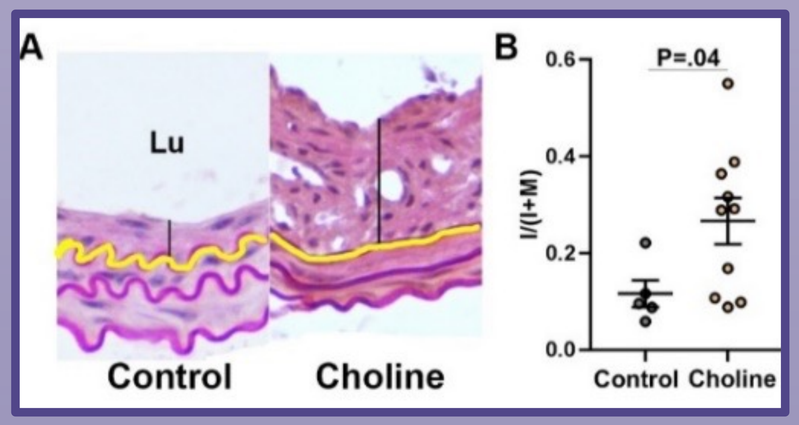


Fig 1. Dietary choline exacerbates neointimal hyperplasia after carotid ligation. A. H+E staining of carotid artery sections (20X) showing increased neointimal hyperplasia (vertical lines) in the choline group 4 weeks after ligation. Yellow lines, internal elastic lamina. Lu, Lumen. B. Quantification of hyperplasia, represented by mean intima area (I)/intima+media area (I+M) measured on arterial sections at 35 μM intervals.

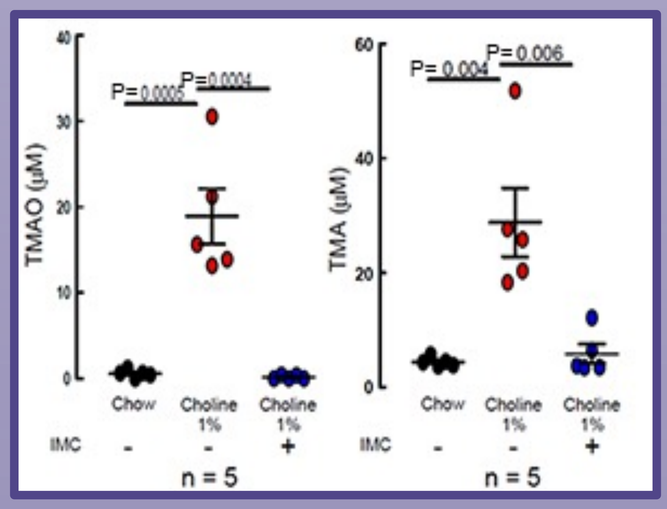


Fig 2. Iodomethylcholine (IMC) blocks TMA production. High choline (1%) diet results in significantly increased conversion of choline to TMA and TMAO. Results are mean ±SEM serum concentrations.

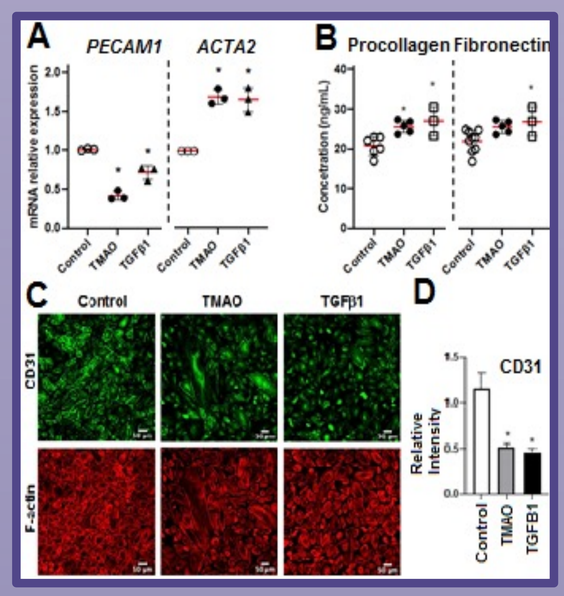


Fig 3. TMAO induces endoMT in endothelial cells. HUVEC incubated with TMAO (200 μM, 24 h) had a significant reduction in CD31 (PECAM1) and concomitant increase in α-SMA (ACTA2) gene expression by qPCR (A) and an increase in the secretion of procollagen Ia1 and fibronectin, an indication of myofibroblast differentiation (B). C. Representative immunofluorescence of HUVEC treated with TMAO (200 μM, 72 h). There is also loss of characteristic cobblestone endothelial cell morphology in the TMAO group by F-actin staining. D. Quantitation of staining by relative intensity. *, P<.05 relative to control.

CONCLUSIONS

Our preliminary findings strongly implicate a novel diet-microbe-host driving neointimal hyperplasia development after vascular surgery.