Gut Microbiota and Atherothrombotic Heart Disease

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Drugs for Bugs
Drugging the Microbiome
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• Dr. Hazen is named as a co-inventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and/or therapeutics.

• Dr. Hazen reports having been paid as a consultant for the following companies: Esperion, and Procter & Gamble.

• Dr. Hazen reports receiving research funds or support from Abbott, Astra Zeneca, Pfizer, Procter & Gamble, Roche, and Takeda.

• Dr. Hazen reports having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics and/or therapeutics from Cleveland Heart Laboratory, Esperion, Frantz Biomarkers, LLC and Siemens.

• Dr. Hazen was the scientific founder of Cleveland Heart Laboratory, and reports having equity in that company.
The microbiome can be considered as our largest endocrine organ.

The microbiome is a "drugable" target.

The microbiome is a filter of our largest environmental exposure - what we eat.

The human body is an integrated circuit between our gut microbes and our human genes.

Some take home concepts:

- Dietary choline and L-carnitine
- Heart Attack
- Heart Failure
- CKD
- Atherosclerosis
- Hepatic FMOs
- TMA
- TMAO
- Gut flora
Diet and Intestinal Microbes are Mechanistically Linked to Atherosclerotic Heart Disease

Meta-organismal pathway:

(i) gut microbe
(ii) host hepatic FMOs

Trimethylamine (TMA)

RA Koeth (2013) *Nature Medicine*
B Bennett (2013) *Cell Metab*
Z Wang (2014) *Eur Heart J*
WHW Tang (2014) *JACC*
RA Koeth (2014) *Cell Metab*
M Warrier (2015) *Cell Reports*
C Organ (2016) *Circ Heart Fail*
W Zhu (2016) *Cell*
TMAO alters macrophage phenotype, EC activation and sterol metabolism in multiple compartments

adapted from:
The gut microbial TMAO pathway contributes to the development of “The Vulnerable Patient”

Elevated plasma levels of TMAO predict incident risk for thrombotic events (myocardial infarction and stroke).

Adjusted for traditional risk factors include age, gender, systolic blood pressure, meds, BMI, LDLc, HDLc, smoking, DM, TG, eGFR, CVD.

N = 4007 sequential subjects undergoing cardiac evaluations, 3-year follow up.

Xinmin Li, PhD

Brief exposure to TMAO enhances human platelet responsiveness to multiple agonists

Washed human platelets

Platelet rich plasma

Zhu et al, Cell (2016)
TMAO enhances stimulus dependent Ca\(^{2+}\) release in platelets

Diet → Choline

↑ TMAO

Change in microbial community composition and function

Platelet

TMAO Receptor?

Platelet Hyperresponsiveness

Carotid artery injury in vivo thrombosis model

- Internal carotid artery cut down
- Vital microscopy imaging of fluorescent labeled platelets

Dietary choline enhances thrombosis susceptibility *in vivo*

Plasma levels of TMAO show a strong correlation with *in vivo* thrombosis rates

Statistical analysis:

- $r = -0.60$
- $p < 0.001$

Graph showing the correlation between the plasma concentration of TMAO and time to cessation of flow (sec).

“Let food be thy medicine and medicine be thy food.”
Hippocrates, Father of Western Medicine
431 B.C.
What are Dietary Sources of Choline/Phosphatidylcholine?

Lekithos *(Greek)* = Egg yolk
Chole *(Greek)* = Bile
Chronic Dietary Choices Impact TMAO Levels

De Filippis et al, *Gut* 2015
Development of inhibitors (and activators) of microbial TMA lyases

Sources of DMB
Olives/Cold-pressed extra virgin olive oil
Grape seed oil
Guinness Lager
Stout
DMB is a non-lethal microbial TMA lyase inhibitor in multiple human commensals

**New concept:** Non-lethal microbial enzyme targeting as a therapeutic

**Small molecule inhibition of microbial choline TMA-lyase activity**

Wang et al, Cell (2015)
Microbial TMA-lyase inhibition reduces TMAO levels in vivo

C57Blk/6J, apoE-/- mice

Wang et al, Cell (2015)
Microbial TMAO lyase inhibition attenuates dietary choline enhanced atherosclerosis

C57BL/6J ApoE-/- mice

(a) Images showing aortic lesions with different treatments:
- Chow
- Choline
- Chow + DMB
- Choline + DMB

(b) Graph showing aortic lesion area in different groups:
- Chow (n=32)
- Choline (n=23)
- Chow + DMB (n=20)
- Choline + DMB (n=20)

Legend:
- Chow: Red dashed line
- Choline: Black bar
- Chow + DMB: Black bar with error bars
- Choline + DMB: Black bar with error bars

$p=0.006$ for Choline vs. Chow + DMB
$p=0.003$ for Choline vs. Chow
$p=0.27$ for Chow vs. Chow + DMB

Wang et al, Cell (2015)
Drugging the Microbiome - Its in Our Future for CVD Therapeutics
