Aortic Valve Calcification & DPP4 Inhibition

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University of Ulsan College of Medicine
Seoul, South Korea
Aortic Stenosis, Atherosclerosis and Skeletal Bone: A Common Link with Calcification and Inflammation?
Conflict of interest disclosure

None

Committee of Scientific Affairs
Calcific Aortic Valve Disease (CAVD)

Normal Aortic Valve

Senile Aortic Stenosis

Severe Aortic Valve Stenosis
1. Target Patient Population

Prevalence of degenerative VHD critically depends on age (USA data)

1. Target Patient Population

Increased Mortality of Degenerative Valvular Heart Disease in Korea

- Coronary AD (left)
- DM (left)
- COPD (left)
- Cancer (right)
- Stroke (right)

Degenerative valvular heart disease
Pneumonia
Genito-urinary disease
Alzheimer
Sepsis
Viral infection
2. Unmet Medical Needs

Aortic valve sclerosis
- 2004
  AV Vmax = 1.9 m/s

Aortic valve sclerosis
- 2009
  AV Vmax = 2.9 m/s

Aortic valve stenosis
- 2012
  AV Vmax = 4.5 m/s
Aortic valve replacement surgery as the only treatment option

Enormous Socio-economic Burden
2. Unmet Medical Needs

Transcatheter Aortic Valve Replacement (TAVR)

High cost (3-5 times of open heart surgery)
TAVR is Available in More Than 65 Countries Around the World

>250,000 total implants to date
Estimated Global TAVR Procedure Growth

In the next 10 years, TAVR growth will increase X4!

SOURCE: Credit Suisse TAVI Comment – January 8, 2015. ASP assumption for 2024 and 2025 based on analyst model. Revenue split assumption in 2025 is 45% U.S., 35% EU, 10% Japan, 10% ROW.
TAVR “Underutilization” is Largely Driven by Variation in Health Policy and Reimbursement

**2015 Country TAVR Penetration**
Total TAVR Units / Millions of Inhabitants

- **Germany**
- **Switzerland**
- **France**
- **Austria**
- **U.S.**
- **Netherlands**
- **Nordics**
- **Italy**
- **UK**
- **Belgium**
- **Ireland**
- **Spain**
- **Portugal**
- **Japan**

**SOURCE:** Eurostat, U.S. Census Bureau, Industry estimates
Is Medical Treatment to Prevent CAVD Progression Possible?
Aortic Stenosis

Lipid deposition
Inflammation
Macrophages
T\textsubscript{H} cells

Calcification
Osteoblasts
RANK/RANKL/OPG
Fetuin A

LVH
Renin-angiotensin system
Myocyte apoptosis
Myocardial fibrosis
Progression to heart failure

STATINS

ACE inhibitors
ARB

BISPHOSPHONATES
DENOSUMAB

Osteoporosis

Atherosclerosis

Hypertension

J Am Coll Cardiol 2012;19:1854
Unmet Clinical Need: Medical Tx. of CAVD

Failure of Statin to Prevent CAVD Progression

Randomized controlled Trial

Ezetimibe/simvastatin 10/40 mg
Placebo

Progressive Risk Factors to Disease
Metabolic Syndrome
Obesity
Hypertension
Smoking
Renal Failure
Hyperlipidemia
Male Gender
Oxidative Stress
Age

Normal Aortic Valve
Aortic Valve Sclerosis
Calcific Aortic Valve Disease

2 outcome

Peak Aortic-Jet Velocity

Mean Change (m/sec)

Year 1
Year 2
Last Follow-up

Simvastatin plus ezetimibe
Placebo

NEJM 2008;359:1343
SEAS n = 1873
F/U 52.2 months

NEJM 2005;352:2389
SALTIRE n = 155
F/U 25 months

Circulation 2010;121:306
ASTRONOMER n = 269
F/U 3.5 years

AS activity – distinct from atherosclerosis and skeletal bone metabolism
Pathologic Processes within the Valve During Development of Aortic Stenosis

Medical Treatment Based on Precise Molecular Mechanisms?
Paradigm Shift: Aortic Valve as a Metabolically Active Tissue?
Cellular Architectures of the Aortic Valve

Aortic Side (outflow)
- Endothelial Cells
- Interstitial Cells (VIC)
- Collagen
- Glycosaminoglycans (GAGs)
- Interstitial Cells (VIC)
- Elastin
- Endothelial Cells

Ventricular Side (inflow)
- Fibrosa
- Spongiosa
- Ventricularis

Aortic Aspects
- Collagen fibers in the fibrosa
- Elastin fibers in the ventricularis

