Uromodulin exerts potent preventive and therapeutic effects in WKY rats with anti-GBM glomerulonephritis via IL-6 activation

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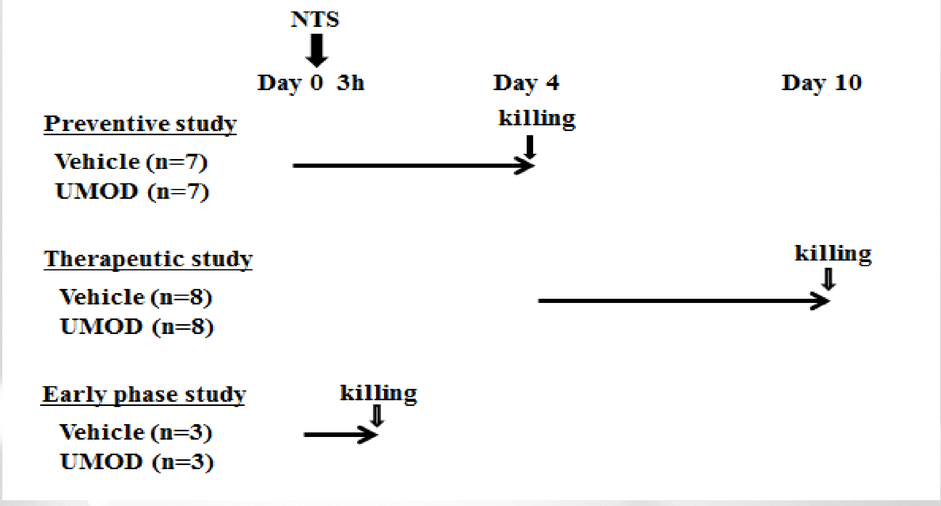
INTRODUCTION

Uromodulin (UMOD), also known as Tamm-Horsfall protein, is an 80-90kDa glycoprotein, isolated from the urine of pregnant women by Muchmore and Decker in 1985. UMOD is exclusively produced in the thick ascending limb (TAL) in Henle's loop. It was reported that administration of UMOD was highly immunogenic. Although there have been several reports that showed the immunosuppressive effects of UMOD on T cell and the ability of binding renal cytokines and lymphokines, other studies suggested pro-inflammatory roles of UMOD in activating neutrophils, monocytes, and dendritic cells, so the role of UMOD in inflammatory states remains unclear. IL-6 has been known as a pleiotropic cytokine. Anti-inflammatory effects of IL-6 have been reported, especially in rat glomerular basement membrane (GBM) glomerulonephritis model. In this model, IL-6 exerts pro-inflammatory effects by regulating macrophages activation. In this study, we showed that preventive and therapeutic effects of UMOD in rat GBM glomerulonephritis induced by NTS in WKY via IL-6 activation. Furthermore, we demonstrated its ability to stimulate production of IL-6 from normal human mesangial cells (NHMCs), human umbilical vein endothelial cells (HUVECs), THP-1 cells and human kidney cells (HK-2).

MATERIAL AND METHODS



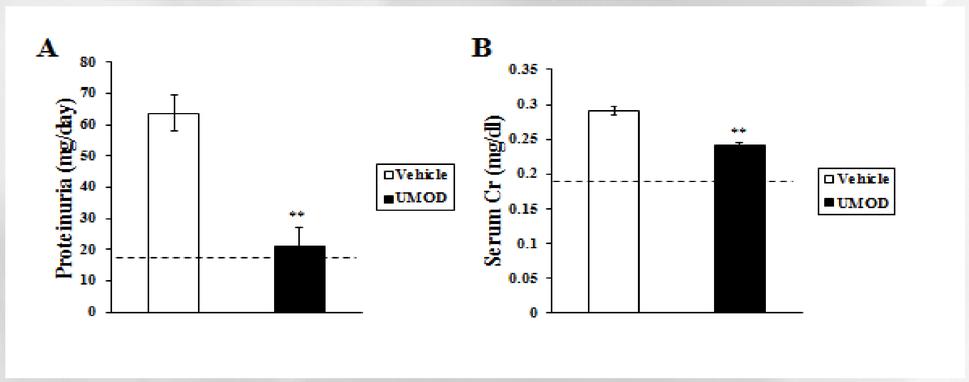
Experimental design.

(*in vivo*) A total of 36 female WKY rats were injected intravenously with 20μl of NTS on day 0. The rats in preventive study were given either UMOD (5μg/ml) or vehicle just before NTS injection and sacrificed on day 4. The rats in therapeutic study were given either UMOD (5μg/ml) or vehicle on day 4 and sacrificed on day 10. The rats in early phase study were given either UMOD (5μg/ml) or vehicle just before NTS injection and sacrificed 3 hours later inducing nephritis.

