Abdominal Aortic Aneurysm
The Search For Therapies
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- No disclosures
Outline

• What is abdominal aortic aneurysm - AAA?
• What causes AAA and how is it treated?
• How are smoking and nicotine related?
• How do we approach the search for new treatments?
• What are some treatments we are investigating that show promise?
Aorta

- Largest artery in the body – elastic artery
- Comes directly out of the heart (aortic valve)
- Supplies all organs via branches
- Subject to some of the largest vessel pressure fluctuations – designed to rebound
- Wall is made up of multiple layers
  - Intima – thin single layer of endothelial cells
  - Media – thick layer of smooth muscle cells with elastic tissue/matrix between
  - Adventitia – layer of fibroblasts, collagen, supporting elements
Aortic Aneurysm

Definition: 50% increase over normal diameter (depends on age, patient size, anatomic location)
Abdominal Aortic Aneurysm = AAA

- Significant cause of morbidity and mortality
- Improving surgical techniques
- *No current medicine-based treatment*
AAA

- A common problem of the elderly.
  - > age 50, and increases with age
- 75% of all “atherosclerotic” aneurysms
- Located in infrarenal (below kidneys) aorta (95%)
- Large gender disparity
  - Men:Women = 9:1
  - + genetic predisposition (12-fold in males)
- Risk factors: male, age, smoking, lipids, family history
  - NOT DIABETES
- Mural thrombus (blood clot) often present
- Usually no symptoms.
  - Rarely rupture or tear if < 5.0 cm
- Rapid growth suggests higher risk of rupture
- Diagnosis?
  - Ultrasound, palpation, CT/MR
  - Often found incidentally
- Treatment?
  - No effective medical therapy yet available
  - Open repair vs. stent-grafting once it reaches large size
Why are AAA dangerous?

- Complications
  - Abdominal pain
  - Atheroembolism – cholesterol and clot moving downstream to smaller arteries
  - Rupture (rarely dissection/tearing) – can be sudden

- Danger Signs: low blood pressure, tender mass, pain
Medical Therapies

Eleven randomized-controlled clinical trials to slow AAA, to date

- Blood pressure meds?
- Fibrate for cholesterol lowering?
- Antibiotics to decrease matrix breakdown – or treat C. pneumonia infection?
- Mast cell inhibitor (pemirolast)?
- Anti-platelet agent (ticagrelor)?
- Others underway!

So far – nothing has worked

Golledge et al. JIM 2020, 288: 6-22
What’s wrong at the cellular/tissue level?

- Endothelial cells – damaged, missing
- Elastic tissue – degrades and fragments
- Smooth muscle cells – die and disappear
- Immune cells – white cells infiltrate
- Clot forms on interior surface
Smoking, Nicotine and AAA

• **Smoking** = strongest modifiable risk factor
  – 90% of AAA patient relate smoking history
  – Only lung cancer has stronger disease association
  – Cessation is **only** therapy known to decrease expansion rate
  – Numerous proposed mechanisms

• **Nicotine** – addictive major component of tobacco smoke
  – Stimulant & parasympathetic alkaloid
  – Substantial data supports role in vascular inflammation
  – Augments murine aortic stiffness – predisposing to AAA
  – Infused and vaped nicotine augment murine model AAA


**SCIREQ Vaping System**

Vascular Discovery Poster 2019
Recently submitted for publication
Toxins can alter DNA – epigenetic modifications
- DNA methylation can silence or activate genes
- Changes can be durable across generations (e.g. imprinted genes)

Cigarette Smoke - one of the strongest modulators of DNA methylation
- Prenatal smoke alters DNA methylation in offspring, persists to adulthood
- DNA variations in human cord blood correlate with cotinine (nicotine metabolite)
- Thousands of differentially methylated regions in DNA in human blood (smokers vs. non
  - Most revert with cessation
  - Some durable for up to 30 years

Nicotine
- Can pass through placenta and alter DNA
- Perinatal maternal nicotine (rats) induced asthma in F1 and F2 offspring
  - Durable changes in DNA methylation in ovary, testis and lung
  - Also alters offspring vascular contractility, heightens oxidative stress, can increase aortic response to AngII

