Pathophysiology of Atherosclerosis

פרופ' שמואל בנאי
המרכז הרפואי תל אביב

Banai S.
Acute anterior wall MI
Recent large Antero-Septal Myocardial Infarction
Acute Myocardial Infarction
Estimated prevalence of cardiovascular disease in Americans 20 years of age and older

From American Heart Association: 2003 Heart and Stroke Statistical Update.

Dallas, American Heart Association, 2003.
עליה בתוחלת חיים בארה"ב בינו השנים 1970-2000

Between 1970 and 2000, life expectancy in the United States increased by 6.0 years overall, with 3.9 years of the increase due to reductions in mortality from cardiovascular causes. The data are from the Centers for Disease Control and Prevention.
התפלגות סיבות המוות העיקריות בישראל 2004 - 1990

שנה

אחוז מכלל מקרי המוות

מחלת לב

מחלת לב כרונית

סרטן

CVD

CHD

cancer

Banai S.
موت ממחלות לב כוניות בקבוצות גיל ומין
ישראל 1994 – 2004

גברים

נשים

> 45 % in 10 years !!!

Banai S.
International mortality trends in men, coronary heart disease

Per 100,000

BHF Heartstats (WHO statistics - Men aged 35 - 74, Standardised)

International mortality trends in men, coronary heart disease

Per 100,000

BHF Heartstats (WHO statistics - Men aged 35 - 74, Standardised)

International mortality trends in men, coronary heart disease

Per 100,000

BHF Heartstats (WHO statistics - Men aged 35 - 74, Standardised)
Age adjusted STEMI rate per 100,000 in the US

NIS* database

(1,352,574 patients in a 20% sample of community and specialty hospitals)

* Nationwide Inpatient Sample

Mohaved, MR. World Congress of Cardiology. Buenos Aires, 21.5.08
Normal coronary Artery
LDL disruption of endothelial integrity

1. Native LDL enters the bloodstream and encounters endothelial cells.
2. Endothelial injury occurs, leading to the release of oxygen free radicals.
3. Oxygen free radicals oxidize LDL, converting it to oxidatively modified LDL.
4. Resident monocyte/macrophages engulf oxidatively modified LDL, forming foam cells.
Leukocyte interactions with the artery wall in hypercholesterolemic nonhuman primates

Adhesion of mononuclear phagocytes to the intact endothelium 12 days after initiating a hypercholesterolemic diet

Interdigitations and intimate association of the monocyte with the endothelium when a monocyte appears to diapedese between two endothelial cells to enter the intima

Schematic of the evolution of the atherosclerotic plaque
Atherosclerosis Involves More Than Just Lipids

Unstable Plaque
- Few SMCs
- Thin fibrous cap
- Eroded endothelium
- Activated macrophages

Stable Plaque
- More SMCs
- Thick fibrous cap
- Intact endothelium
- Lack of inflammatory cells
- Foam cells

Adapted from Libby: Circulation 1995;91:2844–2850
Vascular inflammation and the vulnerable plaque

**Plaque Stability**
- Amount of SMCs
- Collagen synthesis by SMCs

**Plaque Vulnerability**
- Amount of macrophages
- Collagen degradation by MMPs
- Activated T lymphocyte accumulation
- Interferon-γ produced by T lymphocytes inhibits SMC collagen synthesis
The stable atherosclerotic plaque

Thick, VSMC-rich fibrous cap
Plaque Growth From Fatty to Fibrous
Evolves by Cycles Of:

- Disruption
- Non occlusive mural thrombus formation
- Healing

OR

- Hemorrhage into the plaque
- Healing and organization
Plaque growth

- Site of previous plaque rupture
- Resolving thrombus
- Recruitment of new VSMCs

- Lipid core
The healed atherosclerotic plaque
Lipid-rich core with hemorrhage

Dark-red collagen surrounding lipid hemorrhagic cores

Healed plaque rupture

Dense collagen (type 1) that forms fibrous cap is lighter reddish-yellow is disrupted (arrow), with newer greenish type III collagen on right and above rupture site

SMC formation within collagenous proteoglycan-rich neointima showing clear demarcation, with more fibrous regions of old plaque to right

Burke AP, Virmani R: *Circulation* 2001;103:934
The vulnerable (rupture-prone, unstable) plaque

- Size of atheromatous core
- Thickness of fibrous cap
- Inflammation in fibrous cap
Plaque Rupture

