Mechanisms Linking Angiotensin II & Atherogenesis

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Underlying Culprit: Inflammation

“...a pivotal role for inflammation in all phases of atherosclerosis from the initiation of the fatty streak to the culmination in acute coronary syndrome (plaque rupture).”

Hypertension

Dyslipidemia

Current Concepts

Lipoproteins
LDL-Chol.

Pressor Hormones
Ang II, NE, AVP

Inflammation

Oxidative
Stress

Vasoconstriction

Vascular and Cardiac
Remodeling

Atherosclerosis

Hypertension

Vascular Endothelial Dysfunction

END STAGE
VASCULAR DISEASE
Risk Factors, Oxidative Stress, and Early Atherosclerosis

Hypertension
Hyperglycemia/Diabetes
Hypercholesterolemia
Oscillatory Shear Stress

ICAM-1
VCAM-1

TNFα
IL-1
All

Mφ
Lox, MPO
Nox

MCP-1

Ox-LDL

Endothelial Cells

Neointimal SMC Foam Cells

Migration Proliferation

Smooth Muscle Cells Fibroblasts

Managing Global Risks

Metabolic Syndrome

Truncal Obesity
(waist ≥ 40 M; ≥35 F)

Insulin Resistance
(fasting glucose > 110 mg/dL)

Dyslipidemia
TG (150 mg/dL)
HDL-C (M < 40 mg/dL; F mg/dL)
Small dense LDL

Hypertension (≥ 130/85 mm Hg)

Hypercoagulability

Endothelial and Vascular Dysfunction
PATHOPHYSIOLOGY: Metabolic Syndrome

Abdominal Obesity → Resistin → Angiotensin II → TNF-α → Insulin Resistance

RLP’s, FFA’s → VLDL → Endothelial Dysfunction → Inflammation

ROS, Caveolin → Dyslipidemia

Insulin Resistance → PAI-1 → GP IIb/IIa → Hypertension

AGE’s → Hyper-coagulability

IL → PAI-1 → TF → PAI-1

↓ NO → ROS

Adapted from R Vogel, MD
Vascular Actions of Ang II

- Increase in superoxide anion production;
- Smooth muscle proliferation;
- Decrease in EDRF
- Increase monocyte adherence to endothelium
- Inhibition of plasminogen activation;
- Lipoxygenase production by macrophages;
- Increase oxidase LDL;
- Increase activity of NADH/NADPH oxidase.
Hypercholesterolemia Augments Vasoconstrictor Responses to Ang II

Nickenig et al. Circulation 1999;100:2131-2134
Statins Reduced Vasoconstrictor Responses to Ang II in Normotensive Hypercholesterolemic Subjects

Nickenig et al. Circulation 1999;100:2131-2134
Effect of Cholesterol and Statins on Platelet AT$_1$ Receptor Density

Nickenig et al. Circulation 1999;100:2131-2134
Ang II and Atherosclerosis

Angiotensin II

Vasoactive

Proto-oncogenes
- c-fos
- c-jun
- c-myc

Growth Factors
- TGF-B1
- PDGF
- PAI-1
- bFGF

Vasoconstriction

Hypertension

Macrophages

Endothelial NO

Lipoygenase

Ang II-LDL

0x-LDL

LDL Accumulation

Foam Cell Formation

Apoptosis

Occlusive Coronary Artery Atherosclerosis

Endothelial Cell Dysfunction

VSMC Proliferation

Extracellular Matrix

VSMC Ca²⁺
Ang II and Atherosclerosis

- Lipoproteinemia
- Enhanced Lp Transport
- EC Activation-Dysfunction
- Accumulation of modified and reassembled Lp
- Hyperplasia of modified ECM
- Lp Trapping
- Foam Cell
- Losartan (?)

monocytes' chemoattractants

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cardioprotection.pr4

MONOCYTES ADHERENCE TO VASCULAR ENDOTHELium

[mRen2]27 Transgenic Hypertensive Rats

Number of monocytes adhered to vascular endothelium (Hautchen technique).