Smoke-Nicotine – Transgenerational Effects
Approach to AAA Research

1. Animal models
2. Analysis: candidate identification and confirmation
3. In vitro mechanisms
4. Test in animal models
5. Check translational potential
Animal Models – flawed mirror

- Some available murine AAA model options (ATVB 2004;24:429-434)

<table>
<thead>
<tr>
<th>Mode of AAA Induction</th>
<th>Characteristics</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>Genetically Determined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blotchy</td>
<td>MD</td>
<td></td>
</tr>
<tr>
<td>Lox deficiency</td>
<td>MD</td>
<td>Elastin and collagen cross-linking defect with death from aneurysmal rupture in full-term fetus</td>
</tr>
<tr>
<td>MMP-3 or TIMP-1 deficiencies</td>
<td>MD</td>
<td>Medial degeneration that occurred in both the thoracic and abdominal segment</td>
</tr>
<tr>
<td>LDL receptor−/−</td>
<td>MD</td>
<td>Aneurysms localized to the suprarenal segment in mice fed diets enriched with saturated fat, cholesterol, and cholate</td>
</tr>
<tr>
<td>ApoE−/−</td>
<td>MD, A</td>
<td></td>
</tr>
<tr>
<td>ApoE−/− × eNOS−/−</td>
<td>MD, T, A</td>
<td></td>
</tr>
<tr>
<td>SMC-specific LRP−/− × LDL receptor−/−</td>
<td>MD, A</td>
<td>Large AAAs in abdominal aorta with aortic arch thickening and vessel elongation.</td>
</tr>
<tr>
<td>Transgenic mice overexpressing renin and angiotensinogen</td>
<td>MD</td>
<td>Rupture of abdominal and thoracic aorta aneurysms within 10 days of increased salt intake</td>
</tr>
<tr>
<td>Chemically Induced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elastase</td>
<td>MD, I</td>
<td>Infusion into infrarenal aorta leads to delayed dilation and inflammation.</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>MD, I</td>
<td>Progressive dilation and inflammation at site of application.</td>
</tr>
<tr>
<td>AngII infusion into</td>
<td>MD, I, T, A</td>
<td>AAAs form in the supra-renal aorta.</td>
</tr>
<tr>
<td>LDL receptor−/−</td>
<td></td>
<td></td>
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<tr>
<td>ApoE−/− mice</td>
<td>MD, I, T, A</td>
<td></td>
</tr>
<tr>
<td>C57BL/6 mice</td>
<td>MD, I, T</td>
<td></td>
</tr>
</tbody>
</table>

MD indicates medial degeneration; I, inflammatory components; T, thrombus; A, atherosclerosis.
Animal Models

• Angiotensin II/ApoE-/- Mice
  – Pros: technically simple, reproducible, human-like pathophysiology, high incidence (in males)
  – Cons: “dissection model”, supra-renal – above kidneys
Animal Models

- PPE Model (porcine pancreatic elastase)
  - Pros: recapitulates human risk profiles, infra-renal, 100% incidence, human-appearing tissue pathology
  - Cons: technically difficult, no atherosclerotic/hypertensive aspects
Some Approaches to AAA – Work From Our Lab

- **Reduce** pressure differential
- **Strengthen the aorta with BioGlue**
  - Circulation. 2015 May 19;131(20):1783-95
Some Approaches to AAA – Work From Our Lab

- Use **microRNAs** (tiny non-coding RNAs) or **lncRNAs** (long-non-coding RNAs) to alter cellular inflammation, aortic stiffness, smooth muscle death
  - Clin Invest. 2012 Feb;122(2):497-506
  - Nat Commun. 2014 Oct 31;5:5214
  - Circulation 2018 Oct 9;138(15):1551-1568
Some Approaches to AAA – Work From Our Lab

- Use migrating **stem cells** from a porous scaffold to help restore the aorta
  - Biomater Sci. 2021 Oct 12;9(20):6903-6914
From Biology to Drugs

- Remember nicotine?
- Treating mice with nicotine increases the severity of AAA in their offspring!

