# COMP-Angiopoietin-1 ameliorates streptozotocin-induced renal inflammation

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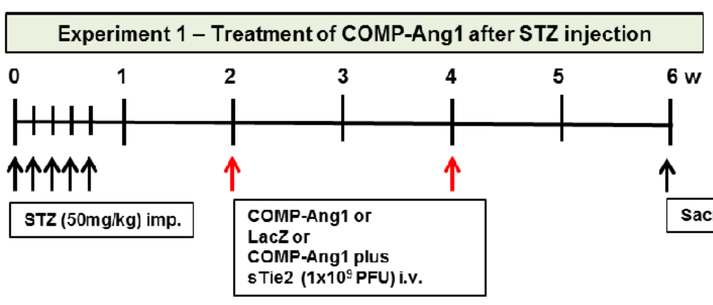
## Abstract

### INTRODUCTION AND AIMS

An inflammatory process is commonly seen as underlying the pathogenesis of diabetic nephropathy. Streptozotocin (STZ)-induced diabetes elevated inflammatory immunity.
Angiopoietin-1 (Ang1) plays essential roles in regulating vascular growth, development, maturation, permeability, and inflammation. We have developed a soluble, stable, and potent Ang1 variant, cartilage oligomeric matrix protein (COMP)-Ang1. However, Effects of COMP-Ang1 in STZ-induced renal inflammation remain to be clarified.

### METHODS




In this study, Streptozotocin-induced diabetes mice were treated with recombinant adonovirus expressing either COMP-Ang1 or LacZ or COMP-Ang1 plus sTie2. We evaluated inflammatory molecule such as intercellular adhesion molecule (ICAM)-1, vascular adhesion molecule (VCAM)-1, monocyte/macrophage infiltration and signaling pathway.

### RESULTS

COMP-Ang1 reduced renal expression of ICAM-1 and VCAM-1. COMP-Ang1 suppressed MCP-1 expression and macrophage infiltration in STZ-induced diabetes mice in kidney tissue. COMP-Ang1 also diminished nuclear factor-kB expression. COMP-Ang1 regulated Akt phosphorylation in the kidney tissue.

### CONCLUSIONS

These results demonstrate that COMP-Ang1 treatment can provide a novel therapeutic approach for STZ-induced inflammation through regulation of inflammatory molecule expression in renal.

## Results

### COMP-Ang1 decreases STZ-induced renal ICAM-1 protein expression

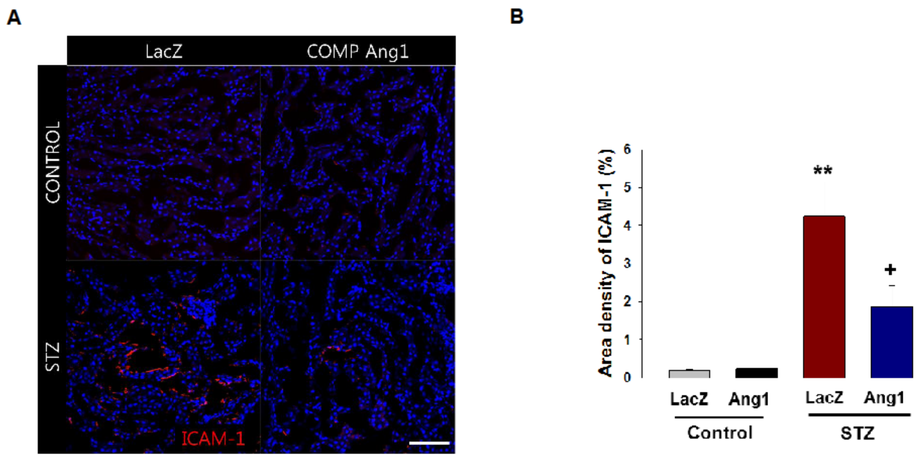



**Figure 1. Effects of COMP-Ang1 on the protein expression of ICAM-1 in STZ-induced mice.** (A) Immunofluorescence study of ICAM-1 in kidney. Kidneys were harvested at 6wk after STZ injection. Tissues were fixed in 4% formaldehyde solution and kidney sections were then stained with ICAM-1 antibody. Scale bar = 100 μm. (B) Quantitative score of ICAM-1 in kidney. Bar graph shows the area density of the positively stained area to the tatal field (0.22μm$^2$). *n* = 5 for each experimental group. Data are expressed as mean ± SD. **, *P* < 0.01 versus control buffer+ LacZ-treated mice; +, *P* < 0.05 versus STZ+LacZ-treated mice.

