Vascular Calcification Increased Cardiovascular Events

~ 20% young
~ 60% middle
~ 90% elderly

5-year mortality

Framingham Risk

Coronary Calcium

Age
Women had a greater probability of death than men in each strata of vascular calcification.

Raggi P. et al, Journal of Women’s Health 13; 3 2004
Calcification of the Aortic Arch
Risk Factors and Association With Coronary Heart Disease,
Stroke, and Peripheral Vascular Disease

**JAMA. 2000;283:2810-2815**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Men</th>
<th>None (n = 54824)</th>
<th>Present (n = 1092)</th>
<th>Women</th>
<th>None (n = 58803)</th>
<th>Present (n = 1590)</th>
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<td>Age, y†</td>
<td></td>
<td>45.8 (11.1)</td>
<td>60.6 (10.8)</td>
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<td>46.4 (10.8)</td>
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<td>Body mass index, kg/m²†</td>
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<td>25.8 (3.4)</td>
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<td>24.5 (4.5)</td>
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<td>Serum cholesterol, mmol/L†</td>
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<td>5.9 (1.1)</td>
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<td>14</td>
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*Data based on patients enrolled in the northern California Kaiser Permanente Medical Care Program between 1964 and 1973 who had multiple health checkups. Values are expressed as percentages unless otherwise indicated.†Values are expressed as mean (SD).‡To convert cholesterol from mmol/L to mg/dL, divide by 0.0259.$Diagnosed as having hypertension if patient had a systolic blood pressure higher than 140 mm Hg and diastolic blood pressure higher than 90 mm Hg, or if self-reported or if diagnosed by a physician, or if a patient self-reported his/her use of antihypertension medication.
§Diagnosed as having diabetes if self-reported or if diagnosed by a physician, or if a patient self-reported his/her use of insulin or hypoglycemic agents.
New Concept of Vascular Calcification Phenotype Change in VSMC

Expression in atherosclerotic plaque

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Intimal xanthoma</th>
<th>Fibrous cap atheroma</th>
<th>Fibrocalcific plaque</th>
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<tr>
<td>BSP</td>
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</tr>
<tr>
<td>BMP-2</td>
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<tr>
<td>ON</td>
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</table>

RANKL System

- Bone metabolism
- Signaling between T cell and dendritic cell
- Mammary gland development
RANKL increases calcification of HASMC

\[\text{Med} \quad \text{Dexamethasone, } \beta \text{-Glycerophosphate, Ascorbate...} \]

\[\text{HASMC} \quad \text{14 days} \quad \text{Alizarin Red Staining} \]

\[\text{Med} \quad \text{Med + RANKL} \]

\[\text{Nodules / well} \quad 0 \quad 10 \quad 20 \quad 30 \quad 40 \quad 50 \]

\[\text{No treat.} \quad \text{Med.} \quad \text{Med + RL} \]

\[\text{ALP mRNA} (\% \text{Med}) \quad 90 \quad 95 \quad 100 \quad 105 \quad 110 \quad 115 \quad 120 \]

\[\text{Med} \quad \text{Med + RL} \]

Osako et al. Circ Res 2010
RANKL system is present in vascular cells

RANKL induces BMP-2 expression in HAEC:

Osako et al. Circ Res 2010
BMP-2 is a potent osteogenic inducer in HASMC

BMP-2 is the main osteogenic protein induced by RANKL

Osako et al. Circ Res 2010
RANKL induces bone-related protein expression in HASMC

Osako et al. Circ Res 2010
High Fat Diet increases vascular calcification in Estrogen deficiency mice

Osako et al. Circ Res 2010
High fat diet increases RANKL system expression in aorta of Estrogen deficiency

![Graph showing RANKL/18S mRNA, OPG/18S mRNA, and PECAM/18S mRNA expression levels with statistical symbols (*) and (#) indicating significance.]

HF
OVX
E2

in situ hybridization

Osako et al. Circ Res 2010
What triggers RANKL activation in vasculature?

OVX/High Fat Diet
ApoE-/-
3 months

↑ RANKL system

Calcification

RANKL
RANK
BMP-2
MGP

BMP-2
Estrogen

cbfa1, msx2
ALP, OPN, OC
Calcification induction in VSMC by Ang II.

Inhibition of RANKL decreases the effect of Ang II in calcification.

Osako et al. ATVB 2013
Vascular calcification after Ang II infusion in ovariectomized ApoE\(^{-/-}\) mice.

Ang II exacerbates the calcification incidence in this HF/OVX ApoE\(^{-/-}\) mice.

The ARB (Olmesartan 3 mg/kg/day) treatment inhibits calcification and calcification-related gene expression

Osako et al. ATVB 2013
RANKL induces AT1R and ACE expression via ERK phosphorylation.

Osako et al. ATVB 2013
VASCULAR CALCIFICATION

Activated Endothelium

RANKL

RAS

Calcification: Osteoblast-like cell
ALP, OPN, OC

Osako et al. ATVB 2013
Clinical Evidence:

ARB inhibits vascular calcification

- ACE-I/ARB treatment in type 1 diabetes patients with albuminuria is associated with lower odds of progression of coronary artery calcification
  

- Prognostic factors for progression of early- and late-stage calcific aortic valve disease in Japanese:
  
The Japanese Aortic Stenosis Study (JASS) Retrospective Analysis
  

→ Calcific aortic valve disease Initiation of ARB treatment during the early stage may be effective
What is unmet medical needs in Adult Common Diseases?

