A novel dipeptidyl peptidase-IV inhibitor gemigliptin has Anti-inflammatory effects in Endothelial cells and Monocytic cells via Akt- and AMPK-dependent mechanisms

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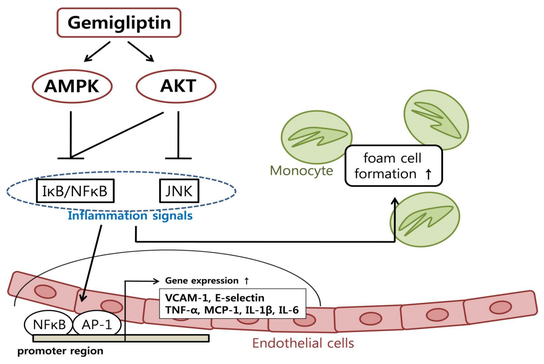
Graphic Abstracts



Objective

Dipeptidyl peptidase-IV (DPP-IV) inhibitors have received attention as a therapeutic target of inflammatory diseases such as atherosclerosis. DPP-IV inhibitors were generally considered as inflammatory modulators, because they can prevent the glucagon-like peptide-1 (GLP-1) degradation by enzymatic action of DPP-IV. In experiment, we investigated the effects of gemigliptin, which is a new DPP-IV inhibitor, in regard to endothelial dysfunction.

Introduction

Atherosclerosis is a chronic inflammatory disease of the artery walls that originates from the interaction between endothelial cells, lipoproteins, and inflammatory cells, leading to the formation of plaque. Although cardiovascular disease is the main cause of mortality in patients with diabetes, there are no effective strategies to prevent the progression of atherosclerosis through modulation of the inflammatory process.

DPP-IV inhibitors are a novel therapeutic option for patients with type 2 diabetes. Their pharmacologic action is based on reduced cleavage of GLP-1 by DPP-IV, thus preserving the insulinotropic action of this peptide. Although DPP-IV inhibitors mainly regulate blood glucose, the wide distribution of DPP-IV in the cardiovascular system, including in the endothelium, coronary smooth muscle cells, and cardiomyocytes, suggests its potential role in cardiovascular diseases.

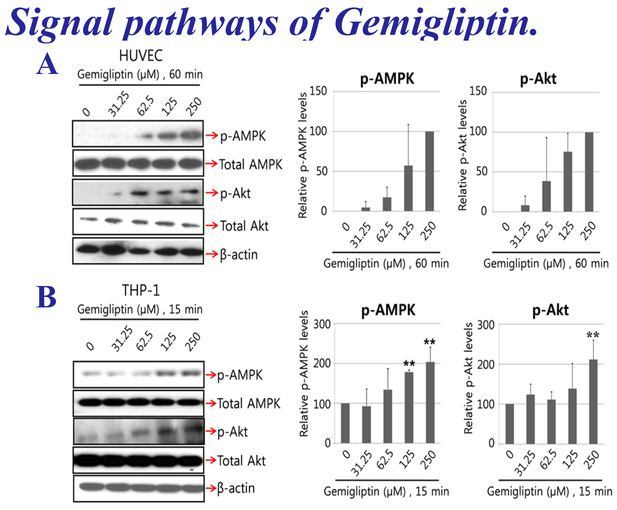
Methods

Foam cell formation and Oil red O staining; THP-1 cells were incubated with PMA, and then stimulated with LDL, LPS, and other additives. Lipid accumulation was analyzed using Oil red O solution.

Adhesion ability of HUVECs to monocytic THP-1 cells; HUVECs were stimulated with LPS, gemigliptin, and other additives. THP-1 cells were incubated with BCECF/AM, green fluorescence. They were co-incubated, and the levels of attached THP-1 cells were analyzed using a fluorescence microscope.

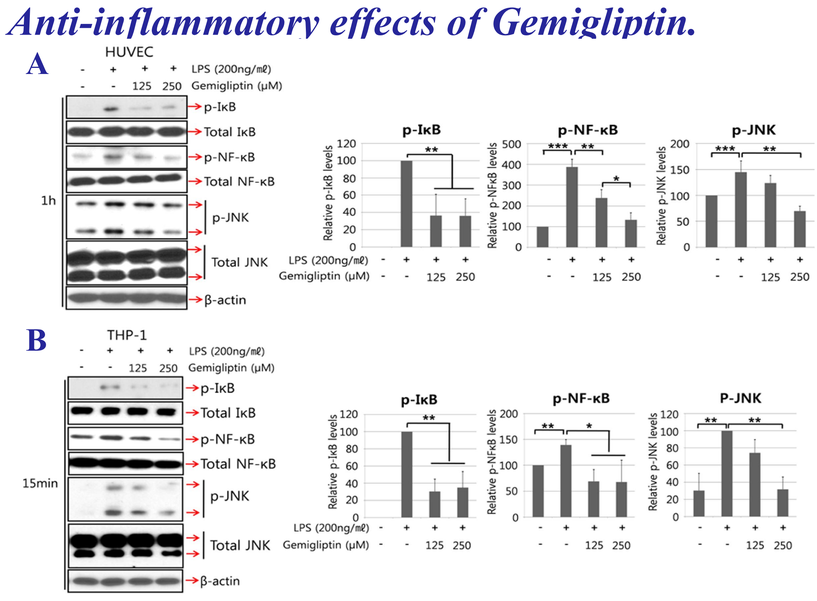
Results

Signal pathways of Gemigliptin.