(*in vitro*) NHMCs, HUVECs, THP-1 and HK-2 were used Ex *vivo* investigation to estimate IL-6 producing property under UMOD. NHMCs in primary culture were purchased from Lonza (Basel, Switzerland), and HUVECs were purchased from PromoCell (Heidelberg, Germany). They were grown in DMEM/F12. THP-1 cells (JCRBO 0112.1) were obtained from the Human Science Research Resources Bank (HSRRB, Osaka, Japan) and were grown in RPMI 1640 medium with. All medium contained 10% FBS, 1% streptomycin-penicillin mixture, 44 mM $NaHCO_3$, and 14mM HEPES and cultured in an atmosphere of 5% CO_2-95% air at 37℃ in a humidified incubator. As the preemptive treatment, the medium adapted to each cell (setting as the control) or UMOD (1μg/ml or 5μg/ml,) was added to each well 1 hour before 10 μg/ml of TNF-α stimulation. After 24 hours culturing, the supernatant (arranged to 100μl/well), was taken, then IL-6 was measured by enzyme-linked immunosorbent assay (ELISA).

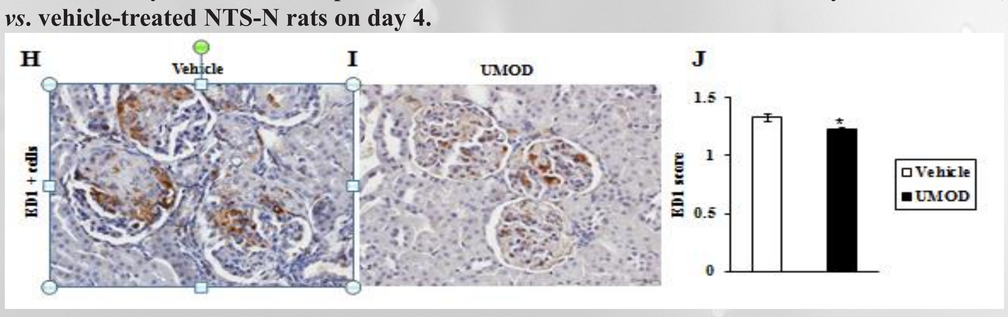
RESULTS

Preventive Study



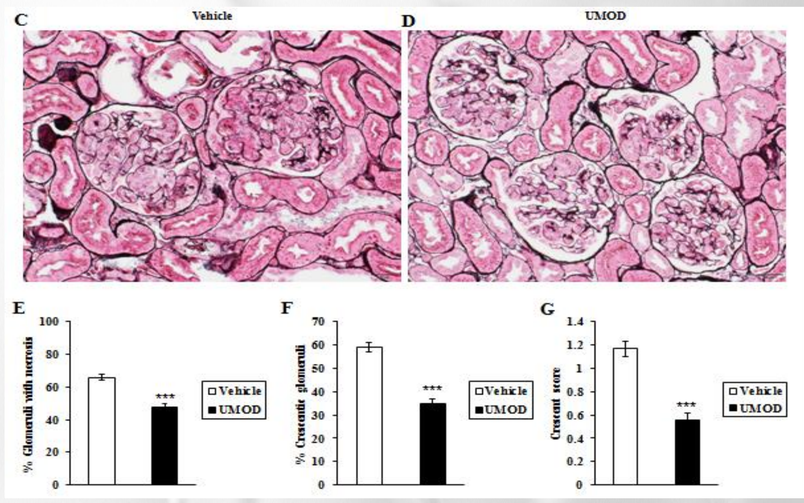
UMOD treatment reduces proteinuria and sCr level in preventive study.

(A) Proteinuria and (B) serum Cr level in rats with NTS-N treated with either vehicle or UMOD on day 4. Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{**}P<0.01$, *vs.* vehicle-treated NTS-N rats on day 4.