### COMP-Ang1 decreases STZ-induced renal ICAM-1 and VCAM-1 protein expression

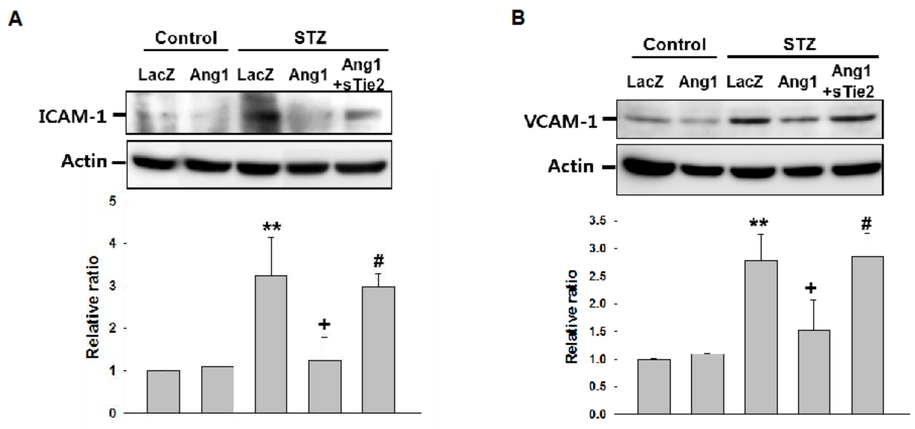



**Figure 2. Immunoblot analyses of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in kidney.** Kidneys were harvested 6 wk after STZ or control buffer injection. Mice were pretreated with 1 x 10$^8$ PFU Ade-sTie2-Fc 24 h before treatment with 1 x 10$^8$ PFU Ade-COMP-Ang1. Blots were probed with an anti-ICAM-1 or VCAM-1 antibody. The membrane was stripped and reprobed with an anti-Actin antibody to control for protein loading in each lane. Results were similar from six independent experiments. Densitometric analyses are presented as the relative ratio of ICAM-1 or VCAM-1 to Actin. The relative ratio measured in kidneys treated with control buffer plus LacZ is arbitrarily presented as 1. Results from three independent experiments were similar. Data are expressed as mean ± s.d. **, *P*<0.01 versus control buffer plus vehicle; +, *P* < 0.05 versus STZ plus LacZ-treated mice. #, *P*< 0.05 versus STZ plus COMP-Ang1.

### COMP-Ang1 suppresses STZ-induced F4/80-positive macrophage infiltration in the kidney

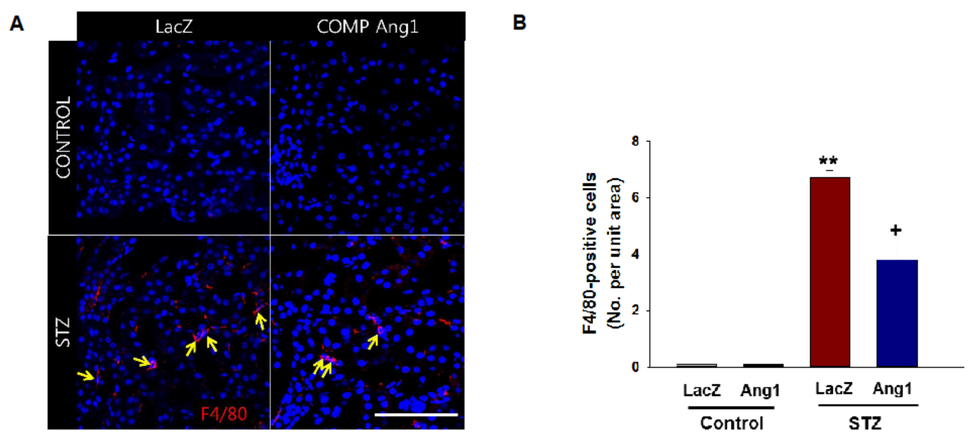



**Figure 3. Immunofluorescence study of F4/80-positive macrophages in kidney.**
(A) Immunofluorescence study of F4/80 in kidney. Kidneys were harvested at 6wk after STZ injection. Tissues were fixed in 4% formaldehyde solution and kidney sections were then stained with F4/80 antibody. Scale bar = 100 μm. (B) Quantitative score of F4/80 in kidney. Value is shown as number per high power field. *n* = 5 for each experimental group. Data are expressed as mean ± SD. **, *P* < 0.01 versus control buffer+ LacZ-treated mice; +, *P* < 0.05 versus STZ+LacZ-treated mice.

