Keeping the Small Aortic Aneurysm Small

John A. Curci, MD, FACS
Associate Professor of Vascular and Endovascular Surgery
Director, Aortic Aneurysm Research Laboratories, Washington University
Principal Investigator [Biomarkers Core], N-TA³CT

Washington University in St. Louis
School of Medicine

Markey Lecture 2012
Abdominal Aortic Aneurysms

- Present in 5-9% of the population over 65 years of age
- 45,000 elective operations each year in US
- 15,000 deaths from rupture each year in US
Abdominal Aortic Aneurysms
Overview

- Brief Clinical Background on AAA
- The Development of a New Therapy
  - Human Pathobiology
  - Lab Models
  - Clinical Trials
- Understanding the Nuance of the Disease
  - Models
    - Detailed mechanisms
    - Clinical correlation
  - Systems Complexity
# Etiology of AAAs

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>5.57</td>
</tr>
<tr>
<td>Male Gender</td>
<td>2.68</td>
</tr>
<tr>
<td>Family Hx</td>
<td>1.94</td>
</tr>
<tr>
<td>Age Over 65</td>
<td>1.71</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.15</td>
</tr>
<tr>
<td>Smoking &lt;20 Pk-Yrs</td>
<td>2.75</td>
</tr>
<tr>
<td>Smoking &gt;50 Pk-Yrs</td>
<td>9.55</td>
</tr>
</tbody>
</table>

Natural History of Abdominal Aortic Aneurysms

- **Stage I**: Aneurysm Development
- **Stage II**: Gradual Expansion
- **Stage III**: Rapid Expansion and Rupture

Risk of Aneurysm Rupture vs. Abdominal Aortic Diameter

- 2.0-2.5 cm
- 3.0 cm
- 5.5 cm
Contemporary Therapy for AAA

<5.5 cm

>5.5 cm
FEATURES OF CLINICAL DISEASE

Therapeutic Development
Histopathology of Abdominal Aortic Aneurysms

Chronic Transmural Inflammation
Destructive Remodeling of the Elastic Media
Inflammation in AAA

- a) Normal aorta
- b) AAA with focal infiltrate
- c) CD3+
- d) CD45+
- e) CD68+
- f) HLA DR, DP, DQ+
Metalloprotease Milieu
Increased Gelatinase B (MMP-9) in Human AAAs

J Clin Invest 1995; 96:318-326
Increased Plasma MMP-9 in Patients with AAAs

A

B

Plasma MMP-9 Concentration, ng/ml

Normal Controls Aortoiliac Occlusive Disease Abdominal Aortic Aneurysm

ULN

Normal Controls n = 5 Aortoiliac Occlusive Disease n = 15 Abdominal Aortic Aneurysm n = 25

P < 0.05

J Vasc Int Radiol 2000; 11:1345-1352
Exquisite Localization
Medial SMC Depletion in Human AAAs

Normal Aorta
199.5 +/- 14.9 SMC/HPF

Aortic Atherosclerosis
176.4 +/- 13.9 SMC/HPF

Aortic Aneurysm
50.9 +/- 6.1 SMC/HPF

Lopez-Candales et al., Am J Pathol 1997; 150:993-1007
SMC Senescence in Human AAAs

Liao et al., J Surg Res 2000; 92:85-95
Features of Aneurysmal Degeneration

Genetic Predisposition

Proteolytic Degradation of Elastin and Collagen

Atherosclerosis and Expansive Remodeling

Medial SMC Depletion: Apoptosis and Senescence

Inflammation, Immunity, and Infection

Impaired Connective Tissue Repair and Diminished Tensile Strength

Mural Thrombus, Medial Ischemia, and Neovascularization

Oxidative Stress and Free Radical Damage

Altered Geometry and Elevated Biomechanical Wall Stresses
A Vicious Cycle

• Medial Elastic Fiber
  • Typically absent
  • Relatively unique
  • Elastolytic enzymes
  • Central pathophysiology?

