New Insights into the Pathophysiology of the Acute Coronary Syndromes

Peter Libby
Brigham & Women’s Hospital
Harvard Medical School

Carl J. Wiggers Lecture
April 26, 2007

Carl J. Wiggers

Wiggers’ Diagram

Left ventricular pressure-volume loop

PROTECTION OF THE SKELETAL MUSCLE

PETER LIBBY
The First Description of Paradoxical Ventricular Motion During Acute Coronary Ischemia

THE EFFECT OF CORONARY OCCLUSION ON MYOCARDIAL CONTRACTIONS

ROBERT TUNNARD and CARL J. WIGGERS

From the Department of Physiology, Wayne State University, Detroit, MI.

Received for publication March 10, 1953.

A. Tissue of a main coronary branch is followed by an increasing zone of ischæmic damage which indicates progressive restriction of circulation to the extent that approximately within a minute the muscle fibers during isometric compression, remain stretched during rapid ejection and shorten quickly during isometric voluntary to short, the muscle is completely arrested.

Molecular Mechanisms of the Acute Coronary Syndromes

Peter Libby
Brigham & Women’s Hospital
Harvard Medical School

Carl J. Wiggers Lecture
April 26, 2007

Case Presentation – “J.S.”

61M who presents with epigastric pain.
Generally healthy and active, runs 5 miles qod
No DM, HTN, hyperlipidemia, tobacco use, or FHx
Episode of abdominal / epigastric pain ~ 2 weeks before presentation while running, improved with rest and OTC proton-pump inhibitor
Similar episode on day of presentation, with nausea, vomiting while running.

JS: Electrocardiogram @ Suburban Clinic

JS: Electrocardiogram @ BWH
Case Presentation – “J.S.”

Cardiac TnI 1.18
CK 234 / CK-MB 8.6

JS: Left coronary artery

JS: Right coronary artery

JS: Right coronary intervention

JS: Right coronary artery
Case Presentation – “J.S.”

- CK peak 688 / CK-MB 40.9 / cTnI 11.13

Case Presentation – “J.S.”

- He had an otherwise uneventful post-PCI course
- Discharged on Aspirin, clopidogrel, atorvastatin, lisinopril and metoprolol.
- Seen in follow up after 6 weeks without symptoms.

Traditional View of Human Atherogenesis

- Ischemic Heart Disease
- Cerebrovascular Disease
- Peripheral Vascular Disease

Traditional View

- Ischemic Heart Disease
- Cerebrovascular Disease
- Peripheral Vascular Disease

- Mls often arise from non-critical stenoses

- Post-thrombolysis angiography
- Serial angiographic studies
- Intravascular Ultrasound studies
Stenosis and Coronary Thrombosis

- Studied 60 consecutive patients by QCA during 1st MI post-thrombolysis
- Residual stenosis < 60 % in 28 (47%)

‘Pre-existing coronary stenoses in patients with first myocardial infarction are not necessarily severe.’

Hackett D, Davies G, Maseri A. European Heart J 1988

Severity of coronary artery stenosis before acute MI

Angiography does not show eccentrically remodeled atheroma

From Scott Kinlay, BWH, HMS

JS: Right coronary artery
**Structural Integrity of the Plaque’s Fibrous Cap**

- Depends on interstitial collagen fibrils (types I & III) synthesized by smooth muscle cells

**Plaque rupture**

- Decreased synthesis of interstitial collagens

---

**Interstitial Collagen Production**

Libby & Aikawa
Circulation
2002;106:1396-1398

**Collagen fibril organization into triple helices**

Gly-X (pro)Y (Hyp)

---

**Cytokines positively and negatively regulate interstitial collagen synthesis by human smooth muscle cells**

(Amento, Ehsani, Palmer, Libby
Arteriosclerosis
11:1166, 1991)

---

**Plaque rupture**

- Increased *degradation* of extracellular matrix
However, so far no direct evidence links collagenases with regulation of the collagen content of atheroma.

Therefore, we tested directly the hypothesis that collagenolysis critically influences collagen accumulation in atheroma in vivo using genetically altered mice.
Collagenase-resistant mutant mice

- Mutation at the specific collagenase cleavage site on type I collagen (“knock-in”)

GLY (775) - ILE (776) collagenase cleavage site
GLY (775) - PRO (776)

Deguchi, Libby, Rabkin, Sakata, Chin, Hill, Lawler, Voro, Schoen, Krane, Aikawa
Circulation 2004;110:1953

Experimental protocol

Backcrossed >7 generations into C57BL/6.
Collagenase-resistant mutant (R/R) / apo E (-/-) mice
Wild-type collagen (+/+) / apo E (+/-) mice

1 month

1 month

Analysis of Atheroma

Does collagenolysis influence collagen accumulation in atheroma?