Circulation 2011;124:1783
Sources of Aortic Valve Osteoprogenitors

1) Circulating osteoprogenitors

2) EM transition of valvular endothelial cell (VEC)

3) Osteogenic transdifferentiation of valvular interstitial cell (VIC)
Circulating Calcifying Cells in the Bone-Vascular Axis

Bone remodeling

Epidemiology

Medications

VSMCs

Intimal

Homing to atherosclerosis

Osteocytes

Monostephils

Osteoblasts

Osteoclast

Mononuclear phagocytes

Osteoblast

Osteoclast

Hemangioblast

CD34

HSC

Monocyte

Monostephil

MCC

EPC

MSC

Mesenchymal osteoprogenitor

MCC

EPC

MSC

Mesenchymal osteoprogenitor

Homing to atherosclerosis

Osteo-/angiogenesis

Bone fracture

Callus formation

VASCULATURE

Circulation 2012;125:2772
Interaction between VEC and VIC

Rajamannan et al  NIH Working Group: Calcific Aortic Valve Disease

Circulation 2011;124:1783
Interaction between VEC and VIC
Including Endothelial-to-Mesenchymal transition

Valve Homeostasis

A
qVIC
αSMA (-)
Vimentin (+)

B
aVIC
αSMA (+)
Vimentin (+)

C
Calcification

CAVD

D
VEC
VE-Cadherin (+)
αSMA (-)

E
eVIC
VE-Cadherin (-)
αSMA (+)

EndMT

Atherosclerosis 2015;242:251
Osteogenic Differentiation of VICs: Role of Interaction between VECs and VICs

1) Circulating osteoprogenitors

2) EM transition of valvular endothelial cell (VEC)

3) Osteogenic transdifferentiation of valvular interstitial cell (VIC)

_Circ Res_ 2013;113:198
Interaction between VEC and VIC: Different Pathway?

A  Hemodynamic Flow Across the Aortic Valve

<table>
<thead>
<tr>
<th>Systole</th>
<th>Aortic Valve Closure</th>
<th>Diastole</th>
</tr>
</thead>
<tbody>
<tr>
<td>outflow</td>
<td>pressure</td>
<td>end diastolic pressure</td>
</tr>
<tr>
<td>inflow</td>
<td></td>
<td>echo-cardiographic view of aortic valve leaflet in end diastole</td>
</tr>
</tbody>
</table>

oscillatory shear stress | tensile stretch | bending stretch |

pulsatile shear stress | tensile stretch | bending stretch |

Circulation;2011;124:1783
Interaction between VEC and VIC: Different Pathway?

A Normal aortic valve

B Calcific aortic valve disease

C

D

Calcified nodules
T cell
Macrophage

Circ 2017;135:1951
cDNA microarray (Aortic Valve Tissues)
Non-CAVD vs CAVD

Elevation of DPP-4 in CAVD Valve Tissue

Basic Cell Research
In vitro cellular mechanism

Clinical Application
Solution
Novel treatment

In vivo studies using animal disease model

www.abdn.ac.uk

www.dbi.illinois.edu
Gene expression profiles of aortic valve tissue of patients with CAVD

**RESULTS:**

<table>
<thead>
<tr>
<th></th>
<th>Non-CAVD</th>
<th>CAVD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#1</td>
<td>#2</td>
</tr>
<tr>
<td></td>
<td>#1</td>
<td>#2</td>
</tr>
</tbody>
</table>

- **Calcification**
  - SPPI
  - CHSL1
  - IGSL
  - ALPL
  - MAP2K3
  - TNFRSF11A
  - CTSHRC1
  - VDR
  - RUNX3
  - RUNX1
  - GREM1
  - RUNC2
  - TGF2
  - WNT3
  - DRK2

- **Fibrosis**
  - ACTA2
  - SHNA1
  - AGT
  - CCL5
  - MAP3K3
  - SERPAH1
  - PLA2G4A
  - SERP1A1
  - SERP1A3
  - MAPK1
  - MAPK14
  - ITGB3
  - COL19
  - CD68
  - COL5A1
  - TGFB1
  - CYR61
  - TNFAIP6
  - CYSLR5
  - IL5RA
  - TNFRSF13A
  - HFE2A
  - TGFB2
  - WNT7A
  - CR7
  - CCL4
  - MST3
  - ACK1
  - DPP4

- **Inflammation**

**Aortic Valve Tissues of patients with CAVD**

"Up-regulation of genes related with Calcification/Fibrosis/Inflammation"