• Inflammatory Cells
  • Chronic Inflammation
  • Th1 vs Th2
  • Protease elaboration/activation

• VSMC
  • Diminished capacity for matrix repair ()? Cell loss
  • May elaborate proteases
  • May elaborate cytokines
Therapeutic Development

MODELING ABDOMINAL AORTIC ANEURYSMS
Studies on Human AAA Tissues Represent “End-Stage” Disease
Elastase-Induced AAAs in Mice

Pyo et al., J Clin Invest 2000; 105:1641-1649
Elastase-Induced AAAs in Mice

Pyo et al., J Clin Invest 2000; 105:1641-1649
Tetracycline Derivatives as MMP Inhibitors

DOXYCYCLINE

MMP-Inhibiting Activity

Antibiotic Activity
Treatment with Doxycycline and MMP-9 Deficiency Both Inhibit Elastase-Induced AAAs

Pyo et al., J Clin Invest 2000; 105:1641-1649
WT Bone Marrow Rescues AAA-Resistant Phenotype of MMP-9 KO Mice

MMP-9 KO Bone Marrow Confers AAA-Resistant Phenotype Upon WT Mice

Pyo et al., J Clin Invest 2000; 105:1641-1649
## MMP Inhibition in Animal Models of Aortic Aneurysm

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>MMP Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastase-Induced AAAs (Rats, Mice)</td>
<td>Doxycycline, MMP-9 Knockout, Chemically-Modified TCNs, Hydroxamates (BB94, RS132908), Indomethacin (indirect), Simvastatin (indirect)</td>
</tr>
<tr>
<td>CaCl₂-Induced AAAs (Mice)</td>
<td>Doxycycline, MMP-9 Knockout, MMP-2, MMP-12 Knockouts, CD4 Knockout, IFN-g Knockout</td>
</tr>
<tr>
<td>Angiotensin-Induced AAAs (Mice)</td>
<td>Doxycycline, MMP-9 Knockout</td>
</tr>
<tr>
<td>Fibrillin-Deficiency (Marfan Mice)</td>
<td>Doxycycline, Losartan</td>
</tr>
</tbody>
</table>
CLINICAL TRANSLATION

Therapeutic Development
Early Clinical Translation

- Effects of Doxycycline *in vivo*, pub 2000
  - Biologic alterations in established AAA
  - Patients for elective open AAA w/ 7 days doxy
    - Reduced MMP activation
    - Reduced MMP production

A.

B.

Normalized mRNA Expression

<table>
<thead>
<tr>
<th>Density Ratio</th>
<th>MMP-2</th>
<th>MMP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAA Dox</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAA Dox</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>
Early Clinical Translation

- Alterations in AAA morphology
  - Mosorin, et. al. in 2001
  - Inhibition of AAA growth after 3 months of therapy
  - Effect persisted beyond initial treatment interval

Table II. Follow-up data*

<table>
<thead>
<tr>
<th></th>
<th>Doycycline group</th>
<th>Placebo group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of aneurysm (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>31.0 (27.5-38.5)</td>
<td>35.0 (31.0-40.0)</td>
<td>NS</td>
</tr>
<tr>
<td>At 6 mo</td>
<td>32.0 (27.5-39.0)</td>
<td>36.0 (32.0-40.0)</td>
<td>NS</td>
</tr>
<tr>
<td>At 12 mo</td>
<td>33.0 (26.7-38.7)</td>
<td>39.0 (33.7-44.5)</td>
<td>.06</td>
</tr>
<tr>
<td>At 18 mo</td>
<td>33.0 (26.7-38.5)</td>
<td>39.0 (30.5-44.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Expansion rate (mm/y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During 0- to 6-mo period</td>
<td>0.0 (0.0-2.0)</td>
<td>0.0 (0.0-2.0)</td>
<td>NS</td>
</tr>
<tr>
<td>During 6- to 12-mo period</td>
<td>0.0 (0.0-2.0)</td>
<td>2.0 (2.0-6.5)</td>
<td>.01</td>
</tr>
<tr>
<td>During 12- to 18-mo period</td>
<td>0.0 (0.0-1.0)</td>
<td>5.0 (2.5-12.0)</td>
<td>.01</td>
</tr>
<tr>
<td>During 0- to 18-mo period (mm)</td>
<td>1.5 (0.0-3.0)</td>
<td>3.0 (0.25-6.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are shown as medians and 25th and 75th interquartile ranges.
More Early Clinical Translation

