Drug discovery of prevention (delay) of diabetic retinopathy using a Korean endemic plant, *Aster Koraiensis* (K83A)

Dr. Kim, Jin Sook

KM Convergence Research Division
Korea Institute of Oriental Medicine (KIOM)
1. Introduction of Diabetic Retinopathy
   (1) The Structure of Eye and Retina
   (2) Biochemistry and Molecular cell Biology of D. Retinopathy

2. Activity and Mechanism of K83A
   (1) HPLC fingerprint
   (2) Screening: in vitro assay, cell-based assay, in vivo assay
   (3) Animal (SDT rats) study
   (4) Mode of Action in human retinal endothelial cell (HREC)

3. Conclusion
Introduction of Diabetic Retinopathy

Retinopathy is the most common microvascular complication of diabetes, and it remains a major cause of visual impairment worldwide.

Epidemiology study: at 20 years after diagnosis;

patients with type 1 diabetes … most
Insulin-dependent patients with type 2 diabetes … ~ 80%
Non-insulin-dependent patients with type 2 diabetes … ~ 50%

Diabetic Retinopathy
The retina is a thin nervous tissue that lines the back of the eye.

Retina is made up of seven principal cell types (ganglion cells, amacrine cells, bipolar cells, horizontal cells, con- / rod-photoreceptors, and mueller glia) arranged into three layers.

(* Neuronal cells located in GCL and INL are damaged and finally undergo apoptotic cell death)

The retina is also perfused by blood vessels which are made up of vascular endothelial cells (red) and pericytes (blue). Pericyte is attached to the endothelial cell.
Retinal pericyte plays important role for the maintenance of capillary integrity, vascular tone, vessel stabilization and structural support to endothelium.

(* Loss of pericyte and subsequent formation of acellular capillaries are increased)
The pathogenesis of diabetic complication is multifactorial.

1. Overproduction of Advanced Glycation Endproducts (AGEs)
2. Activation of Aldose Reductase-related Polyol pathway (AR)
3. Activation of Protein Kinase C (PKC) isomers
4. Increased Hexosamine Pathway Flux

Four main molecular mechanisms finally induced oxidative stress have been implicated in glucose-mediated vascular and neuronal damage.

Neuronal and vascular dysfunctions in retina induced by AGEs overproduction

AGEs were accumulated in the neural retina and vascular cells of diabetic animals.

AGEs are also directly linked with the apoptotic cell death of retinal pericytes and neuronal cells.

The loss of retinal pericyte leads to the development of microaneurysms, retinal hemorrhages and neovascularization.

Neuronal degeneration of the retina is also a critical factor of diabetic retinopathy.
Ganglion cell death causes permanent impairment of visual function.

Enhanced apoptosis of the retinal pericyte and neuronal cell is also associated with NF-κB.
Under chronic diabetic condition, the reaction of glycation accelerates, toxic product such as AGEs begin to over produce and over accumulate, and retinal vessels and retinal neuronal cells are injured.

As a result, microvascular lesions, such as pericyte loss, acellular capillaries, microaneurysms, blood retinal barrier dysfunction (leakage) and neuronal death are appeared.

If these pathological process can not be prevented and/or delayed, the persons with diabetes will suffer from poor eyesight and finally lose the sight of eye(s)
Our strategy

It has been well known that current commercial antidiabetic can not completely treat DM and also prevent (delay) the progression of diabetic complications.

So I think that diabetes patients need to be cared with combination therapy of antidiabetic drug and drug of antidiabetic complication for the prevention (delay) of the progression of diabetic complications.

We tried to discover herbal medicine which have no hypoglycemic action, but reduce development of diabetic complications.
2. Activity and Mechanism of K83A

(1) HPLC fingerprint

(2) Screening

Inhibition of AGE formation
  Non-enzymatic reaction
  Cell-based assay (human retinal pigment epithelial cell line)
  Inhibition of cross-link of already produced AGEs and proteins
  Inhibition of hyaloid-retinal vessel dilation in larval zebrafish

(3) Animal (SDT rats) study

Preventive effect on diabetic retinopathy

(4) Mode of Action in human retinal endothelial cell (HREC)
Chemical Constituents from the Aerial Parts of *Aster koraiensis* with Protein Glycation and Aldose Reductase Inhibitory Activities

Jun Lee,† Yun Mi Lee,† Byong Won Lee,‡ Joo-Hwan Kim,§ and Jin Sook Kim*†

*J. Nat. Prod. 2012, 75, 267-270*

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Extract of the aerial parts of *Aster koraiensis* reduced development of diabetic nephropathy via anti-apoptosis of podocytes in streptozotocin-induced diabetic rats

