Progress in Atherosclerosis Research - Impact on Treatments and Biomarkers

Department of Cardiology
Nagoya University School of Medicine

Xian Wu Cheng MD, PhD

Disclosures (None)
Angiotensin type 1 receptor (AT1R) antagonism on atherosclerosis-associated vasa vasorum.

Identification of new biomarker for atherosclerosis-based coronary artery disease (CAD).
Atherosclerotic plaque growth and rupture

Normal  Plaque growth  Plaque rupture
Atherosclerotic plaque and vasa vasorum

Stable  Vulnerable
Key players in neovascularization processes

TLR2/4 → MMP-2/-9

SDF-1/ CXCR4 → Neovascularization → EPCs

TLR: toll-like receptor; EPC: endothelial progenitor cell; CXCR4, CXC chemokine receptor; SDF-1: stromal cell-derived factor-1; MMP: matrix metalloproteinase
Aging reduces MMP-2 expression and EPC recruitment in response to ischemia

§ P < 0.01, † P < 0.01 vs. corresponding controls

MMP-2 deficiency impairs exercise-induced neovascularization in advanced age

WT-Non-ST  WT-ST
KO-Non-ST  KO+ST

Capillary/myofiber ratio

* P < 0.05 vs. corresponding controls
N.S.: no significant difference

Cheng XW Murohara T et al. Circulation 2010
Hypothesis

AT1R antagonism

SDF-1/CXCR4

TLR2/4

PI3K

MCP-1/osteopontin

MMP2/9

Angiogenesis

Macrophages

AT1R: angiotensin II type 1 receptor;
MCP-1: macrophage chemoattractant protein-1; PI3K: phosphatidylinositol 3-
Goal

To investigate the protective effects and the mechanism of action of angiotensin II type 1 receptor antagonism therapy on atherosclerotic plaque growth and instability in apolipoprotein E-deficient (ApoE\(^{-/-}\)) mice with a special focus on plaque neovascularization.
Exp: Protocol (1):
Early atherosclerotic lesion formation

ApoE−/− mice (n = 26)

High fat diet (12 weeks)

Birth

10 weeks

4 Weeks 8 weeks 12 weeks

0.5% Carboxymethyl cellulose (CMC, CONT)
Olmesartan (1 mg/kg/d: OLM)
Sampling procedure

Aortic sinus
Thoracic
Abdominal
Microscopy images show whole aortas of ApoE^{−/−} mice treated with or without olmesartan
Olmesartan inhibits atherosclerotic lesion formation
OLM treatment reduces neovessel in the atherosclerotic plaques of ApoE$^{-/-}$ mice

OIM inhibits macrophage infiltration and inflammatory chemokine expressions.
OLM inhibits targeted gene expressions in atherosclerotic plaques

AR, aortic root; TA, thoracic aorta; AA, abdominal aorta; TLR, toll-like receptor
SDF-1, stromal-derived factor-1; CXCR4, CXC chemokine receptor
Localization of MMP-2/-9 in macrophages and SMCs of atherogenic plaques
OLM inhibits aorta-ring angiogenic action

Aortic ring microvessel sprouting area ratio (%)

P = 0.003

OLM inhibits aorta-ring angiogenic action.
OLM inhibits the levels of MMP-2/-9 mRNAs in bone marrow-derived EPCs

EPC identification

Non-staining  CD-31

C-Kit  Merged

MMP genlatinolytic activity

P = 0.04
P = 0.007

Relative mRNA abundance (% of control)

P = 0.01
P < 0.001

MMP-9  MMP-2

OLM inhibits the levels of MMP-2/-9 mRNAs in bone marrow-derived EPCs
Exp: Protocol (2)

- ApoE<sup>−/−</sup>/MMP-2<sup>+/+</sup> (n = 13)
- ApoE<sup>−/−</sup>/MMP-2<sup>−/−</sup> (n = 12)

Birth

10 weeks

4 Weeks 8 weeks 12 weeks

21 weeks

High fat diet (12 weeks)
MMP-2 deficiency reduced fat accumulation around aortas in ApoE\(^{-/-}\) mice
MMP-2 deficiency reduces atherosclerotic plaques and neovessel formation