"Up-regulation of DPP-4 and its related genes"

Up-regulation of DPP4 in aortic valve tissues of patients with CAVD
High DPP-4 protein expression in the CAVD aortic valves

DPP-4 is elevated in the calcified lesion of aortic valves
High DPP-4 protein expression in the VICs

Functional relationship b/w endothelial dysfunction and DPP-4 induction in VICs
Enhancement of osteogenic differentiation of hVICs from CAVD patients

**RESULTS:**

DPP-4 induced the osteogenic change of VICs

<table>
<thead>
<tr>
<th>hVICs + OM</th>
<th>Non-CAVD</th>
<th>CAVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitagliptin</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AR</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Osteogenic markers**

- **AR positive area** (% of control)
  - Non-CAVD: Vehicle - Sitagliptin
  - CAVD: Vehicle - Sitagliptin
  - Significant differences: p<0.01

- **Calcium (μg/mg)**
  - Non-CAVD: Vehicle - Sitagliptin
  - CAVD: Vehicle - Sitagliptin
  - Significant differences: p<0.001

- **Relative mRNA expression (Fold change to Vehicle of Normal)**
  - hALP
    - Non-CAVD: Vehicle - Sitagliptin
    - CAVD: Vehicle - Sitagliptin
    - Significant differences: p=0.033
  - hRUNX2
    - Non-CAVD: Vehicle - Sitagliptin
    - CAVD: Vehicle - Sitagliptin
    - Significant differences: p=0.036
  - hSp7
    - Non-CAVD: Vehicle - Sitagliptin
    - CAVD: Vehicle - Sitagliptin
    - Significant differences: p=0.027
  - hBGLAP
    - Non-CAVD: Vehicle - Sitagliptin
    - CAVD: Vehicle - Sitagliptin
    - Significant differences: p=0.024
Induction mechanism of DPP-4 in the hVICs

Endothelial NO deficiency up-regulates DPP-4 expression in VICs

\[ \text{hVICs} \]

<table>
<thead>
<tr>
<th>DETA-NO (μM)</th>
<th>0</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>hDPP-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-130kDa
-100kDa
-55kDa
-40kDa

\[ \text{DPP-4 promoter} \]

-882 -684 -674 -42 +18

- NF-κB binding site

WT GGGAAATTC
MT GCCAAATTC

\[ \text{hDPP-4 promoter activity} \]

- Fold change to Control

- DETA-NO (μM): 0, 10, 100

- WT promoter
- MT promoter

- p<0.001
Induction mechanism of DPP-4 in the hVICs

RESULTS:

Endothelial NO deficiency up-regulates DPP-4 expression via NF-κB activation in VICs

Circulation 2017;135:1935
Aortic valve pathology in eNOS knock out mouse

Endothelial NO deficiency results in calcification with DPP4 over-expression
RESULTS:  Inhibition of osteogenic differentiation of mVICs by DPP-4 inhibition

Osteogenic changes of mVICs from eNOS KO mice is inhibited by DPP-4 inhibition
Valve calcification associated with the DPP-4 level in vivo is inhibited by DPP-4 inhibitor
Potential mechanism of DPP-4 action in the hVICs

“Regulatory function of IGF-1 on osteoblasts and vascular smooth muscle cells”

- Maintenance of bone mass through a direct action on osteoblasts, which express insulin and IGF1 receptors (Nature Medicine, 2012)
- Maintenance of differentiated phenotype in vascular smooth muscle cells (JBC, 2004)
IGF-1 inhibits osteogenic differentiation of hVICS

pERK- and pAkt-mediated IGF-1 action on osteogenic differentiation of hVICS

Circulation 2017;135:1935
Decreased IGF-1 expression in the hVICS of patients with CAVD

**Graph:**
- **hIGF-1 mRNA expression** (Fold change to control)
  - **Non-CAVD**
  - **CAVD**
  - **p = 0.015**

**Images:**
- **DAPI**
- **hIGF-1**
- **Merge**

**Diagram:**
- **Healthy AV**
  - **CAVD**
  - **IGF-1 production**
  - **Inhibition of osteogenic differentiation of VIC**
  - **DPP-4**
  - **IGFBP3**
  - **PI3K**
  - **ERK**
  - **AKT**