Disrupted plaque with occlusive thrombus
a. Monocyte
b. Infiltrating monocytes / macrophages
c. RBC's and platelets
d. Extracellular matrix
e. Smooth muscle cell
f. Endothelial cell
g. MMPs

A. THIN FIBROUS CAP
B. LARGE LIPID CORE
h. Ox-LDL
i. Apoptotic macrophages
j. T-cell
Plaque Rupture
הMaxLengthות טרשת בוערך כלל

Lesion

Normal Injury Fibrofatty stage and Lipid-laden foam cell formation Stenosis Cap rupture Thrombosis
Heart Attack Should be Treated Early

Say, 50 years before it happens
WOMEN & HEART DISEASE

Is your biggest worry breast cancer? Think again. ONE OUT OF THREE women will die of heart disease. What you can do to protect yourself.
LEADING CAUSES OF DEATH FOR AMERICAN WOMEN (2002)

- Heart Disease: 356,000
- Stroke: 100,000
- Chronic Obstructive Pulmonary Disease: 68,000
- Lung Cancer: 64,000
- Breast Cancer: 42,000
Mortality Rates in Women

Major risk factors for CVD

- High LDL cholesterol levels
- Low HDL cholesterol levels
- High triglyceride levels
- Obesity
- Insulin resistance
- Hypertension
- Smoking
- Diabetes mellitus
- Family history

Metabolic syndrome
Prevents TF synthesis by apoptotic macrophages loaded with ox-LDL

Anti Inflammatory

Normalize endothelial function

Enhance reverse cholesterol transport from vessel-wall atheroma to the liver

Anti Thrombotic

Inhibit SMC and Macrophages apoptosis

Pro Fibrinolytic

Anti oxidant

HDL
Endothelial Dysfunction

Platelets Reactivity

Plaque Progression

Hyper coagulability

ox-LDL
Acute Coronary Syndromes (ACS)

The clinical manifestation of coronary atherothrombosis

- A spectrum of conditions in which an atherosclerotic coronary plaque is ruptured and subsequently an intraluminal thrombus is formed

- Majority of patients with ACS have no prior symptom
Atherothrombosis

The thrombotic complications of atherosclerosis
<table>
<thead>
<tr>
<th>Atherosclerosis</th>
<th>Inflammation</th>
<th>Atherothrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A multifactorial systemic</td>
<td>The link between atherosclerosis and thrombosis</td>
<td>The thrombotic complications of atherosclerosis</td>
</tr>
<tr>
<td>inflammatory disease</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Inflammation

The link between atherosclerosis and thrombosis

- Responsible for plaque progression
- Responsible for plaque rupture
- The cause for acute coronary syndromes
ACS - Rupture of the Fibrous Cap

- Ongoing Inflammation at the site of Plaque Disruption
Vulnerable plaque may not be black or white
Vulnerable Plaque
((Rupture-prone

Vulnerable Blood
(Prone to thrombosis)

Vulnerable Patient
(Unstable, high-risk patient)
Vulnerable Plaque and Patient Risk

Vulnerable Blood
- Hypercoagulability
- Increased platelet activation and aggregation
- Increased coagulation factors
- Decreased fibrinolysis
- Increased thrombogenic factors

Vulnerable Patient

Vulnerable Plaque
- Active inflammation
- Cap thickness
- Lipid core size
- Endothelial denudation
- Injured plaque

Vulnerable Myocardium
- Myocardial ischemia
- Electrophysiological disorders
- Myocarditis

The Challenge: Stabilizing the vulnerable plaque

- The treatment of CAD must be aimed at stabilising the vulnerable plaques, which are at risk of becoming a site for acute thrombosis
Vascular inflammation and activation:
The target for lipid lowering
LDL lowering

- Reduce the incidence of coronary events and stroke by changing the quality of the plaque
- Produces only modest improvements in the luminal caliber of fixed atherosclerotic lesions
Lipid lowering and qualitative changes in the plaque

- Reduces macrophage accumulation
- Reduces proteolytic activity and expression (MMPs)
- Reduces TF expression and activity
- Improves SMCs activation
- Reduces ECs activation
For every 30-mg/dL change in LDL-C, the relative risk for CHD is changed in proportion by 30%. The relative risk is set at 1.0 for LDL-C=40 mg/dL.
The optimal low-density lipoprotein is 50 to 70 mg/dl: lower is better. O'Keefe, JR et al. J Am Coll Card. 2004; 2;43(11):2142-6