Col+/+ / apoE+-

Collagen resistance promotes collagen accumulation in mouse atheroma

Col+/+ / apoE+-

Collagen area

p<0.05

%Collagen / intima

p=0.01

Collagen / SMC mm²/cells

p<0.01

Fukumori, Deguchi, Libby, Rabkin, Sakata, Chin, Hill, Lawler, Voro, Schoen, Krane, Aikawa
Circulation 2004;110:1953
This study in genetically altered mice provides the first **direct evidence** that links collagenolysis with control of the collagen content of atheroma.

**Experimental protocol**

Mmp 13−/− mouse backcrossed 7 generations into C57BL6 background, then crossed with atherosclerosis-susceptible apoE−/− mice

- Birth
- Genotyping
- 4 weeks
- High-cholesterol feeding for 5 or 10 weeks

*Deguchi et al. Circulation 2005; 112:2708*

**Which proteinases control the collagen content of atheroma?**

- MMP-1?
- MMP-8?
- MMP-13?
- MT-1-MMP...

*Deguchi et al. Circulation 2005; 112:2708*

**MMP-13/collagenase-3 deficiency does not affect atheroma burden in apoE−/− mice**

*Deguchi et al. Circulation 2005; 112:2708*

**MMP-13/collagenase-3 deficiency increases fibrillar collagen in mouse atheroma**

*Deguchi et al. Circulation 2005; 112:2708*

**Collagenases critically influence collagen accumulation in mouse atheromata in vivo**

*P. Libby*
**Work in Progress**

MMP-14 deficient / apoE⁻/⁻ mice
MMP-8 deficient / apoE⁻/⁻ mice
TIMP-3 deficient / apoE⁻/⁻ mice
MMP-8 & 13 deficient / apoE⁻/⁻ mice

1 month  
Western diet  
5, 10 weeks

**Analysis of Atheroma**

- Many matrix-degrading proteases participate in atherogenesis
- Proteolysis may predispose to plaque disruption and thrombosis
- Can we monitor protease activity in vivo?

---

**Inflammation in Atherosclerosis**

**Visualizing Matrix Metalloproteinase Activity in Macrophages In Vivo**

Anna Deguchi, MD, PhD; Munenori Aikawa, MD, PhD.; Ichiro Hamajima, MD; 

Elena Aikawa, MD, PhD; Dingding Xue, MD; Yasuko Protetchin, PhD; 

Hirofumi Waseda, MD, PhD; Peter Libby, MD

**Background:** Matrix metalloproteinases (MMPs) in inflamed atherosclerotic plaques may contribute to intracellular matrix remodeling and the onset of atherothrombotic complications.

**Methodology:** To test the hypothesis that spatial and temporal changes in MMP activity are associated with plaque stability.

**Results:** MMP-13 generation within Macrophages (MMP-13/MMP-8) and MMP-13 inhibition (MMP-13/3 deficient) may be affected by a high cholesterol diet. 5-10 weeks and apoE⁻/⁻ mice as controls. The presence of MMP-13 microinjected into peritoneal macrophages by MMP-13 inhibitor shows reduced MMP-13 activity in apoE⁻/⁻ mice. MMP-13 deficient mice as controls. MMP-13 activity in apoE⁻/⁻ mice at 5-10 weeks shows reduced MMP-13 activity in apoE⁻/⁻ mice as controls.

**Conclusion:** These results suggest the feasibility of non-invasively imaging the proteolytic activity of MMPs in vivo, an approach that may improve inflammatory and fibrotic states of atherosclerotic plaques, thereby reducing plaque instability.

---

**Near Infrared Fluorescent (NIRF) probes**

The NIRF probes are optically inactive in their native, quenched state and produce very low background fluorescence.

Upon enzymatic cleavage of specific peptide sequences by the protease (e.g., MMP-2), the NIRF probe elicits fluorescence (e.g., Cy5.5) with hundreds-fold amplification.


---

**Cleavage of an MMP-13-activatable agent in vitro**

Graph Deleted Unpublished Data

---

**Imaging of MMP-13 by Near Infrared Fluorescence in Mouse Atheromata**

Aikawa M et al., unpublished data
Near IR Fluorescence Catheter

Intra-arterial Protease (Cat B) Imaging in vivo in Watanabe Rabbits

Proteinases Participate in the Pathogenesis of the Acute Coronary Syndromes

Plaque rupture with thrombosis

Thrombosis of a disrupted atheroma, the cause of most acute coronary syndromes, results from:
- Weakening of the fibrous cap
- Thrombogenicity of the lipid core

CD40 and tissue factor in atheroma

Molecular Mechanisms of the Unstable Coronary Syndromes

The “New Biology” of Atherosclerosis
- Unstable coronary atheromata are often not the “tight” stenoses
- Stabilization of lesions, by medical therapy, provides a new therapeutic target beyond revascularization

How do we “stabilize” atherosclerotic plaques?
- Smoking Cessation
- Diet
- Physical activity
- Choosing your parents wisely

Lipid-Lowering Prevents Clinical Events

How does lipid-lowering improve patient outcome?
How does lipid-lowering improve patient outcome?