Eunjin Sohn, Junghyun Kim, Chan-Sik Kim, Young Sook Kim, Dae Sik Jang, Jin Sook Kim †

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*Biochemical and Biophysical Research Communications 391 (2010) 733–738*
HPLC fingerprint: Aster Koraiensis

Korean endemic plant. In Korea, its young leaves have been used as spring greens. The roots are used as a medical herb for antibacterial treatment and against asthma and some cancers. The aerial parts of Aster koraiensis 80% Ethanolic extract of aerial part of A. koraiensis (K83A)

<table>
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<th>Time (min)</th>
<th>Flow rate (ml/min)</th>
<th>Acetonitrile (%)</th>
<th>0.1% Acetic acid in water (%)</th>
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<td>25</td>
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<td>83</td>
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<td>60</td>
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<td>81</td>
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<tr>
<td>70</td>
<td>1.0</td>
<td>40</td>
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② Screening

Inhibition of advanced glycation endproducts (AGEs) formation and cross-link of already produced AGEs and proteins

<table>
<thead>
<tr>
<th></th>
<th>IC_{50%} (ug/ml)</th>
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<tr>
<td></td>
<td>K83A</td>
<td>71.04±1.11</td>
<td>K83A</td>
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<tr>
<td></td>
<td>Aminoguanidin</td>
<td>82.50±1.10</td>
<td>Aminoguanidin</td>
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**Humane Retinal pigment epithelial cell**

<table>
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<tr>
<th>(kDa)</th>
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</table>

**Aminoguanidin**

\[
\begin{align*}
\text{HG (25 mM)} + \text{BSA (500 \mu g/ml)}
\end{align*}
\]
Inhibition of hyaloid-retinal vessel dilation induced by high-glucose in larval zebrafish

Zebrasfish is one of vertebrates. Retinal vascular change in HG-exposed zebrafish may be similar to that of mammalian model.

<table>
<thead>
<tr>
<th>Group</th>
<th>NOR</th>
<th>HG (130mM)</th>
<th>K83A (1 μg/ml)</th>
<th>K83A (5 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel diameter (AU)</td>
<td>7.02±0.17</td>
<td>11.88±0.54###</td>
<td>9.31±0.35***</td>
<td>9.27±0.29***</td>
</tr>
</tbody>
</table>

Mean±SEM

###p<0.001 vs. Nor
***p<0.001 vs. HG
Animal study: Preventive effect on diabetic retinopathy

Animal model:
Spontaneous type 2 diabetic Torii (SDT) rat is a representative type 2 DM model that closely exhibits the features of human DR.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage (mg/BW Kg)</th>
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<tbody>
<tr>
<td>Nor</td>
<td>-</td>
</tr>
<tr>
<td>DM</td>
<td>-</td>
</tr>
<tr>
<td>MET</td>
<td>350mg/kg</td>
</tr>
<tr>
<td>MET+K83A</td>
<td>Metformin (350mg/kg) + K83A(100mg/kg)</td>
</tr>
<tr>
<td>K83A-25</td>
<td>25mg/kg</td>
</tr>
<tr>
<td>K83A-50</td>
<td>50mg/kg</td>
</tr>
<tr>
<td>K83A-100</td>
<td>100mg/kg</td>
</tr>
</tbody>
</table>
Inhibitory effect on AGEs accumulations in retinal vessels

NOR | DM | MET | MET+K83A
---|---|---|---
K83A-25 | K83A-50 | K83A-100

Fluorescence intensity (AU)

* # #
Inhibitory effect on blood-retinal barrier (BRB) leakage in retinal vessels

Leakage of blood-retinal barrier (BRB) is caused to formation of acellular capillaries
Inhibitory effect on acellular capillaries formation in retinal vessels

![Image showing cellular capillaries](image)

Pericyte

Endothelial cell

Acellular capillary

- NOR
- DM
- MET
- MET + K83A
- K83A - 25
- K83A - 50
- K83A - 100

Acellular capillaries (No. / mm²)

![Graph showing data](graph)
Inhibitory effect on Tight junction protein (occludin) loss in whole retina

TJPs form the closest contact between adjacent cells (retinal pericyte and endothelial cells), and maintain normal cellular functions by regulating cellular permeability.
Inhibitory effect on Apoptosis in retinal neuronal cells
Inhibitory effect on Bax and Bcl2 staining in retinal cryosection (A) and expressions of Bax and BCL2 ratio in whole retina (B)

(A)

BAX

(B)