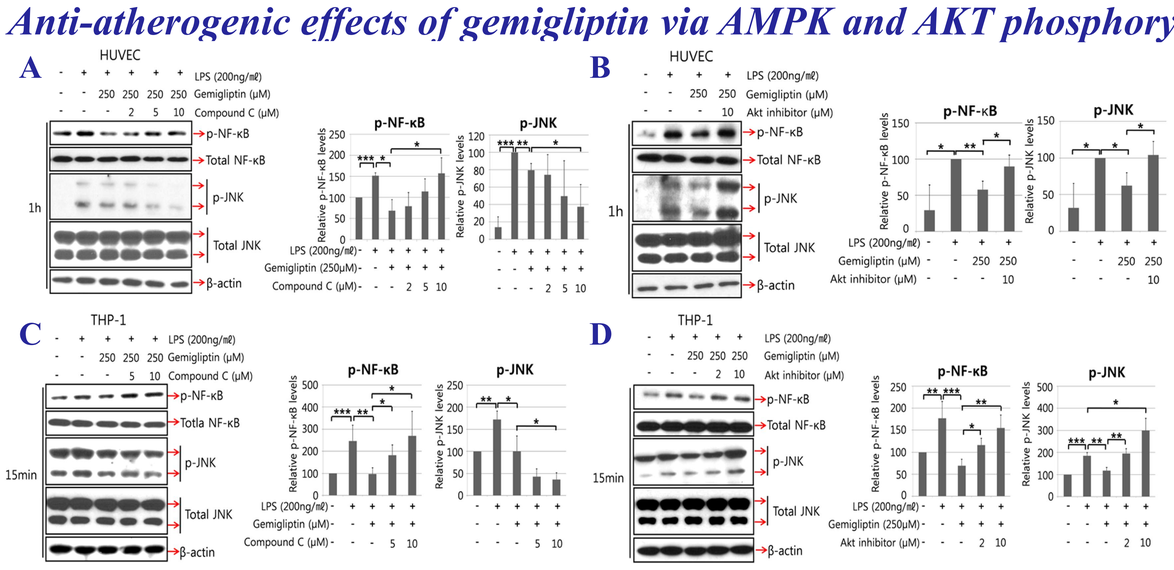
Gemigliptin importantly induced AMPK and Akt phosphorylation in a dose defendant manner. (A) HUVECs and (B) THP-1 cells were stimulated with various doses of gemigliptin for indicated times, and analyzed for phosphorylated AMPK and AKT level by Western blotting. Graphs were obtained from three separate experiments. Error bars represent mean ± SD (* $P < 0.05$, ** $P < 0.005$, ANOVA).

Anti-inflammatory effects of Gemigliptin.



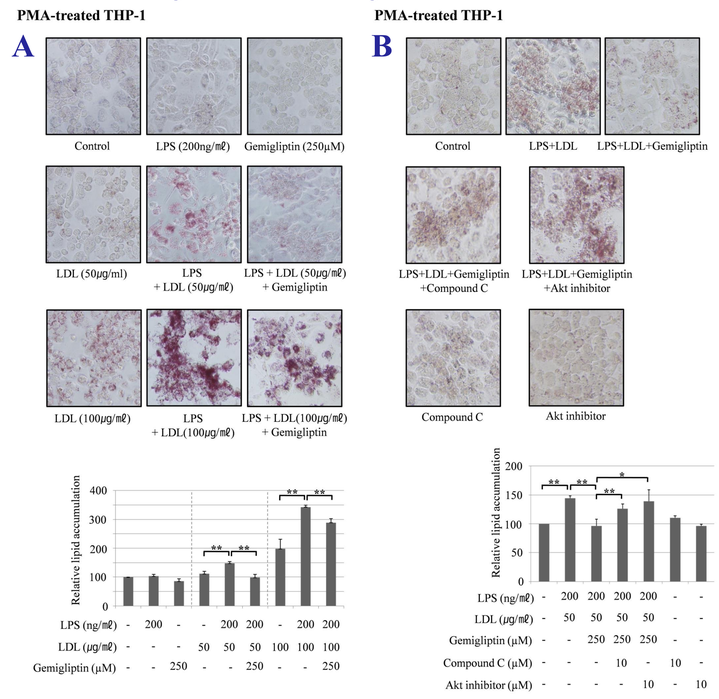
Gemigliptin significantly inhibited LPS-induced NF-κB and JNK phosphorylation, which are represent atherogenic pathways. (A) HUVECs and (B) THP-1 cells were stimulated with LPS or/and gemigliptin, and analyzed for phosphorylated IκB, NF-κB and JNK levels by Western blotting. Graphs were obtained from three separate experiments. Error bars represent mean ± SD (* $P < 0.05$, ** $P < 0.005$, ANOVA).