**Circulation 2017;135:1935**
In Vivo Rabbit Model of CAVD
In Vivo Rabbit Model of CAVD

- Normal chow
- 1% cholesterol diet + VitD
- 1% cholesterol diet + VitD + 4mg/kg sitagliptin
- 1% cholesterol diet + VitD + 8mg/kg sitagliptin
- 1% cholesterol diet + VitD + 15mg/kg sitagliptin
- 1% cholesterol diet + VitD + 30mg/kg sitagliptin
- 1% cholesterol diet + VitD + 60mg/kg sitagliptin (n=8 each)

*J Am Coll Cardiol* 2003;41:1211–1217
In Vivo Rabbit Model of CAVD
In Vivo Rabbit Model of CAVD

1.26 m/s With Sitagliptin

0.86 m/s
In vivo effect of DPP-4 inhibitor on valve calcification in rabbit AS model (I)

DPP-4 inhibitor can prevent CAVD development or progression
Rabbit CAVD model induced by high cholesterol diet with vitamin D2 supplementation

**RESULTS:**

Alterations in aortic valve area of rabbits treated with different doses of Sitagliptin

Circulation 2017;135:1935
**RESULTS:**

*In vivo effect of DPP-4 inhibitor on valve calcification in rabbit AS model (II)*

<table>
<thead>
<tr>
<th>Sitagliptin (mg/kg):</th>
<th>Normal diet</th>
<th>Chol+Vit.D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>H.E</th>
<th>MT</th>
<th>AR</th>
<th>Macrophage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HE</strong></td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td><strong>MT</strong></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
<tr>
<td><strong>AR</strong></td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
<tr>
<td><strong>Macrophage</strong></td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
<td><img src="image16" alt="Image" /></td>
</tr>
</tbody>
</table>

**Plasma DPP-4 activity (pmole/min/ml)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0w</td>
<td>0</td>
</tr>
<tr>
<td>3w</td>
<td>0</td>
</tr>
<tr>
<td>6w</td>
<td>0</td>
</tr>
<tr>
<td>12w</td>
<td>0</td>
</tr>
</tbody>
</table>

*ND* indicates not detected.

**Plasma IGF-1 (ng/ml)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0w</td>
<td>0</td>
</tr>
<tr>
<td>3w</td>
<td>0</td>
</tr>
<tr>
<td>6w</td>
<td>0</td>
</tr>
<tr>
<td>12w</td>
<td>0</td>
</tr>
</tbody>
</table>

*ND* indicates not detected.

*P values: p<0.01, p<0.001.*
Interaction between VEC and VIC: Different Pathway?

Circulation 2017;135:1935
Unresolved Issues

1) Association with other potential mechanisms:
   - Circulating osteoprogenitors
   - EM transition

2) Adequate dose and drug of DPP4 inhibitors
   - role of different tissue distribution

3) Proof of concept study / RCT
Cardiac valves are metabolically active tissues!
Multifunctional protein
1) Protease
2) Binding with fibronectin & adenosine deaminase (ADA)
3) Cell surface co-receptor activity to mediate viral entry
4) Regulation of intracellular signal transduction coupled to control of cell migration & proliferation
Heart Distribution of DPP-4 Inhibitors

Heart/plasma ratio

- 1hr
- 4hr

A | B | C | D | E
---|---|---|---|---
| 0 | 2 | 8 | 6 | 12
| 2 | 4 | 10 | 8 | 16
| 4 | 6 | 12 | 10 | 20

Error bars represent standard deviation.
Heart Distribution of DPP-4 Inhibitors

![Graph showing concentration levels of various samples.](image)
Class 1: vildagliptin, saxagliptin
Class 2: alogliptin, linagliptin
Class 3: sitagliptin, tenegliptin
Interaction with Non-substrate Binding S2 Extensive Site

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPP-4 inhibition, IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vildagliptin</td>
<td>29.2 nmol/L</td>
</tr>
<tr>
<td>Saxagliptin</td>
<td>6.3</td>
</tr>
<tr>
<td>Alogliptin</td>
<td>4.9</td>
</tr>
<tr>
<td>Linagliptin</td>
<td>0.6</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>10.3</td>
</tr>
<tr>
<td>Teneligliptin</td>
<td>1.9</td>
</tr>
</tbody>
</table>
Unique Drug-specific Effects of Different DPP-4 Inhibitors

Linagliptin but not Sitagliptin inhibited transforming growth factor-β2-induced endothelial DPP-4 activity and the endothelial-mesenchymal transition