Experimental Atherosclerosis
Cynomolgus Monkeys (Macaca Fascicularis)

- Spontaneous atherosclerosis (Prathap, 1973);
- Diet-induced atherosclerosis bears high similarity with human lesions;
- Coronary lesions similar to humans (Stary and Manilow, 1982)
- Lesion progression from initial foam cell accumulation (Small et al., 1984)
- Carotid atherosclerosis correlates with plasma lipid concentrations (Kaplan et al., 1984)
- Plasma LDL uptake increased in aortas with diet-induced fatty streaks (Ghosh et al. 1987)
Aorta Fatty Streak in Cynomolgus Monkeys

- Fatty streak confined to intima
- Composed of macrophage foam cells
Losartan and Prevention of Atherosclerosis

Strawn et al. Circulation, 2000

Control

Losartan

Coronary Artery Intima

* p < 0.01

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Monocyte-Derived Macrophages from Hypercholesterolemic Patients

Hayek et al., BBRC 273:418, 2000
Angiotensin II AT$_1$ Receptor Blockade Reduces Monocyte CD11b Expression in Patients with Coronary Artery Disease
Can we translate basic mechanisms of the role of Ang II in atherogenesis to prevention of vascular disease?
THE LIFE TRIAL:
Stroke End-Points

Comparative Adjusted Risk Reduction (%)
Hypertension and Strokes

Stroke Subtypes

- Hemorrhagic: 26%
  - ICH: 13%
  - SAH: 13%
- Ischemic: 71%
  - Lacunar: 19%
  - Thromboembolic: 6%
  - Cardioembolic: 14%
- Unknown: 32%
- Other: 3%

Classification of Strokes in the LI FE

LOSARTAN AND CARDIOVASCULAR REMODELING

**Angiotensin II (AII) and LVH**

- **AT1 Cardiac myocyte**
  1. Hypertrophy
  2. Gene reprogramming
  3. Necrosis

- **AT1 Cardiac fibroblast**
  1. Proliferation
  2. Gene upregulation

**Cardiac dysfunction**

1. Hypertrophy
2. Gene reprogramming
3. Necrosis

**LIFE: Losartan vs. Atenolol Reduced Carotid Artery Hypertrophy**

- **LIFE Echo: Change in LV Mass Index**
  - *p=0.021, adjusted for baseline LVMI and baseline & in treatment BP*

- **Association of Carotid Atherosclerosis with LV Mass in Employed Adults**

- **LIFE: Losartan vs. Atenolol Reduced Carotid Artery Hypertrophy**
  - Intima-medial thickness—change from baseline at year 3
  - *p<0.05

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Thrombosis

Platelets in Hypertension

1. Morphological Changes
   - Increased volume
   - Change in shape
   - Reduced life span

2. Biochemical Changes
   - Elevated free Ca++
   - Increased sensitivity to catecholamines
   - Higher density of $\alpha_2$ adrenoreceptors
   - Decreased content of catecholamines and serotonin
   - Reduced uptake of catecholamines
   - Reduced nitric oxide

3. Functional Changes
   - Increased aggregability
   - Increased adhesiveness
   - Increased spontaneous aggregation

Losartan but not Candesartan Interacts with the Platelet TxA$_2$ receptor

Li et al. J Pharmacol Exp Ther 1997; 281(3):1065-1070.
Changes in TRA-EC$_{50}$ Extent

*P < 0.001*

Levy et al. Am J of Cardiology, 2000
Novel Metabolite Inhibits TxA₂ and COX-2

Losartan

P-450 Pathway

EXP-3174

EXP-3179

Ferrario, 2003
Effect of EXP3179 on Platelet Aggregation and COX-2

Losartan Inhibits Platelet Aggregation in Man (via EXP-3179?)