- CD31⁺-FITC
- Oil-red O
- Plaque Base
  - $P = 0.028$
- Neovessl density
  - $MMP-2^{+/+}$ vs $MMP-2^{-/-}$
  - $ApoE^{-/-}$
- TLR mRNA/GAPDH
  - $P = 0.004$
  - $P = 0.016$
- ApoE
  - $MMP-2^{+/+}$ vs $MMP-2^{-/-}$
OLM inhibits Ang II-induced MMP expression via TRL signaling pathway in HUVECs

HUVEC, human umbilical vein endothelial cell; Ang II, angiotensin II; LY294002, PI3K inhibitor; U1024, extracellular signal-regulated kinase inhibitor AG490, janus kinase/sinal transducer and activator of transcription 3 inhibitor.
Observations

- Olmesartan lessened the levels of TLR2/4 and SDF-1/CXCR4 genes and MMP-2/-9 protein and activity in the atherosclerotic plaques.

- Olmesartan reduced diet-induced atherosclerotic plaque neovessel density and plaque instability in Apo E^{-/-} mice.
Proposed mechanisms

AT1R → O2⁻ → PI3K → MCP-1/Osteopontin → MMP-2/-9 → Angiogenesis/Plaque growth/Plaque rupture

Cheng XW, Murohara T. Hypertension 2011

Olmesartan
Conclusion

Our findings suggest that olmesartan exerts inhibitory effect on TLR2-mediated MMP-2/-9 expression and activity and angiogenic action, leading to the enhancement of atherogenic plaque stability and protection of its disruption in ApoE−/− mouse model without lipid lowering effect.
Exercise rescues vascular action in response to hypoxia in aged animals and humans.

Identification of new biomarker for atherosclerosis-based coronary artery disease (CAD).
The properties of cysteine protease: cathepsins (Cats)

Cats generally known as functioned in lysosomes, were discovered in the half of the 20\textsuperscript{th} century. There are 11 human Cats (B,C,F,H,K,L,O,S,V,W,and X) that belong to papain subfamily of cysteinyln proteases. Cystatin C (CystC) is one of the major endogenous inhibitor of cathepsins.

Previously, we have reported that CatK, which is one of the most potent mammalian collagenase, was overexpressed in the failing myocardium of humans and rats with hypertension.
CatK expression in the balloon-injured carotid artery and failing myocardium of rat

In situ hybridization

Control | Balloon-injured

Immunostaining

Control | Hypertensive-HF

Rat

Illustration of cathepsin function in pathogenesis of atherosclerosis-based vascular disease and its implications

Vascular SMCs and ECs inflammatory cells

Imbalance of cathepsins and cystatin C
Proteolytic activity in vasculature

Inflammatory cytokines
Neural hormones
Various stress

December 2011
(next issue online December 14)

Editor's Picks FREE

Cysteine Protease Cathepsins in Atherosclerosis-Based Vascular Disease and Its Complications.
Full Text | PDF

Cheng XW and Murohara T Hypertension 2011.
Circulating CatK levels might represent a novel marker of patients with CAD that predict potential atherosclerotic plaque.
Study Protocol: 3

Subjects:

257 CAD vs 100 controls
(admitted for scheduled RFCA between Mar. 2009 - Dec. 2010)

Definition

- Coronary angiography (at least one major artery 50% > stenosis)

Exclusion criteria

- Dilated or hypertrophic cardiomyopathy
- Valvular heart diseases
- Congenital heart disease
- Renal failure on hemodialysis
- Congestive heart failure
Methods

Laboratory measurements

- CatK, CystC
- Intact procollagen type I N-terminal propeptide (I-PINP), Carboxy-terminal telopeptide of collagen type I (ICTP, either as an index of collagen synthesis or degradation, respectively)
- High-sensitivity C-reactive protein (hs-CRP)
- Interleukin (IL)-1β level

