Pathogenesis of vulnerable plaque

Andrew Newby
Wei-chun Huang, Nick Jenkins, Sarah George, Jason Johnson, Karina di Gregoli, Buket Reel, Rebecca Salter, Graciela Sala-Newby
Questions

• What is a vulnerable plaque?
• What effect does it have in patients?
• What are the mechanisms?
• Is there an animal model?
• What can we do about it?
Atherosclerotic plaque is a highly prevalent, slowly developing cause of angina pectoris and a risk factor for MI and stroke.

I  Type II  Type III  Type IV

Stary HC An Atlas of Atherosclerosis
Stroke and MI (heart attack) are mainly caused by plaque rupture or endothelial erosion.

Plaque rupture – 85% of male MI

Surface erosion
- 40% of female MI
- smokers

Davies, MJ Heart 2000; 83:361-6
Vulnerable plaque - definitions and assumptions

• Plaque with a high probability to rupture or erode hence provoke thrombosis
• Predictor of MI and stroke
• Similar structural characteristics as plaques with recent thrombus
• Formed by similar mechanisms as other plaques only more so!

Are these assumptions valid?
Does Vulnerable Plaque predict MI

In 697 STEMI patients, thin-cap fibroatheromas were more likely to give rise to MACE in next 3 years. However, most MACE were new episodes of angina. MI was too infrequent for statistical power.

Vulnerable plaque was a good predictor of subsequent MACE but the situation for MI is still unclear.

Stone, NEJM;364:226
Transient ischaemic attacks predicts stroke – carotid endarterectomy

Ulcerated plaque with thrombus
What features of VP are predictive?

In 818 carotid plaques from AtheroExpress

- Haemorrhage and micro-vessels were predictors of MACE
- Lipid core size, macrophages, smooth muscle cells and collagen were not predictive

Hellings, Circulation 2010;121:1941
Why does plaque rupture occur?

Plaque rupture like bridge collapse results from the chance interplay of intrinsic weakness and external forces.

Davies MJ
Circulation
Heart 2000; 83:361-6
Factors that influence MI and stroke

Cap weakening
Reduced endothelial adhesion (erosion)
Coagulation factors
Smoking, infections (size of thrombus)

Haemodynamics
i.e. flow patterns
Blood pressure

Blood
Flow
Vessel wall
What makes plaque likely to rupture?

Foamy macrophages

Thin fibrous cap (<65 μm), large lipid core, lots of foam cells, loss of collagen from the cap or core, microvessels

Davies, M Circulation 1996; Falk, E JACC 2006;47:C7-12
How do mechanical factors influence MI?

Hypertension, arterial stiffness = increased pressure wave = high stress

Weak shoulder = High strain

Big lipid core = high strain
Why do plaques rupture when they do?

- **Fibrous plaque**
- **Cap thins by proteolysis**
- **Thin, weak cap**

**Tensile Strength**

**Arterial pulse wave** = Strain on plaque cap

- **Young**
  - Low stress on cap
- **Arterial stiffening, hypertension**
  - High stress on cap
- **Running for the bus**
  - Cap ruptures

**Statins**

**BP lowering**
Extent of thrombosis determines severity of outcome

Partial occlusion – NSTEMI  Total occlusion – STEMI

Davies, MJ Heart 2000; 83:361-6
Autoantigens promote T-cell activation

TCC, T-cell chemokines, CXCL9, 10, 11, CD40L, OX40L
Various damage associated molecular patterns are recognised by Toll-like receptors

CD16 +/-

Foam Cell (M1)

Cell injury, altered matrix components

Foam cell and smooth muscle apoptosis and matrix remodelling ensues

Free cholesterol activates the inflammasome
Foam cells make proteases that destroy collagen

van der Wal Cardiovasc Res 1999;41:334-344
Which proteases are important - rabbit foam cells

Protein levels (arbitrary units)

MMP-10, -25 should be added

Chase et al. ATVB 2002;22:765-771
What about MMP-12, MMP-14 and TIMP-3 in man?

Gerard Pasterkamp
Vincent Scholtes

AtheroExpress Biobank (n>2000)
<table>
<thead>
<tr>
<th>Staining and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-14 IgG</td>
</tr>
<tr>
<td>COX-2 IgG</td>
</tr>
<tr>
<td>MMP-12 IgG</td>
</tr>
<tr>
<td>NF-κB IgG</td>
</tr>
<tr>
<td>CD206 IgG</td>
</tr>
<tr>
<td>TIMP-3 IgG</td>
</tr>
</tbody>
</table>
Histological correlates of macrophage MMP-12, MMP-14 and TIMP-3 expression

Johnson, Jenkins et al, unpublished
# Correlation between parameters

<table>
<thead>
<tr>
<th></th>
<th>MMP-14</th>
<th>TIMP-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P value</td>
<td>rho</td>
</tr>
<tr>
<td>Collagen semiquantitative</td>
<td>0.000</td>
<td>-.409</td>
</tr>
<tr>
<td>SMC semiquantitative</td>
<td>0.000</td>
<td>-.623</td>
</tr>
<tr>
<td>SMC quantitative</td>
<td>0.000</td>
<td>-.466</td>
</tr>
<tr>
<td>% fat / atheroma</td>
<td>0.000</td>
<td>.665</td>
</tr>
<tr>
<td>Macrophage semiquantitative</td>
<td>0.000</td>
<td>.567</td>
</tr>
<tr>
<td>Macrophage quantitative</td>
<td>0.026</td>
<td>.453</td>
</tr>
<tr>
<td>Thrombus</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Collaboration with Vincent Scholtes and Gerard Pasterkamp
MMP-14 and TIMP-3 don’t co-localise
MMP-12 and MMP-14 don’t co-localise

Johnson, Jenkins et al, unpublished
Hypoxia and MMP-12 expression *in vivo*

Sala-Newby, Johnson et al, unpublished
Outcome studies

• Plaques from 236 patients following endarterectomy.
• 3 year follow up.
• Primary outcome was any vascular event or vascular intervention.
• Sections stained for MMP-12, MMP-14 or TIMP-3 and CD68.
• Ratio positive for MMP-12, MMP-14 or TIMP-3 was quantified.