Differential Actions
Blockade of TxA₂ Receptors

Control  Candesartan  Valsartan  Losartan  EXP-3174  EXP-3179

EC₅₀, nM

The Origin of Atherosclerosis

Monocytes leave the blood stream to become macrophages engulfing antigens, debris, and lipids.

Monocytes

Bone Marrow Origin

Summary

The existence of a bone marrow renin-angiotensin system (RAS) in the bone marrow stromal cells (MSC) was investigated to determine whether the RAS in stromal cells may contribute to a local bone marrow RAS. The mRNA for renin, angiotensinogen, angiotensinogen converting enzyme (ACE), and angiotensin (Ang) II and its AT$_1$ and AT$_2$ receptors were present in BMC, and human BMC ACE mRNA was only detected in BMC. Two-color flow cytometry analysis showed that monocytes/macrophages and cultured bone marrow stromal cells (MC) were positively stained by anti-AT$_1$ and anti-AT$_2$ antibodies, and anti-Ang II, as well as binding of Ang II to AT$_1$ and AT$_2$ receptors, in BMC. Furthermore, Ang II treatment of BMC was detected by radioimmunoassay and conditioned culture media. The presence of Ang II receptors in both bone marrow stromal cells and BMC and the de novo synthesis of Ang II by MSC are supported by a potential autocrine-paracrine mechanism for local RAS mediation regulation of haeostatic response.
Is the BONE MARROW the SOURCE of Atherogenesis?

Bone marrow RAS promotes differentiation of monocytes into Macrophages

Ang II

Monocyte

Vessel Lumen

LDL

Endothelium

Intima

Modified LDL

MCP-1

Macrophage

Modified LDL

LDL

Macrophage

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STEPS TO EVALUATING THE HYPOTHESIS

1. Does Ang II modulate hematopoiesis?
2. Is it a function of a local RAS?
3. Do dyslipidemia affect the bone marrow RAS?
Ang II Immuno Reactive Products
Rat Bone Marrow

Hematopoietic Lineage

Stroma Osteoclasts
Expression of RAS Components

RAT BONE MARROW

Rat unfractionated bone marrow cells

AT$_{1a}$  AT$_{1b}$  AT$_2$  ACE2  Ren  Ao  ACE  AT$_{1b}$

28S rRNA

Rat bone marrow stromal cells

AT$_{1a}$  AT$_{1b}$  AT$_2$  ACE2  Ren  Ao  ACE  AT$_{1b}$  ACE2

28S rRNA

% cells exhibiting Ang II binding

Strawn et al. British Journal of Haematology, 126, 120–126, 2004
BONE MARROW – PRIMATES-

CD34⁺ Progenitor Cells Express AT₁ Receptors

CD34⁺ Cell

AT₁ Receptor Immunodetection on CD34⁺ Cell
Acetylated LDL Increases Angiotensin II Binding to CD34⁺-Derived Myeloid Precursors

72 h incubation with 1.0 mg/mL Ac-LDL, Sudan IV, 400X

Strawn et al., Arterio Thromb Vasc Biol 2003; 23:a-56-57
Induction of Hypercholesterolemia Increases Bone Marrow Hematopoietic Precursor Cell Proliferation

Strawn, Richmond & Ferrario, 2005
Immunodetectable Ang II and AT₁ Receptor Expression Increased by Cholesterol Feeding in Cynomolgus Monkey Bone Marrow Cells

Strawn, Richmond & Ferrario, 2005
Ang II-stimulated arachidonic acid release from HS-5 human bone marrow stromal cells

* p<0.0001 vs Control, ∞ p<0.001 vs Ang II
N = 21 experiments

SUMMARY

- Hyperlipidemia alters hematopoiesis, generating activated monocytic phenotypes

- The bone marrow RAS is activated by hyperlipidemia

- One result of bone marrow RAS activation by hyperlipidemia is the generation of activated monocytes that participate in atherogenesis

- The bone marrow RAS may be a target for diminishing end-organ pathology where inflammation is an early initiator
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