Echocardiography

- Left atrial (LA) dimension
- Left ventricular ejection fraction (LVEF)
## Patients Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CAD (-) (n=100)</th>
<th>CAD (+) (n=257)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>60.1±5.08</td>
<td>62.8±6.96</td>
<td>N.S.</td>
</tr>
<tr>
<td>Male (%)</td>
<td>61</td>
<td>67.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>BMI</td>
<td>23.5±2.42</td>
<td>24.3±5.21</td>
<td>N.S.</td>
</tr>
<tr>
<td>HT (%)</td>
<td>16.0</td>
<td>30.2</td>
<td>***</td>
</tr>
<tr>
<td>DM (%)</td>
<td>13.0</td>
<td>57.5</td>
<td>***</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>29.0%</td>
<td>28.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>101.2±25.6</td>
<td>123.0±27.6</td>
<td>***</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>8.0</td>
<td>35.2</td>
<td>***</td>
</tr>
<tr>
<td>ARBs (%)</td>
<td>5.0</td>
<td>24.5</td>
<td>***</td>
</tr>
<tr>
<td>CCB (%)</td>
<td>7.0</td>
<td>29.1</td>
<td>***</td>
</tr>
<tr>
<td>ACEI (%)</td>
<td>3.0</td>
<td>7.5</td>
<td>***</td>
</tr>
<tr>
<td>Insulin (%)</td>
<td>0.0</td>
<td>25.1%</td>
<td>***</td>
</tr>
</tbody>
</table>

BMI, body mass index; HT, Hypertension; DM, diabetes mellitus; LDL, low-density lipoproteins; ARB, angiotensin II receptor blocker; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitor.
The levels of serum Cat K, ICTP, I-PINP, and IL-1β in CAD and non-CAD patients

ICTP: linked carboxy-terminal telopeptide of collagen type I
I-PINP: intact procollagen type I N-terminal propeptide

***P < 0.001, vs CAD(-)
The correlations of circulating Cat K and ICTP and ICTP/I-PINP

- For circulating Cat K vs. ICTP:
  \[ P < 0.0001, r = 0.7144, \]
  \[ Y = 33.8227 + 10.8668x, \quad n = 206 \]

- For circulating Cat K vs. ICTP/I-PINP:
  \[ P < 0.001, r = 0.5235, \]
  \[ Y = 49.4874 + 200.162x, \quad n = 206 \]
Representative images of serial conventional and integrated backscatter (IB) intravascular images

**Green**: fibrous volume

**Blue**: lipid volume

**Red**: Calcification: red

Serum Cat K: 156 pg/ml

Serum Cat K: 72 pg/ml
IVUS analysis shows that the correlations of serum Cat K and plaque and fibrous volumes in CAD patients

\[
P = 0.04116, \ r = 0.2387, \\
Y = 48.8731 + 1.08591x, \ n = 58
\]

\[
P = 0.07327, \ r = 0.1999, \\
Y = 167.557 - 1.0558x, \ n = 58
\]
Receiver Operator Characteristic (ROC) curve for logistic regression models

- CatK: 0.901
- ICTP: 0.875
- I-PINP: 0.850
- LAD: 0.801
- ANP: 0.790
- I-PINP/ICTP ratio: 0.703
- IL-1β: 0.652
- Cystatin C: 0.557
The levels of Cat K, collagen, and elastin in the aortic plaques of mice

PSR: picrosirous red staining for collagen
EVG: elastica van Gieson staining for elastin

Cheng XW et al. (2011 AHA in Orlando: unpublished data)
Summary

- Patients with CAD had significantly higher plasma CatK levels as well as IL-1β and ICTP levels than control subjects.

- Plasma CatK levels were correlated positively with ICTP, and IL-1β.

- Stepwise Logistic regression analysis revealed that, among age, gender, CatK, and collagen markers, CatK, and I-PINP/ICTP ratio were independently associated with CAD.
Cat expressions in cardiovascular and valve cells

CFC, cardiac fibroblast; CMC, cardiomyocyte
SMC, smooth muscle cell; ECs, endothelial cell.

Cats B, K, L, S
Cystatin C

Valve
Myocardium
Vascular cells
Macrophages: Cats B, K, L, S, V
ECs: Cats B, K, L, S Cystatin C

Cheng XW, Murohara T (Review) Circulation 2011
Proposed mechanisms underlying the regulation of CatK expression and releasing in atrium with AF

Mito = mitochondria; ER = endoplasmic reticulum
Xao = xanthine oxidase

Cheng XW, Murohara T (Review) Circulation 2011 (accept)
These findings suggest that serum CatK levels represent a novel marker of patients with CAD and predict potential atherosclerotic plaque.
Collaborative Researchers

Harvard University  Nagoya University  Hamamatsu University

Peter Libby  Guo-Ping Shi  Masafumi Kuzuya

Michigan University  Yanbian University

Y. Eugene Chen  Weonsam Kim  Zhao X et al. YMJ 2011

PPAR-γ and mesenteric artery aneurysm (on going)

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