Scholtes, et al, JAHA, in press
Areas of interest show a wide variation in MMP-12 positivity.

Scholtes, et al, JAHA, 2012
Macrophage MMP-12 expression associates with poor prognosis

Scholtes, et al, JAHA, 2012
Macrophage MMP-12 expression associates with poor prognosis

Too much MMP-12 can kill you!

Scholtes, et al, JAHA, 2012

<table>
<thead>
<tr>
<th>MMP12 ratio</th>
<th>Major endpoint</th>
<th>Stroke endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>1e quartile</td>
<td>16%</td>
<td>7%</td>
</tr>
<tr>
<td>2e quartile</td>
<td>25%</td>
<td>15%</td>
</tr>
<tr>
<td>3e quartile</td>
<td>24%</td>
<td>14%</td>
</tr>
<tr>
<td>4e quartile</td>
<td>31%</td>
<td>20%</td>
</tr>
<tr>
<td>overall</td>
<td>23%</td>
<td>14%</td>
</tr>
</tbody>
</table>

p = 0.021          p = 0.018
Is there an animal model?

- Reproducible
- Quick
- Easy
- Cheap

- Near to human
- Highly predictive

Publishable
Sites of predilection are similar.

Cheng Circulation.2006;113:2744–2753
Size matters!

3.5 mm

< 65 μm

0.5 mm

< 10 μm

Thombus volume 1000 times less
Jackson et al ATVB 2007; 27:714
Plaque rupture or intraplaque haemorrhage?

‘The most worrisome difference between the pathology in the mouse and the pathology of human disease is the absence of fibrin formation either within the lesion or within the lumen’. Rosenfeld ATVB 2000;20:2587

200 times smaller, less PAI-1= quicker thrombolysis
Jackson et al ATVB 2007; 27:714
BCA, 8 week high-fat diet

62% of all animals: 1.05 buried caps/plaque
Neither occur in aortic sinus


p<.000001
MMP-12 may reduce plaque stability by increased macrophage migration

Johnson et. al. PNAS 2005;102:15575-80

<table>
<thead>
<tr>
<th></th>
<th>ApoE-/-: MMP-12+/+</th>
<th>ApoE-/-: MMP-12-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque area (x10^3 mm^2)</td>
<td>116 ± 12</td>
<td>56 ± 7*</td>
</tr>
<tr>
<td>Buried fibrous layers</td>
<td>1.33 ± 0.21</td>
<td>0.55 ± 0.14*</td>
</tr>
<tr>
<td>SMC (%)</td>
<td>9 ± 1</td>
<td>23 ± 3*</td>
</tr>
<tr>
<td>Macrophage (%)</td>
<td>32 ± 4</td>
<td>15 ± 4*</td>
</tr>
</tbody>
</table>
Development of Selective Inhibitors and Substrate of Matrix Metalloproteinase-12*

Received for publication, January 10, 2006, and in revised form, February 13, 2006 Published, JBC Papers in Press, February 15, 2006, DOI 10.1074/jbc.M600222200

Laurent Devel†, Vassilis Rogakos§, Arnaud David‡, Anastasios Makaritis§, Fabrice Beau†, Philippe Cuniasse†, Athanasios Yiotakis§, and Vincent Dive‡†

© 2006 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.

RXP470.1

Jason Johnson
BHF, IRF

<table>
<thead>
<tr>
<th>Compound 1</th>
<th>Ki (nM)</th>
<th>MMP-1</th>
<th>MMP-2</th>
<th>MMP-3</th>
<th>MMP-7</th>
<th>MMP-8</th>
<th>MMP-9</th>
<th>MMP-11</th>
<th>MMP-12</th>
<th>MMP-13</th>
<th>MMP-14</th>
<th>ACE</th>
<th>NEP</th>
<th>TACE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-3</td>
<td>67000</td>
<td>192</td>
<td>40</td>
<td>626</td>
<td>271</td>
<td>1255</td>
<td>18400</td>
<td>0.19</td>
<td>49</td>
<td>140</td>
<td>&gt;100000</td>
<td>&gt;100000</td>
<td>&gt;100000</td>
<td></td>
</tr>
<tr>
<td>Selectivity/MMP-12</td>
<td>350000</td>
<td>1011</td>
<td>211</td>
<td>3295</td>
<td>1426</td>
<td>6658</td>
<td>96842</td>
<td>1</td>
<td>259</td>
<td>737</td>
<td>&gt;500000</td>
<td>&gt;500000</td>
<td>&gt;500000</td>
<td></td>
</tr>
</tbody>
</table>
Vulnerable plaque – what can we do about it?

- Reduce all known risk factors (lifestyle changes, quit smoking, statin, BP lowering)
- Reduce thrombotic consequences (aspirin)
- Prophylactic angioplasty?
- Decrease plaque inflammation (NHR, cytokine/chemokine inhibitors, immunotherapy)
- Prevent apoptosis (?)
- Prevent loss of collagen (protease inhibitor)