### COMP-Ang1 increases renal Akt phosphorylation through Tie2 in STZ mice

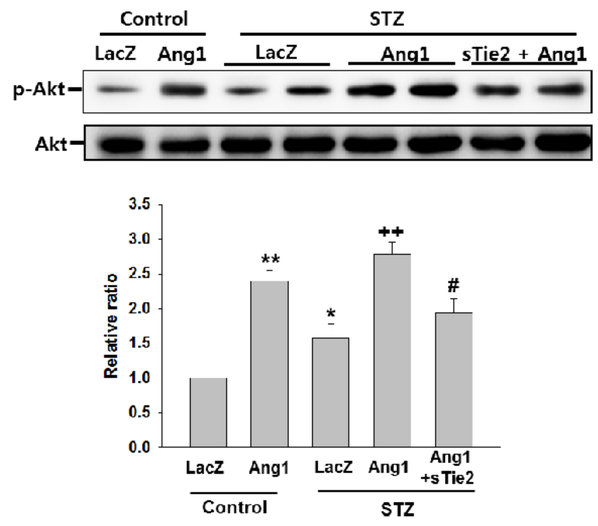



**Figure 4. Immunoblot analyses of Akt and phosphor-Akt from the kidneys of STZ-induced mice.** Kidneys were harvested 6 wk after STZ or control buffer injection. Mice were pretreated with 1 x 10$^8$ PFU Ade-sTie2-Fc 24 h before treatment with 1 x 10$^8$ PFU Ade-COMP-Ang1.
Blots were probed with an anti-phospho-Akt (p-Akt) antibody. The membrane was stripped and reprobed with an anti-Akt antibody to control for protein loading in each lane. Results were similar from six independent experiments. Densitometric analyses are presented as the relative ratio of p-Akt to Akt. The relative ratio measured in kidneys treated with control buffer plus LacZ is arbitrarily presented as 1. Results from three independent experiments were similar. Data are expressed as mean ± s.d. *,*P*<0.05 versus control buffer plus vehicle; **, *P*<0.01 versus control buffer plus vehicle; ++, *P* < 0.01 versus STZ plus LacZ-treated mice. #, *P*< 0.05 versus STZ plus COMP-Ang1.
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### COMP-Ang1 regulates the phosphorylation of p65 of nuclear factor kappa- B

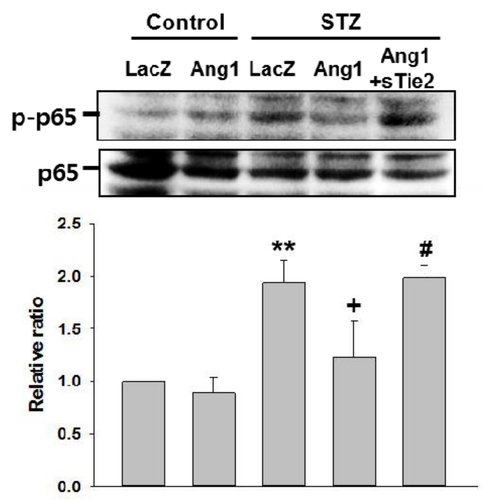


**Figure 5. A representative immunoblot photograph of phospho p65 expression in kidney after treatment of STZ.** Data from densitometric analyses of phospho p65 is presented as the relative ratio of each protein to p65. The relative ratio measured from control buffer plus LacZ treated mice is arbitarily presented as 1. Data are expressed as mean ± S.D. from three independent experiments. **, *p*<0.01 *versus* control buffer plus LacZ- treated mice; +, *p*< 0.01 versus STZ plus LacZ-treated mice; #, *P*< 0.05 versus STZ plus COMP-Ang1-treated mice.

## Conclusion

COMP-Ang1 treatment can provide a novel therapeutic approach for STZ-induced inflammation through regulation of inflammatory molecule expression in renal.

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