Vascular Discovery - Oral Presentation, Boston 2021
Treating parents with nicotine alters aortic DNA of the offspring. ESPECIALLY TRANSCRIPTION FACTOR DNA! (Bind to DNA and activate it to start transcribing genes)
From Biology to Drugs

• So… of these modified transcription factors (TFs), which ones might contribute to AAA?

• SOFTWARE TOOL CHEA3 predicts which TFs target genes from a list

• Use the list of genes that rise or fall with AAA in mice! (Microarray data)
  • Identified candidate IRF8
IRF8? What’s that?

- IRFs are “interferon response factors”
- Activate genes in response to gamma interferon – a signaling molecule for the immune system
- IRF8 is expressed in immune cells and stressed smooth muscle cells, very important for turning on monocytes and macrophages (key cells in AAA) and can regulate SMC structure, function, and death
From Biology to Drugs – chemicals that might affect IRF8

Top Interacting Chemicals

- Lipopolysaccharides
- (+)-JQ1 compound
- Tetrachlorodibenzo-dioxin
- Benzo(a)pyrene
- Acetaminophen
- Mercuric Chloride
- Mercury
- Tetradecanoylphorbol Acetate
- Tobacco Smoke Pollution
- Calcimycin

Interactions

Comparative Toxicogenomics Database

Inflammmatory Transcriptome Profiling of Human Monocytes Exposed Acutely to Cigarette Smoke
From Biology to Drugs

- What drugs might downregulate IRF8?
- Use DSigDB – a database that contains expression profiles from cells perturbed by thousands of compounds.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source</th>
<th>Chemical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>alprostadil</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>bromocriptine</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>lycorine</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>15-delta prostaglandin J2</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>Prestwick-983</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>dihydroergocristine</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>dihydroergotamine</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>clonidine</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>8-azaguanine</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>securinine</td>
</tr>
<tr>
<td>IRF8</td>
<td>D3(DOWN)</td>
<td>mebendazole</td>
</tr>
</tbody>
</table>

Also high on the list for chemicals that alter AAA-related genes from our data.
OK, so what’s securinine?

• One of the 50 fundamental traditional Chinese herbal remedies - 一叶秋 (yī yè qiū)

• Alkaloid found in Securinega suffruticosa and Phyllanthus niruri

• Used with some efficacy in neurological disorders like ALS, Parkinson’s disease, Alzheimer’s disease, polio

• Thought to be helpful in colon cancer, acute leukemia

• Has neurological anti-inflammatory properties:
  – Mediators Inflamm. 2017;2017:8302636

• Warning: promotes seizures if too much is used
Securinine in cells

• Reduces IRF8 and inflammatory markers in smooth muscle cells and monocytes
Securinine in cells

- Reduces key AAA inflammatory gene interleukin-6 in macrophages compared with nicotine, and reduces the effects of nicotine
Securinine in Model AAA

- Reduces AAA growth in the PPE model if given after induction
Securinine in Model AAA

- Reduces gene expression for aortic inflammation, some macrophage markers and IRF8 (vs. DMSO vehicle) at 28 days
Securinine – Issues Remain

• Several mice died during the project due to seizures, frequent issue during treatments
• Key to find a dose/regimen that will be effective without causing these side-effects
• Confirm with trial in other AAA models
• Attempt to find other candidate drugs with similar effects on AAA without the pro-convulsant down-side
• Once efficacy established can start thinking about larger mammal trials (e.g. minipigs)
Conclusions

- Abdominal aortic aneurysm remains a vascular scourge with no medical treatment.
- Smoking (and likely vaping) not only increases the risk of AAA but may increase disease risk in future generations.
- Numerous approaches are being studied – difficulties include differences between humans and animals, delivery methods, side effects, and especially efficacy.
- Support from research institutes and groups like CARE will be crucial for future success.
Other Funding

- Collaborators
  - Philip Tsao
  - Ron Dalman
  - Lars Maegdefessel
  - Tom Quertermous
  - Joseph Wu

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  - Joscha Mulorz
  - Pireyatharsheny Mulorz
  - Yae Hyun Rhee
  - Markus Wagenhaeuser
  - Alicia Deng
Q & A

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