Adapted from –

The Optimal low-density lipoprotein is 50 to 70 mg/dl: lower is better. O'Keefe, JR et al. J Am Coll Card. 2004; 2;43(11):2142-6
Relationship between LDL-C levels and change in percent atheroma volume for several IVUS trials

<table>
<thead>
<tr>
<th>Median change in Percent Atheroma Volume (%)</th>
<th>Mean LDL-C (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>-0.6</td>
<td>60</td>
</tr>
<tr>
<td>1.2</td>
<td>70</td>
</tr>
<tr>
<td>1.8</td>
<td>80</td>
</tr>
<tr>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>110</td>
<td>120</td>
</tr>
</tbody>
</table>

$R^2 = 0.97$
P < 0.001

Ref: Nissen S et al. JAMA 2006; 295: e-publication ahead of print
Atherosclerosis

Vascular Inflammation

and the

(Renin-Angiotensin System (RAS

Banai S.
The Renin-Angiotensin System as a Risk Factor and Therapeutic Target for Cardiovascular Diseases

The RAS activity may represent an ideal target for pharmaceutical treatment in a number of cardiovascular diseases, including:

- Hypertension
- Congestive heart failure
- Renal disease
- Stroke
- Myocardial infarction
- Atherosclerosis
The Renin-Angiotensin System as a Risk Factor and Therapeutic Target for Cardiovascular Diseases

The RAS activity may represent an ideal target for pharmaceutical treatment in a number of cardiovascular diseases, including:

- Atherosclerosis/Atherothrombosis
  - Hypertension
  - Congestive heart failure
  - Renal disease
  - Stroke
- Myocardial infarction
Several pathways of Ang II generation

Local Ang II synthesis is independent of ACE

Angiotensinogen (Liver)

- Renin inhibitor
- Bradykinin
- ACE inhibitor
- Peptides
- AT₁ receptor blocker
- Chymase

Angiotensin I

Angiotensin II

AT₁

AT₂

Different roles of $AT_1$ and $AT_2$ receptors

Angiotensin II

$AT_1$
- Vasoconstriction
- Vascular cell proliferation
- Aldosterone secretion
- Cardiac myocyte hypertrophy
- Increased sympathetic tone

$AT_2$
- Vasodilation
- Antiproliferation
- Apoptosis
- Anti oxidant action

The biologic functions of AngII under physiologic conditions

- Homeostasis of the cardiovascular system
- Blood Pressure
- Perfusion pressure of a number of organs
- Salt and water balance
- Cellular growth and replication

The RAS and Atherosclerosis

- Angiotensin II is a most important bioactive factor involved in the development and progression of atherosclerosis.

- The pro-inflammatory effects of Ang II are mediated by the AT$_1$-R, whereas AT$_2$-R seems to confer vascular-protection.
Atherosclerosis, Vascular Inflammation and the Renin-Angiotensin System

The RAS, through the actions of Ang II:

Production of reactive oxygen species (ROS) in the vessel wall

- Enhances vascular oxidant tone to produce EC dysfunction
- Enhances vascular LDL oxidation

- Upregulate vascular cell adhesion molecule-1 (VCAM-1) on ECs
- Increase the expression of the proinflammatory cytokines interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1)

- Promotes macrophages migration into the intima


Proatherogenic mechanisms for Ang II produced in the vessel wall

Brasier AR, ATVB. 2002;22:1257
The Pro-Atherogenic role of the RAS

- Ang II is proatherogenic, especially in the presence of hyperlipidemia
- Locally produced Ang II synergizes with oxidized lipid to perpetuate atherosclerotic vascular inflammation

Therefore, RAS antagonists prevent atherosclerosis by reducing vascular inflammation

Oxidative Stress

- During normal cellular metabolism, several enzyme systems reduce molecular oxygen, resulting in formation of a variety of reactive oxygen species, including superoxide (O$_2^-$), hydroxyl radical (HO·), hypochlorous acid (HOCl), lipid radicals, and hydrogen peroxide (H$_2$O$_2$)

- ROS play a critical role in the normal functioning of cells. For example, the normal growth of vascular SMC.
Oxidative Stress

- Excessive production of ROS, outstripping antioxidant defense systems = oxidant stress

- Oxidant stress has been implicated in many pathophysiological conditions in the CVS, including:
  - cigarette smoking
  - hypercholesterolemia
  - diabetes
  - hypertension
  - and heart failure
Untoward events that occur as a consequence of oxidant stress