BCL2

K83A-25
K83A-50
K83A-100

Bax signal intensity (AU)

Bax / BCL2 ratio (AU)
Mode of action in Human retinal endothelial cells (HREC)

Anti-apoptotic effect of 83A in HREC under HG condition

![Image of cell images and bar graph showing Tunel assay results]
Inhibition of mitochondrial damage in HERC under HG condition

**HG + K83A (μg/ml)**

Cleaved Caspase-9

Cleaved Caspase-3

Bax

Bcl-2

β-actin

**Bax**

**Bcl-2**

**Superoxide**

**SOD**

Superoxide anion level (RLU x μg protein⁻¹)

SOD (Superoxide Dismutase) activity (RLU x mg protein⁻¹)
Inhibition of oxidative phosphorylation (OXPHOS) complexes in mitochondria in HERC under HG condition

- Complex V-ATP5A 54kD
- Complex III-UQCRC2 48kD
- Complex II-SDHB 29kD
- Complex IV-COX II 22kD
- Complex I-NDUFB8 18kD

β-actin
Inhibition on mitochondrial superoxide of live-HERC under HG condition

MitoTracker® Green | MitoSOX | Merge
---|---|---
Con

HG

HG+83A
0.01 μg/ml

HG+K83A
0.1 μg/ml

HG+K83A
1 μg/ml

Fluorescence Intensity of MitoSOX Red (% of control)

**

#
Inhibition of VEGF, NF-κB and p-IκB expressions in HREC under HG condition

**VEGF ELISA**

- Released VEGF (pg/ml) under HG + K83A (μg/ml)
- VEGF Western Blot for HG + K83A (μg/ml)
- p-NF-κB Western Blot for HG + K83A (μg/ml)
- p-IκB Western Blot for HG + 83A (μg/ml)
- VEGF/β-actin Western Blot for HG + K83A (μg/ml)
- p-NF-κB/β-actin Western Blot for HG + K83A (μg/ml)
- Released VEGF (pg/ml) under HG + K83A (μg/ml)

* and # indicate significant differences compared to control (Con) and HG, respectively.
4. Conclusion

O2 → HG → AGEs

NADPH oxidase

PKC-δ

ROS

Activated Bax

Cytochrome C

Caspase-9, 3

Apoptosis

Oxidative stress

Activated Bcl2

OXPHOS complex I

Iκ-B

NF-κB

VEGF

Acellular capillaries

Leakage, Non-perfusion

Breakage of TJ protein

Angiogenesis

Neuronal cell death

DR

NADPH oxidase component protein p47 phox

means the potential therapeutic targets of 83A
Thank you
### Animal study

The value of plasma chemistry of experimental rats

<table>
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</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>534.7±25.9</td>
<td>364.7±25.7</td>
<td>364.4±39.2</td>
<td>378.1±21.9</td>
<td>372.5±29.2</td>
<td>369.7±40.8</td>
<td>355.3±40.6</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>164.8±55.2</td>
<td>416.9±71.3</td>
<td>478.7±183.1</td>
<td>489.00±148.3</td>
<td>478.7±152.2</td>
<td>371.1±93.6</td>
<td>331.2±134.7</td>
</tr>
</tbody>
</table>

* indicates statistical significance.
World First Approval for **Fenofibrate**® (PPARα agonist) in Diabetic Retinopathy (approved. Nov. 2013, Australia)

### Fenofibrate plus simvastatin in type 2 diabetes (ACCORD-Eye; NCT00542178)

<table>
<thead>
<tr>
<th>Standard Glycaemic therapy</th>
<th>Intensive Glycaemic control</th>
<th>Standard lipid Therapy (Statin+ Placebo)</th>
<th>Standard lipid Therapy (Statin+ fenofibrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.4</td>
<td>7.3</td>
<td>10.2</td>
<td>6.5</td>
</tr>
</tbody>
</table>

- **Progression of diabetic retinopathy (%)**
- **Primary End point**: Progression of DR or Development of PDR
- **Stage of DR at baseline**: Mild-to-moderate NPDR
- **Size of Effect**: Reduced risk of progression by 40%
- **Comment**: Additive effects of fenofibrate plus simvastatin

### Fenofibrate in type 2 diabetes (FIELD; ISRCTN647833481)

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Fenofibrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9%</td>
<td>3.4%</td>
</tr>
</tbody>
</table>

- **Size of Effect**: Reduced risk of progression and macular edema by one third
- **Comment**: Fenofibrate reduces need for laser treatment

*N ENGL J MED (2012) 366; 13, 1227-1239*