Uprgulation of Semicarbazide-Sensitive Amine Oxidase (SSAO) in Endothelial Cells Is the Mechanism of Oxidative Vascular Injury in Diabetes: Blockade by UD-014, a Novel SSAO Selective Inhibitor

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Introduction
Semicarbazide-sensitive amine oxidase (SSAO) / vascular adhesion protein-1 (VAP-1) expressed on the endothelium catalyzes deamination of primary monamines.

SSAO

CH₃NH₂ + O₂ + H₂O ➔ HCHO + H₂O₂ + NH₃
CH₂OHCH₂NH₂ + O₂ ➔ CH₂OHCHO + H₂O + NH₃

This enzyme also mediates leukocyte rolling, adhesion and transmigration through the endothelium. SSAO activity is shed from the endothelium into plasma and is known to be elevated in diabetes mellitus[1]. Thus, SSAO has been considered to be one of the causative factors to induce microvascular injury in diabetes[2]. Our recent study found that UD-014, a novel SSAO selective inhibitor, ameliorated albuminuria in the streptozotocin-induced diabetic nephropathy model in rats (Refer to S337A1 and ADA2017). However, its mechanism remains unclear. The aim of this study is to clarify the mechanism of action of UD-014 using glomerular microvascular endothelial cells (GMVECs) focusing on the vascular injury induced by oxidative stress.

Materials and Methods

- Human GMVECs (DS Pharma BioMedical) were cultured in exclusive culture medium with 10% FBS in type I collagen coated culture ware. Cells were subjected to one day culture before the experiments.
- Methylamine-induced cytotoxicity assay was performed. Cell survival was detected by a WST-8 reduction assay.
- In quantitative RT-PCR analysis, the threshold cycle (Ct) was used for determining the relative expression level of each gene, by normalizing to the Ct of GAPDH.
- In Immunocytochemistry, cells were fixed by 4% PFA, permeabilized with 0.1% Triton X-100, immunostained with anti-human SSAO antibody (Santa Cruz) and Alexa488-conjugated secondary antibody, and imaged with a fluorescent microscope.

Methylation Metabolites Cytotoxicity

UD-014 in vitro profile

<table>
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<th>IC₅₀ [mM]</th>
<th>SSAO</th>
<th>MAO-A</th>
<th>MAO-B</th>
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<tr>
<td>UD-014</td>
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<td>6.10</td>
<td>12.00</td>
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<tr>
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Methylation induced Cytotoxicity

Fig.1 Methylation-induced cytotoxicity during 2-day culture. (A) Methylamine at 1 mM and more induced cytotoxicity in GMVECs. An SSAO inhibitor, UD-014 at 1 μM completely rescued the toxicity. Neither MAO-A (Red) nor MAO-B (Green) inhibitors did. (B) UD-014 rescued the methylamine-induced cytotoxicity concentration-dependently.

Fig.2 Time-dependent cytotoxicity by 1 mM methylamine. The cell death progressed slowly, and 1 μM UD-014 rescued more than 50% of cells in 2-day culture.

Fig.3 Post-treatment with UD-014. Until 20 hrs post-treatment, 1 μM UD-014 rescued more than 50% of cells.

Fig.4 Methylamine metabolites induced endothelial cell toxicity in 2-day culture. (A) Metabolites induced cell toxicity at lower concentrations than methylamine. (B) UD-014 specifically rescued the methylamine-induced cellular toxicity. Methylamine:1 mM, H₂O₂:1 mM, Formaldehyde:0.3 mM.

SSAO mRNA Induction

Fig.5 Methylamine induced SSAO mRNA upregulation. Methylamine induced the mRNA more than 50-fold after 30 hr incubation. UD-014 completely inhibited the induction.

Fig.6 Post-treatment with UD-014. Even after 16 hr post-treatment with 1 μM UD-014 inhibited SSAO mRNA induction.

SSAO protein expression in GMVECs.

(A) Vehicle control. (B) Methylamine treatment at 1 mM for 3 hr. Most cells expressed SSAO proteins. (C) 1 μM UD-014, treated before methylamine addition, inhibited SSAO protein expression efficiently.

SSAO mRNA expression change

SSAO expression

SSAO upregulation may be a key to the damage of vascular endothelial cells in the presence of its substrates and contribute to the microvascular injury in diabetic complication. SSAO may be an intriguing therapeutic target for diabetic nephropathy and chronic kidney diseases. UD-014 may be a useful agent directly protecting the kidney from oxidative stress.

References