Anti-atherogenic effects of gemigliptin via AMPK and AKT phosphorylation.



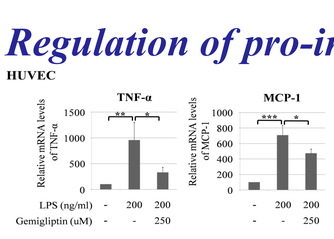
Gemigliptin markedly reduced LPS-mediated pro-inflammatory signaling through AMPK and Akt activation. (A and C) HUVECs and THP-1 cells were treated with LPS and gemigliptin with or without compound C. (B and D) The cells were incubated with LPS and gemigliptin with or without Akt inhibitor, and analyzed by Western blotting using antibodies against p-NF-κB and p-JNK. Graphs were obtained from three separate experiments. Error bars represent mean ± SD. (* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ANOVA).

Foam cell formation of THP-1 cells.



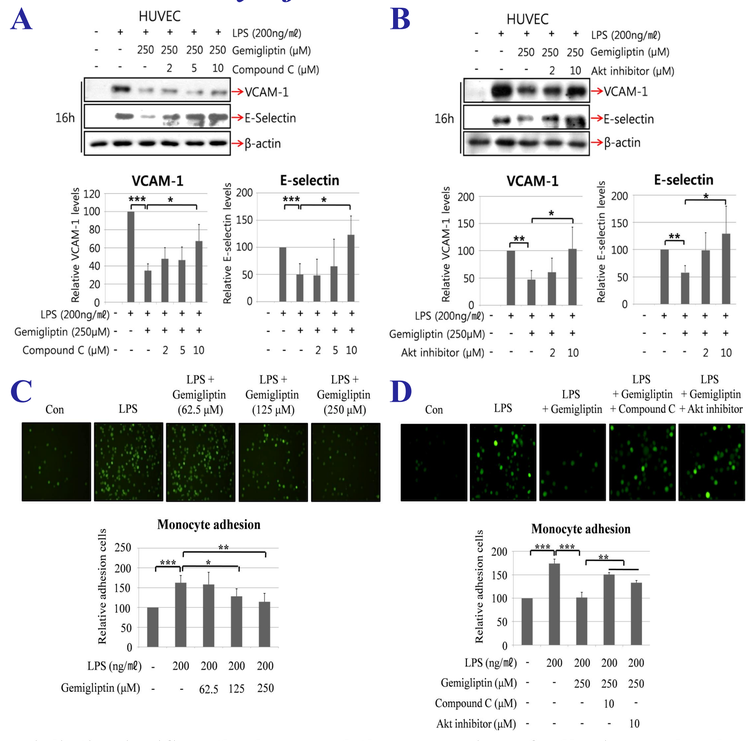
Gemigliptin efficiently inhibited LPS- and LDL-mediated form cell formation in PMA-treated THP-1 cells. (A and B) Lipid accumulation was measured by Oil red-O staining. Results were obtained from six separate experiments. Error bars represent mean ± SD. (* $P < 0.05$, ** $P < 0.005$, ANOVA).

Regulation of pro-inflammatory cytokines.



Gemigliptin blocked LPS-mediated induction of transcript levels for pro-inflammatory cytokines such as TNF-α, MCP-1, IL-1β, and IL-6. Data were obtained from three separate experiments. Results were obtained from three separate experiments. Error bars represent mean ± SD. (* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$, ANOVA).

Adhesion ability of THP-1 cells to HUVECs.



Gemigliptin significantly decreased the expression of adhesion molecules and adhesion ability of THP-1 cells to HUVECs. (A and B) HUVECs were treated with LPS and gemigliptin with or without compound C or Akt inhibitor, and analyzed by Western blotting for VCAM-1 and E-selectin. (C and D) Attachment of labeled THP-1 cells were evaluated by fluorescence microscope and Spectrofluorometer. Graphs were obtained from three separate experiments. Error bars represent mean ± SD (* $P < 0.05$, ** $P < 0.005$, , *** $P < 0.0005$ ANOVA).

Conclusions

Gemigliptin inhibited LPS-induced inflammatory pathways in endothelial cells and monocytic cells through the AMPK and Akt activation. Therefore, gemigliptin might have protection effects for vascular endothelium against inflammatory diseases such as atherosclerosis.

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