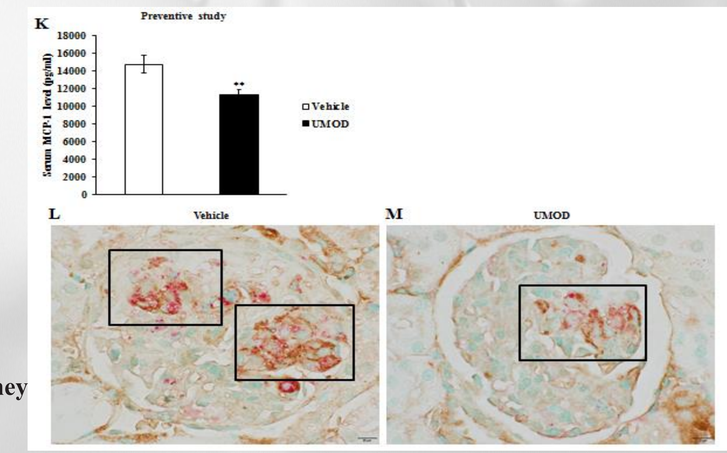
UMOD treatment blockades macrophages infiltration in preventive study.

(H and I) Immunohistochemistry for ED1 and (J) quantification of the cell accumulation in glomerular area. Each group contained 7 rats and 50 glomeruli per rat were evaluated in a blind manner. Original magnification, ×400. Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{***}P<0.001$, *vs.* vehicle-treated NTS-N rats on day 4.



UMOD treatment ameliorates histological findings in preventive study.

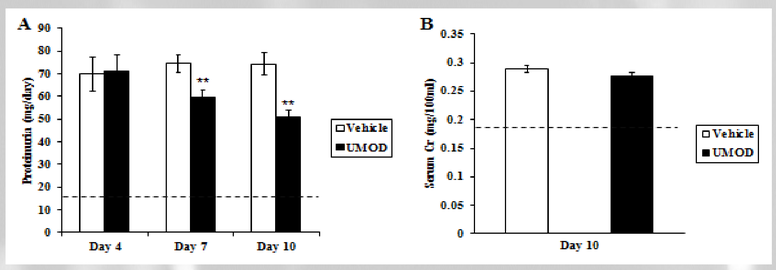
(C and D) Representative photographs of PAM-stained kidney section in each group. Original magnification, ×400. Quantification of the histological findings, including (E) % glomeruli with necrosis, (F) % crescentic glomeruli, and (G) crescent score. Each group contained 7 rats and 100 glomeruli per rat were evaluated in a blind manner. Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{***}P<0.001$, *vs.* vehicle-treated NTS-N rats on day 4.



UMOD treatment reduces serum MCP-1 and its expression located on ED1+ macrophages in renal cortex in preventive study.

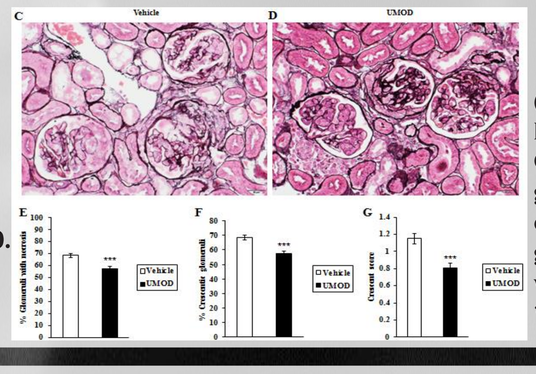
(K) Serum MCP-1 level in rats with NTN treated either vehicle or UMOD in preventive study. (L and M) Kidney sections were stained using two-color immunohistochemistry with ED1 (red) and MCP-1 (brown) in rats with NTS-N treated with either vehicle or UMOD in preventive study. Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{**}P<0.01$ *vs.* vehicle-treated NTS-N rats on day 4. Original magnification, ×1000.

Therapeutic Study



UMOD treatment reduces proteinuria in therapeutic study.

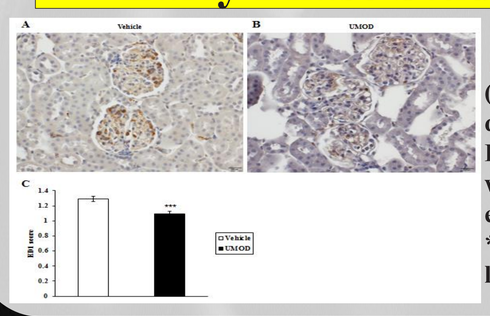
(A) Proteinuria in rats with NTS-N treated with either vehicle or UMOD on day 4, 7 and 10. (B) Serum Cr level in each group on day 10. Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{**}P<0.01$, *vs.* vehicle-treated NTS-N rats on day 10.



UMOD treatment ameliorates histological findings in therapeutic study.