- Phase II Study, Baxter, et.al., 2002
  - 6 months of treatment tolerable
- Hackmann, et. al., 2008
  - Stabilized aneurysm neck dilatation after endograft
  - May have accelerated AAA shrinking
  - Reduced circulating MMP-9
- Lindeman, et. al., 2009 JVS and Circulation
  - Doxy reduces aortic neutrophils and cytotoxic T-cells
Screening with CT Scans
282 Patients

Enrollment and Biomarkers
248 Patients

124 Placebo
124 Doxycycline

Follow-Up Visits Every 3 Months
Biomarker and CT scan @ 6-Month Follow-Up
SF-36 QOL evaluations
Common Termination After ≈3.5 Years
Minimum Follow-Up of 2 Years

National Institute on Aging
Basic Study Design

- Prospective, Randomized, Placebo-controlled
- Doxycycline 100 mg bid
- Include Small AAA
  - 3.5 to 5.0 cm in men
  - 3.5 to 4.5 cm in women
- CT imaging every 6 months
- Statistics to evaluate growth
  - Based on rank order
  - Includes effect of sudden death or AAA repair
  - Sensitivity of >90% to identify a 40% effect on growth.
- Biorepository (imaging and serum/plasma)
Details and Nuance of the Mouse Model

BEYOND MATRIX METALLOPROTEASE: MECHANISMS OF ELASTOLYSIS
Schematic Overview of Events Involved in Elastase-Induced AAAs

- **Molecular Mediators**
  - Matrix Peptides
  - Oxygen Radicals
  - Angiotensin
  - Eicosanoids
  - Chemokines
  - Cytokines

- **Cell Types Involved**
  - EC, SMC, Fibroblasts
  - PMN, Mast Cells
  - Monocytes, T-Cells
  - Macrophages, Th1-Cells
  - SMC, Fibroblasts
  - Macrophages, Th2-Cells
  - SMC, Fibroblasts

- **Phases**
  - Acute Response
  - Transitional Inflammation
  - Chronic Inflammation and Tissue Destruction
  - Persistence or Resolution

- **Histology**
  - Intact Elastic
  - Destruction of Elastic Media
  - Adventitial Fibrosis
  - Degradation of Adventitial Collagen

- **Diameter**
  - Initial Dilatation
  - Secondary Dilatation & Aneurysm Formation

- **ELASTASE PERFUSION**
  - IL-1b, TNF-a, IL-6
  - JNK
  - AP-1
  - NF-kB
  - MMPs & TIMPs
  - Serine Proteases
  - Cathepsins & Cystatin
  - Immunomodulating Cytokines
    - IFN-g, TGF-b
    - IL-4, IL-10
Suppression of Elastase-Induced AAAs by Treatment with a Cysteine Protease Inhibitor (E64)

![Graph showing suppression of AAAs](image)
Neutrophil Proteases in Elastase-Induced AAAs

Significant Suppression in Mice Deficient in Dipeptidyl Peptidase-I (DPPI)

Pagano et al., Proc Natl Acad Sci USA 2007; 104:2855-2860
Cat-S Deficiency Suppresses Development of Elastase-Induced AAAs

- Extent of Aortic Dilatation (%)
- Day 0
- Day 7
- Day 14
- Interval After Elastase Perfusion

- C57Bl/6 Wild-Type
- CatS-Deficient

- NS

- P < 0.001
Suppression of Aortic Wall Inflammation and Elastin Degradation by Cathepsin Inhibition and Cat-S Deficiency
IL-6 is Necessary for Development of Elastase-Induced AAAs

A

B

C
Angiotensin II (AT1 Receptor)

Oxidative Stress and Pro-Inflammatory Signaling

Increased Vascular Wall MMP Production

Localized Disruption of Elastic Lamellae With Focal Dissection

Chronic Inflammation & MMP Expression

Aneurysm Formation
Suppression of Inflammation, Elastin Degradation, and MMP Expression in AT1R (-/-) Mice

A. Histology images showing wild-type and AT1R (-/-) mice after 14 days.

B. Graph showing MMP-2 and MMP-9 expression levels.

C. Graph showing inflammation and elastin content scores.

Legend:
- WT: Wild-type
- AT2a/-: AT2a knockout
- AT1a/-: AT1a knockout

Statistical significance:
- *: P < 0.05
- **: P < 0.01

Scale bars for histology images: 50 μm
AT1R Antagonism Prevents Elastase-Induced AAAs