- Oxidative modifications of DNA
- Lipid oxidation
- Modification of proteins
- Activation of redox sensitive genes, such as: vascular cell adhesion molecule-1 (VCAM-1) intercellular adhesion molecule-1 (ICAM-1) monocyte chemoattractant protein-1 (MCP-1)
- Activation of matrix metalloproteinases (MMPs)

Oxidative stress is involved in the pathogenesis of atherosclerosis

- Under oxidative stress, macrophages generate reactive oxygen species leading to LDL oxidation
AT1 receptor and Oxidative Stress

- AT_{1} receptor activation by angiotensin II leads to production of ROS in the vessel wall and inactivation of nitric oxide
- Loss of nitric oxide via this mechanism leads to endothelial dysfunction, one of the earliest steps in the atherosclerotic process
- Inhibition of AT_{1}R activation by ARBs or ACE-I improves endothelial dysfunction

Mancini JGB: *Circulation*. 1996;94: 258–265
Prasad A: *Circulation*. 2000;101:2349–2354
Schiffrin EL: *Circulation*. 2000;101:1652–1659

Banai S.
Angiotensin II Inhibits Endothelial Cell Motility Through an AT$_1$-Dependent Oxidant-Sensitive Decrement of Nitric Oxide Availability

- The migratory capability of vascular EC plays a pivotal role in the maintenance of vessel wall integrity, and is stimulated by nitric oxide.

- Angiotensin II inhibits EC motility by reducing NO availability.

- Such reduction is due to AT$_1$ receptor-dependent increment in intracellular ROS generation.
AT$_1$ Receptor and all Stages of Atherogenesis

Loss of nitric oxide and formation of peroxynitrite promote atherosclerosis at virtually all stages of the disease.
**AT$_1$ Receptor and all Stages of Atherogenesis**

The earliest stages:

Increased attraction and adhesion of monocytes to the endothelium

- Inflammatory molecules: MCP-1, VCAM-1 are critically important in this process

- Angiotensin II induces their production and secretion via generation of ROS and suppression of nitric oxide
AT$_1$ Receptor and all Stages of Atherogenesis

Fatty streak formation:
- Increased oxidation of LDL
- Uptake of oxLDL by macrophages, and foam cell formation

These processes are promoted by AT$_1$ receptor activation by angiotensin II

The expression of the receptor for oxidized LDL (LOX receptor), is dramatically increased by AT$_1$ receptor activation

Morawietz H: Circulation. 1999;100:899–902
$\text{AT}_1\text{Receptor and all Stages of Atherogenesis}$

**Plaque formation:**

Is propagated by migration and proliferation of vascular SMCs

Oxidant stress induced by angiotensin II plays a major role in stimulating growth and migration of vascular SMCs


Harrison DG: *Clin Cardiol.* 1997;20(suppl II):11–17
AT_1 Receptor and all Stages of Atherogenesis

Plaque rupture:
- Inflammatory events
- Apoptosis
- Accelerated matrix degradation

AT_1 receptor activation via angiotensin II initiates:
- Inflammatory processes such as IL-6 production
- Vascular SMCs apoptosis, a prelude to plaque rupture
- Increases MMP activity, resulting in plaque degradation and ultimately rupture

Schieffer B: Circulation. 2000;101:1372–1378
Increased ACE activity in culprit lesions in ACS

Enhanced ACE activity is related to the causative mechanism of active coronary lesions

ACE activity in coronary tissue obtained from directional coronary atherectomy and in serum of patients with ACS and with stable IHD

Hoshida S, Circulation 2001;103:630
Pre-treatment with AT$_1$R blockade in patients with symptomatic carotid artery stenosis for 4 months before endarterectomy, decreases inflammation and inhibits COX-2/mPGES-1 expression in plaque macrophages.

This effect contributes to plaque stabilization by inhibition of MMP-induced plaque rupture.
Blockade of the AT$_1$R provides a novel form of therapy for plaque stabilization.
Conclusions:

**RAS blockade:**

- attenuates the degree of atherosclerosis
- reduces macrophage accumulation
- increases collagen deposition within the plaque
- reduces the frequency of plaque disruption
The additional benefit of RAS inhibitors beyond the BP lowering effect

- RAS inhibitors improved structural abnormalities and normalized endothelial function of small arteries from patients with essential hypertension.

- None of these effects was found in a parallel group of hypertensive patients treated with β-blockers, despite similar BP lowering.