• Regression of fixed stenoses?

Regression of Human Atherogenesis?

Disproportionate reduction of coronary events and stenosis in lipid-lowering trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Δ Stenosis</th>
<th>Δ Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>FATS (niacin + resin)</td>
<td>-0.9 %</td>
<td>-80 %</td>
</tr>
<tr>
<td>FATS (statin + resin)</td>
<td>-0.7 %</td>
<td>-70 %</td>
</tr>
<tr>
<td>STARS (diet)</td>
<td>-1.1 %</td>
<td>-69 %</td>
</tr>
<tr>
<td>STARS (diet + resin)</td>
<td>-1.9 %</td>
<td>-89 %</td>
</tr>
</tbody>
</table>

After Brown BG.

What accounts for the disparity between degree of coronary artery stenosis and producing the acute coronary syndromes?

The functional state of the atheroma, not merely its size or the degree of luminal encroachment, determines the propensity for development of acute coronary syndromes.

How does lipid-lowering improve patient outcome?

• Regression of fixed stenoses?

• Anti-inflammatory effect?
Does lipid lowering stabilize experimental atheroma?

Lipid lowering in rabbits

- 0.05-0.2% cholesterol
- 0.3% cholesterol
- No cholesterol


Baseline lesions

Macrophages Collagenase

High, 16 m

Dietary lipid lowering increases collagen content of rabbit atheroma

(Picrosirius-red staining after 16 months on a low-lipid diet)
**Dietary Lipid Lowering Reduces Inflammation in Atheromata**

Lipid lowering by diet alone reduces ROS production, oxLDL accumulation, and VCAM-1 and MCP-1 expression in atheroma of cholesterol-fed rabbits.


*Circulation* 2002;106:1390-1396

**Lipid lowering stabilizes atheroma**

In rabbits with diet-induced atherosclerosis, reduced cholesterol consumption:

-Limits inflammation in atheroma
-Improves features of plaques associated with stability
-Reduces plaque thrombogenicity
-Decreases oxidative stress and endothelial dysfunction

**Statin Therapy Reduces C-Reactive Protein**

Mechanisms of benefit of lipid lowering therapy:
Experimental and human studies suggest that lipid-lowering may stabilize plaques and reduce events by limiting inflammation.

COURAGE: Kaplan-Meier Survival Curves

Coronary Intervention for Persistent Occlusion after Myocardial Infarction (OAT)
Judith S. Hochman, M.D., Gervasio A. Lamas, M.D., Christopher E. Butler, M.D., Vladimir Gatzke, M.D., Harmony E. Reynolds, M.D., Stacy J. Abramsky, M.P.H., Sandra Forman, M.A., Witsold-Rupelino, M.D., Aldo P. Maggioni, M.D., Mary White, M.D., Zbigniew Sadownik, M.D., Antonio C. Canovas, M.D., Jaime M. Rankin, M.D., Jean P. Rankin, M.D., Gabriel Stieg, M.D., Alice M. Mavroudis, M.D., George Sapho, M.D., Matthias C. Filatov, M.D., Jonathan Leor, M.D., William Friedrich, M.D., Daniel B. Muni, M.D., M.P.H., Ge nell L. Khattaroud, Ph.D., for the Occluded Artery Trial Investigators

Optimal Medical Therapy with or without PCI for Stable Coronary Disease (COURAGE)
William E. Boden, M.D., Robert A. O’Rourke, M.D., Koon H. Teo, M.B., B.Ch., Ph.D., Pamela M. Harrigan, Ph.D., David J. Maron, M.D., William J. Kastor, M.D., Merrill Knudson, M.D., Marcin Gadek, M.D., Paul Capron, Ph.D., Crystal L. Hams, Ph.D., Bernard R. Chatman, M.D., Leslie Shaw, Ph.D., Gilbert Gosselin, M.D., Shah Navaz, M.D., Lawrence M. Tito, M.D., Gerald Gav, M.D., Alvin S. Blaustein, M.D., David C. Booth, M.D., Eric R. Balse, M.D., John A. Spitz, M.D., M.P.H., Daniel S. Berman, M.D., G.B. John Mancini, M.D., William S. Weintraub, M.D., for the COURAGE Trial Research Group

The Vulnerable Plaque
❤ Local therapies relieve angina (PCI, CABG)
❤ Systemic therapies prevent MI, stroke, and prolong life
The Clinical “Bottom Line” of the “New Biology” of Atherosclerosis

• After revascularization patients should receive life style counseling and medical therapy to modify the biology of the underlying disease and prevent future events