% Increase in Aortic Diameter

<table>
<thead>
<tr>
<th>AAAs:</th>
<th>% Increase</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AngII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydralazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Losartan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant difference**

*[Image of bar chart with comparison of AAAs]*
Limitations of Current Models of AAA

IMPROVING MODELING THROUGH COMPLEXITY
Adding Tobacco Smoke

- Multifactor Model for a Multifactor Disease
- Exposure to Tobacco Smoke
  - Up to 500%-1000% increase in incidence of AAA.
  - Compares to “only” 150%-200% increase in coronary disease.
  - Risk persists long after smoking cessation.
- Models of Pulmonary Emphysema
  - Used smoke exposure of mice for months.
  - Opened new ways of considering the disease processes.
A More Clinically Relevant Model

Modified Smoking Model of AAA

- Inactive Elastase
- Low dose elastase
- Low dose elastase

Percent Diameter Increase

2 cigs/day 6 days/week. Start 2 weeks before perfusion

Bergoeing, JVS 2007, 45(6)
Histology

Low Dose Elastase Only

Low Dose Elastase + Smoke

Bergoeing, JVS 2007, 45(6)
Cured … or Not?

Doxycycline effects overcome with Tobacco Smoke.

Jin & Arif, et. al., ATVB, in publication
Smoke Enhances Model Independently of MMP

- Smoke = larger model aneurysms.

- * P<.05 cf. non-smoke
- † P<.05 cf. wild-type

Jin & Arif, et. al., ATVB, in publication
Non-MMP Enzymes and Smoke

No inhibition with other enzyme classes
- Cathepsins – Cathepsin S
- Serine Proteases – Neutrophil Elastase

Effect appears to transcend simple single enzyme inhibition.

Jin & Arif, et. al., ATVB, in publication
Smoking Cessation

- 6 Weeks of Smoke Exposure for all Mice then stopped.

- Varied interval between smoke exposure and aortic perfusion

- All animals harvested 2 weeks after perfusion.

* P < 0.05 Smoke v. Smoke-free

Jin & Arif, et. al., ATVB, in publication
Altered Leukocyte Function

Jin & Arif, et. al., ATVB, in publication
UNDERSTANDING THE ROLE OF THE SMOOTH MUSCLE CELL IN AAA
The Multifaceted Role of the Vascular Smooth Muscle Cell

Figure 3: Vascular Smooth Muscle Cell products involved in MMP-mediated matrix injury in aneurysms. Products which may promote matrix injury in green and products which may reduce matrix injury in red. uPA=urokinase, PAI=plasminogen activator inhibitor, TIMP=tissue inhibitor of MMP.
Expression Profile of VSMC

Source of SMC: AAA = Abdominal Aortic Aneurysm, NAA = Non-dilated Abdominal Aorta, CEA = Atherosclerotic Plaque
Defining the Phenotype
In vitro Elastolysis

A

Mean ug Elastin Degraded

NAA  CEA  AAA

p<0.001

p=0.001

B

Mean ug Elastin Degraded

AAA  AAA + BB94  AAA + E64  AAA + Aprotinin

p=0.55

p=0.09

p<0.01

F

NAA  AAA

Day 0

Day 10
VSMC and MMP Mediated Elastolysis

3A

VSMC Only

VSMC - U937 Co-culture

3B

3C

MMP-9

Mean Relative Density

n=6

3D

MMP-2

Mean Relative Density

n=6

mRNA Relative Expression

VSMC Only

VSMC - U937 Co-culture
Summary

• Elastolysis of AAA is relatively unique and remarkably anatomically localized
• Matrix destruction results from both chronic inflammation and VSMC dysfunction
• Inhibition of elastolytic enzymes may inhibit the progression of small aortic aneurysms
• Care must be taken to recognize the limitations of animal models of complex human diseases
• No single model is adequate for evaluation of all aspects of pathobiology and all potential therapeutics
Contributors

• Michel Bergoeing, MD
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• Nathan Airhart, MD
• Amy Hackmann, MD
• Paqui Fernandez-Garcia, MD
• Batool Arif
• Jianping Jin
• Kathy Grapperhaus

• RW Thompson, MD
• Kathy Raman, MD, MS
• Elaine Davis, MD
• Robert Mecham, MD

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Washington University in St. Louis

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“It’s fine to discover cures, but, remember, chronic conditions are our bread and butter.”