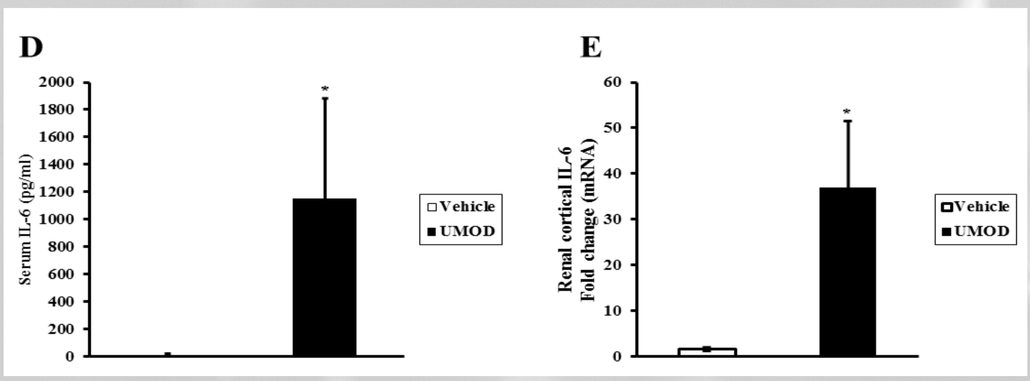
(C and D) Representative photographs of PAM-stained kidney section in each group Original magnification, ×400. Quantification of the histological findings, including (E) % glomeruli with necrosis, (F) % crescentic glomeruli, and (G) crescent score. Each group contained 8 rats and 100 glomeruli per rat were evaluated in a blind manner. Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{***}P<0.001$, *vs.* vehicle-treated NTS-N rats on day 10.

Early Phase Study



UMOD treatment blockades macrophages infiltration in rats with NTS-N in early phase study.

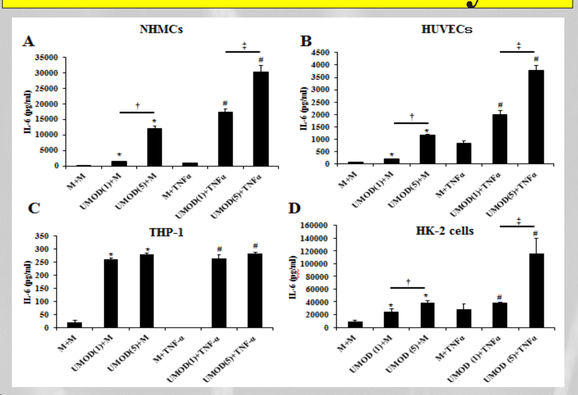
(A and B) Immunohistochemistry for ED1 and (C) quantification of cell accumulation in glomerular area. Each group contained 3 rats and 50 glomeruli per rat were evaluated in a blind manner. Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{***}P<0.001$ *vs.* vehicle-treated NTS-N rats 3 hours later inducing NTN. Original magnification, ×400.



UMOD treatment increases serum IL-6 level and its expression in kidney cortex in early phase study.

(D) Serum IL-6 level and (E) its expression in kidney cortex (RT-PCR).Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{*}P<0.05$, *vs.* vehicle-treated NTS-N rats 3 hours later inducing NTN.

Cell Culture Study



UMOD treatment stimulates IL-6 excretion from NHMCs, HUVECs THP-1 cells. and HK-2 cell.

Supernatant IL-6 level in (A) NHMCs, (B) HUVECs, (C) THP-1 cells, and (D) HK-2 cells. Data were expressed as Mean ± S.E.M. The Mann-Whitney test: $^{*}P<0.05$, cultures pretreated with UMOD *vs.* control. #$P<0.05$, cultures pretreated with UMOD and stimulated by TNF-α *vs.* cultures stimulated by TNF-α. †$P<0.05$, cultures pretreated with UMOD 5 μg/ml *vs.* 1 μg/ml, each culture was stimulated by medium. ‡ $P<0.05$, cultures pretreated with UMOD 5 μg/ml *vs.* 1 μg/ml, each culture was stimulated by TNF-α.

Summary

- UMOD remarkably decreased production of proteinuria and ameliorated histological findings in preventive and therapeutic studies.
- Throughout this study, UMOD blockaded macrophages infiltration into glomerular area.
- IL-6 has been known to have therapeutic effects on rat NTN model by regulating macrophages. In early phase study, serum IL-6 level and its expression in kidney cortex were increased dramatically.
- UMOD exerted the ability to stimulate production of IL-6 from NHMCs, HUVECs, THP-1 cells and HK-2.

CONCLUSION

We demonstrated that UMOD induced impressive reduction of proteinuria and glomerular macrophages infiltration in experimental anti-GBM GN, which might be mediated by systemically and locally in kidneys enhanced IL-6 that has been recognized to have anti-inflammatory effects in this model of GN.

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