1) Do we need drugs forever?

2) Should we take drugs everyday?

3) Can we prevent the onset of DM, hypertension….?

4) Is cost expensive to take DPP4 inhibitors, ARBs, even if these drugs work as organ protectors? Answer is No!!

if vaccine would become realm.
Design of DPP-4 Peptide Vaccine

Food intake

GLP-1

DPP-4

DPP-4 INHIBITOR

Insulin

DPP-4 inhibitor

Blockade of DPP-4 by anti-DPP-4 antibody

Plasma glucose

DPP-4 vaccine

(E1, E2 E3 antigen as candidates)
Screening of DPP-4 antigen (E1, E2, and E3)

E1 or E3 antigen can increase anti-DPP-4 titer.
E3 antigen decreased DPP-4 activity and increased GLP-1.
DPP-4 vaccine did not affect body weight and food consumption in C57Bl/6 mice.
DPP-4 vaccine attenuated plasma glucose level evaluated by Meal Tolerance Test

Meal Tolerance Test

Plasma glucose level (mg/ml)

- DPP4 vaccine
- KLH
- Lean control

*P<0.05 vs. KLH

AUC

Plasma glucose AUC (mg*min/d)

- Lean control
- KLH
- DPP4 vaccine

*P<0.05 vs. Lean
DPP-4 vaccine improved insulin resistance evaluated by ITT or HOMA-IR

ITT

HOMA-IR

*P<0.05 vs. KLH

**P<0.05 vs. KLH
DPP-4 vaccine improved glucose metabolism in KKAy mice (type 2 diabetes model)

*P<0.05 vs. KLH

Plasma glucose AUC (mg/min/dl):
- KLH
- E3 vaccine

Plasma insulin (ng/ml):
- KLH
- E3 vaccine

Graph showing the comparison of plasma glucose and insulin levels between KLH and E3 vaccine treatments.
DPP-4 vaccine increased insulin-positive cells in islet in KKAy mice (type 2 diabetes model)
Peptide vaccines for AngI, AngII and AT1R lowered BP in animal model.

**Vaccine for Hypertension**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Angiotensinogen</th>
<th>Peptide vaccine</th>
</tr>
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<tbody>
<tr>
<td>Renin Inhibitor</td>
<td>→ Renin</td>
<td>← Renin vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Discontinued)</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>→ ACE</td>
<td>← AngII vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Phase II)</td>
</tr>
<tr>
<td>ARB</td>
<td>→ AT1R</td>
<td>← AT1R vaccine</td>
</tr>
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</table>

Peptide vaccines for AngI, AngII and AT1R lowered BP in animal model.
DNA vaccine system

1. Activation of innate immunity
2. T cell response: CTL and helper T cells
3. B cell response: antibody production

Adaptation of DNA vaccine for common diseases (i.e. hypertension, DM)
Ang II DNA Vaccine Using Hepatitis Virus B core protein (HBc)

Present target molecule on the surface of sphere

Self-aggregation sphere formation

Secretion of HBc-AngII fusion protein

This system provides higher immunogenisity as an antigen and efficient antibody production.
Needleless Injection for High Transfection Efficiency

Shima Jet

Kunugiza et al, Gene Therapy 2006
Anti-Ang II antibody was elevated for 6 months.

Anti-Ang II antibody was crossreacted with AngI, but not angiotensinogen.
Decrease in BP was continued up to 6M!

Life Span of rats is about 2 years. 6 months might be equivalent to 20 years in human!?
Ang II DNA Vaccine Decreased Myocardial Infarction & Cerebral Infarction Size

Myocardial Infarction

Cerebral Infarction

Control

Ang II vaccine

Area of fibrosis (%)

Control

Ang 2 vaccine

Ang II Control
Elevated Serum lipoprotein(a) concentration is an independent risk factor for Atherosclerotic disease, such as CHD, PAD, restenosis after angioplasty.

The role of Lp(a) in CVD

At present, no pharmacotherapy to decrease Lp(a) is not existed. Since the homology of apo (a) is too close to that of plasminogen, it is quite difficult to develop the specific drug to decrease Lp(a) alone.

Selection of the epitope

Low homology to plasminogen with high antigenicity (12 amino acids: EAPSEQAPTEQR)

Construct of DNA vaccine for Lp(a)

Kyutoku M et al. Scientific Reports 2013
High titer of anti-apo(a) antibody was observed only in HBc-apo(a) group and additional immunization raised the titer of anti-apo(a) antibody.

Kyutoku M et al. Scientific Reports 2013
Apo(a) vaccination attenuated neointima formation in carotid artery ligation model.

Carotid Ligation Model

-2w 0w 2w 4w 6w 10w 12w 13w 16w

OVX

Ligation

HE stain

Immunized [HBc-apo(a)]

Control [HBc & Saline]

Intima / Media

p < 0.05

Kyutoku M et al. Scientific Reports 2013
Treatment with apo(a) DNA vaccine inhibited the deposition of Lp(a) & Migration of macrophases in blood vessels.

Kyutoku M et al. Scientific Reports 2013
DNA vaccine system

1. Activation of innate immunity
2. T cell response: CTL and helper T cells
3. B cell response: antibody production

Adaptation of DNA vaccine for common diseases (i.e. hypertension, DM)