Schiffrin EL: Circulation. 2000;101:1653
Vascular protective effect of RAS inhibitors

- This vascular-protective effects of RAS inhibitors will translate into improved outcome in hypertensive and CHF patients beyond the effect of blood pressure lowering itself, with reduced morbidity or mortality.
Vascular protective effect of ACE-I and ARB

Ox-LDL is taken into EC via the LOX-1 receptor

Ang II produces EC dysfunction by upregulation of the LOX-1 receptor

Inhibition of Ang II and blockade of the AT$_1$R will improve endothelial function
Conclusions:

- The RAS plays a key role in the development and acceleration of all stages of atherosclerosis, from endothelial dysfunction, lipid accumulation and fatty streak formation, through plaque progression, inflammation to plaque destabilization and rupture.

- Inhibitors of the RAS inhibit LDL oxidation, improve endothelial function, decrease inflammation, and stabilize the atherosclerotic plaque.
Conclusions:

- Therefore, inhibitors of the RAS should be used routinely for primary and secondary prevention of atherosclerosis and atherothrombosis.
Tissue Factor
Tissue factor (TF)

A low molecular weight (45-kDa) membrane-bound glycoprotein,

Binding of TF to factor VIIa is the first step in the extrinsic coagulation cascade
Tissue Factor

A major regulator of coagulation and a critical determinant of thrombin generation in normal hemostasis and in atherothrombotic disease.
The clinical consequences of high intravascular expression of TF are catastrophic

Intraluminal TF activity triggers thrombogenic cascade that underlies the often-lethal thrombotic complications of:

- Atherosclerosis
- Consumptive coagulopathy and hemorrhagic diathesis of systemic infections
- Prothrombotic state of cancer patients
Tissue Factor in human atherosclerotic vessels

- Expressed by monocyte-derived macrophages
- Abundant within the acellular lipid core of the plaques, and in the "shoulder region" close to the lumen
- The source for TF in the lipid core are apoptotic macrophages

Nigel Mackman: ATVB 2004;24:1015
Tissue Factor in the human atherosclerotic plaque

- The high TF content within the lipid-core is the reason for the high thrombogenicity of this component in human coronary arteries.

- Thus, the ability to inhibit TF-dependent procoagulant activity after plaque disruption would likely alleviate many of the acute clinical manifestations of cardiovascular disease.
injury to the atherosclerotic arterial wall

disruption of the luminal surface and the fibrous cap

exposure of TF to circulating factor VII/VIIa

TF:FVIIa complex

proteolytically activation of factor X to factor Xa

factor IX to factor IXa

thrombin generation

fibrin formation  platelet activation

thrombosis
Tissue factor pathway inhibitor (TFPI) the endogenous inhibitor of TF

- The activity of TF:FVIIa complex is regulated by the endogenous inhibitor: TF pathway inhibitor
- The major pool of TFPI is in the endothelium
- TFPI forms an inactive complex consisting of TFPI, TF, factor VIIa, and factor Xa, which inhibits the TF-dependent coagulation cascade
Systemic expression of TF

Increased systemic expression of TF by activated circulating monocytes contribute to the enhanced thrombogenic state in patients with an acute coronary syndrome
Surface Distribution of Monocyte Tissue Factor and hypercoagulability

Individuals with higher availability of surface TF antigen on MNCs, are more susceptible to hypercoagulation

Banai S.

Tissue Factor and ACS

- Plasma TF levels are increased in patients with unstable angina compared to those with stable AP.

- Plaques from patients with ACS have significantly greater concentrations of TF antigen and activity than those from patients with stable angina.

- Systemic TF levels are an important predictor of outcome in patients with ACS.
Tissue Factor and Human Atherosclerosis

TF, by its local and systemic effects, plays a major role in the pathogenesis of advanced human atherosclerosis and its thrombogenicity
Role of TF in thrombus formation after rupture of an atherosclerotic plaque

TF expressed by adventitial cells (blue), EC, and SMC

Blood-borne TF may contribute to thrombus propagation after plaque rupture

TF expressed by foam cells (orange) and in the necrotic core (yellow) of the plaque would be exposed to clotting factors in the blood and initiate clotting

TF is expressed by adventitial cells (blue). EC, and SMC

Nigel Mackman:ATVB 2004;24:1015
Formation of the TF:FVIIa complex initiates clotting by activating FX and FIX. Alternatively, FXI can activate FIXa.

The prothrombinase complex (FVa:FXa) activates prothrombin (PT).

Thrombin activates various proteases and cofactors. Thrombin cleavage of fibrinogen to soluble monomers (SFM), which are cross-linked by FXIIIa, and activation of protease-activated receptors (PARs) on platelets leads to the formation of a clot.
תודה רבה