Sen Shi a, b, c, Keizo Kanasaki a, b, *, Daisuke Koya a, b, **

a Department of Diabetology & Endocrinology, Kanazawa Medical University, Uchinada, Ishikawa, 920-0293, Japan
b Division of Anticipatory Molecular Food Science and Technology, Medical Research Institute, Kanazawa Medical University, Uchinada, 920-0293, Ishikawa, Japan
c The Department of Vascular and Thyroid Surgery, The First Affiliated Hospital of Sichuan Medical University, Luzhou, 646000, PR China

Biochem Biphys Res Commun 2016;471:184-190
Overexpression of Insulin-like Growth Factor-1 in Mice Protects from Myocyte Death after Infarction, Attenuating Ventricular Dilation, Wall Stress, and Cardiac Hypertrophy

Qiong Li,* Baosheng Li,* Xiaowei Wang,* Annarosa Leri,* Kumar P. Jana,* Yu Liu,* Jan Kajstura,* Renato Baserga,‡ and Piero Anversa*

*Department of Medicine, New York Medical College, Valhalla, New York 10595; and ‡Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania 19107
Cardiac-Specific IGF-1 Receptor Transgenic Expression Protects Against Cardiac Fibrosis and Diastolic Dysfunction in a Mouse Model of Diabetic Cardiomyopathy

A

B

Collagen deposition (% collagen area/visual field)

<table>
<thead>
<tr>
<th></th>
<th>Ntg sham</th>
<th>Ntg diabetic</th>
<th>IGF-1R sham</th>
<th>IGF-1R diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bars = 20μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diabetes 2010;59;1512
Pathologic Processes within the Valve During Development of Aortic Stenosis

새로운 세포 기전에 근거한 치료약물의 개발 필요
DPP-4 – IGF1 Axis: 차별화된 target molecule?

- 혈관내피세포 기능 장애
- DPP4 억제제
  - NO
  - NF-κB
- DPP4 발현 증가
  - eNOS
  - DARC 발현 증가
  - TNFα 증가
  - IGF-1 활성 억제
  - 3) Osteogenic differentiation of VICs

- 섬유화
- 석회화
- 판막 및 심근 섬유화
- 대동맥판 협착증
 선행연구결과 (Ⅱ) 
 석회화 동반 대동맥판막협착증 환자 조직 및 혈액에서 증가하는 신규 표적단백질 DPP-4 발굴 및 발현 확인(특허 등록)

Cluster of differentiation (CD) 26 (Dipeptidyl peptidase 4, DPP-4)
- Chemokine 활성화/비활성화 통한 면역반응 조절
- 다양한 기질 활성 억제를 통한 세포반응 조절

Induction of DPP-4 Expression in Human Aortic Valve Cells via Nitric Oxide Deprivation

A

Relative mRNA expression (Fold change to Normal ECs)

hPECAM1

hACTA2

hMYH7

hALP

hDPP-4

heNOS

B

hDPP-4 in CM (ng/ml)

C

hDPP-4 mRNA expression (Fold change to vehicle)

D

hVICs

DETA-NO (µM) 0 10 100

hDPP-4

β-actin

E

Relative hDPP-4 protein level/β-actin (Fold change to vehicle)

hDPP-4 in CM (ng/ml)

Human valvular interstitial cell에서 NO dependent한 DPP-4 발현 증명
Osteogenic Transdifferentiation of Aortic Valve Interstitial Cells

DPP-4 up-regulation via NO deprivation-dependent NF-κB activation
DPP-4 inactivates IGF-1-mediated signaling to induce osteogenesis
In Vivo Experiment Using eNOS\(^{-/-}\) Mouse

Attenuation of in vivo calcification by DPP-4 inhibition
In Vitro Experiment Using Valvular Interstitial Cells of eNOS⁻/⁻ Mouse

Attenuation of in vitro calcification is associated with an increase in the IGF-1 level
RESULTS: Rabbit CAVD model induced by high cholesterol diet with vitamin D2 supplementation

DPP-4 inhibitor prevents development of CAVD
The Low-Risk Journey

STS database 2002-2010 (141,905 pts)

- Low risk (STS <4%): 79.9%
- Intermediate risk (STS 4-8%): 13.9%
- High risk (STS > 8%): 6.2%
Estimated Global TAVR Economic Growth

SOURCE: Credit Suisse TAVI Comment – January 8, 2015. ASP assumption for 2024 and 2025 based on analyst model. Revenue split assumption in 2025 is 45% U.S., 35% EU, 10% Japan, 10% ROW; Morgan Stanley Comment July 6, 2015

In the next 10 years, TAVR economics will increase X4!

© TVT 2016  Transcatheter Valve Therapies (TVT)  A Multidisciplinary Heart Team Approach