UD-014, a novel, selective, orally available, long-acting vascular adhesion protein-1 inhibitor improves albuminuria in a streptozotocin (STZ)-induced diabetic nephropathy model in rats

Masaru Shinohara, Koji Itoh, Tetsuo Kawaguchi, Hiroyoshi Kawada, Akishi Ninomiya, Kenichi Komori, Noriaki Iwase and Shigeru Ushiyama, Pharmaceuticals Research Laboratory, Ube Industries, Ltd., Ube

UBE INDUSTRIES,LTD. 120th Anniversary

1. Abstract

Vascular adhesion protein-1 (VAP-1)/semicarbazide-sensitive amine oxidase (SSAO) is recognized to increase in plasma of patients with inflammation-associated diseases, and is considered a potential therapeutic target for various inflammatory diseases, including diabetic complication.

UD-014 is a novel, potent and orally active SSAO/VAP-1 inhibitor with an extended duration of action. The aim of this study is to investigate efficacy of UD-014 on kidney function in a STZ-induced diabetic nephropathy model in rats. UD-014 inhibited recombinant human SSAO/VAP-1 potently and highly selectively with an IC50 value of 3.2 nM. When orally administrated at single doses (0.1-10 mg/kg) to normal SD rats, UD-014 inhibited plasma SSAO/VAP-1 activity dose-dependently up to 24 hrs. SD rats were subjected to type-1 diabetes by an i.v. injection of streptozotocin (50 mg/mL/kg) and orally administered with UD-014 (1 and 3 mg/kg) once daily for 4 weeks after STZ treatment. Urinary albumin and urinary L-FABP, a marker of proximal tubule disorder and oxidative stress, were reduced significantly by UD-014 treatment. SSAO/VAP-1 activity in the plasma was measured on the last day. SSAO/VAP-1 activity increased about 2-fold in the STZ rats compared to the control rats. UD-014 inhibited the elevated plasma SSAO/VAP-1 activity dose-dependently. We also observed that SSAO/VAP-1 was expressed in the glomeruli in the kidney using immunofluorescence staining, and was co-localized with α-SMA staining, a marker of transformed mesangial cells.

These results suggest that the SSAO/VAP-1 inhibitor, UD-014 has a potential for a therapeutic agent to ameliorate kidney functions in diabetic nephropathy.

2. In vitro Pharmacological profile ~Potency and Selectivity~

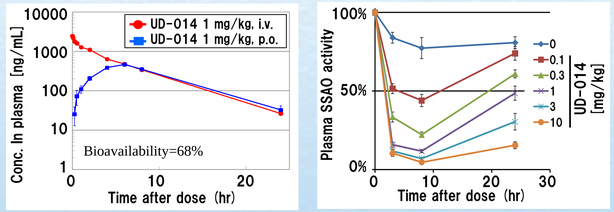
IC_{50} [nM]	Human recombinant			
	SSAO/VAP-1	MAO-A	MAO-B	DAO
UD-014	3.2	6,100	12,000	690
Mofegiline	5.2	550	0.51	ND
Clorgyline	ca.10,000	2.6	4,100	ND
Pargyline	ca.10,000	2,200	73	ND

▶ **UD-014 is a potent and selective inhibitor of SSAO/VAP-1.**

SSAO/VAP-1 activity was determined by a radio-enzymatic procedure using ^{14}C-labelled benzylamine as a substrate. MAO-A/B activity was measured by a fluorescence-based method, using non-labelled benzylamine.

3. PK and PD profile

(A) PK (1 mg/kg, iv, po) (B) Inhibition of plasma SSAO/VAP-1 activity

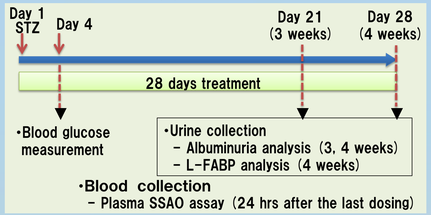


▶ **UD-014 is an orally available, long-acting inhibitor of SSAO/VAP-1.**

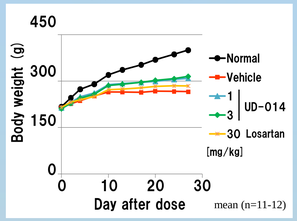
(A) UD-014 plasma concentration curve after administration (iv, po) at 1 mg/kg in SD rats. (B) Plasma SSAO activity after oral doses of 0.1-10 mg/kg in SD rats. SSAO activity was assayed by a radio-enzymatic procedure. Data are presented as the mean±S.D. of 3 animals.

4. Effect of UD-014 on kidney function in STZ-induced diabetic nephropathy model in rats

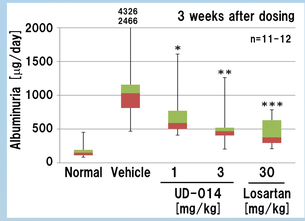
(A) Protocol



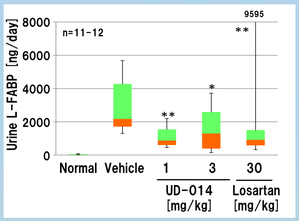
(B) Body weight



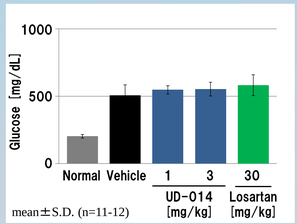
(D) Albuminuria (3-weeks)



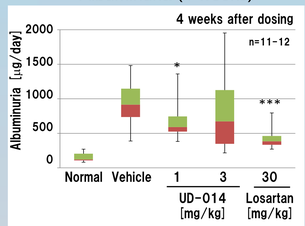
(E) Urine L-FABP (4-weeks)



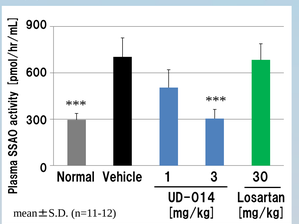
(C) Serum glucose



Albuminuria (4-weeks)



(F) Plasma SSAO/VAP-1 activity

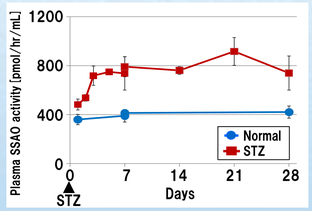


▶ **UD-014 improves kidney function at 1 and 3 mg/kg, q.d. without affecting the body weights and blood glucose.**

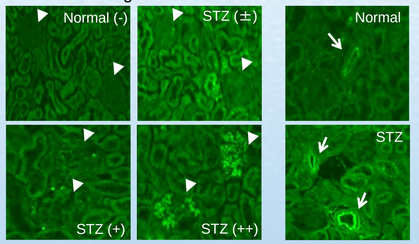
(A) Rats were iv injected with streptozotocin (STZ) on day 1 and administered orally with the compounds q.d. for 28 days. The rats with blood glucose levels of more than 350 mg/dl on day 4 were subjected to further experiments. (B) Body weight from day 1 to 27. (C) Serum glucose on the last day (D) Albuminuria 3 and 4 weeks after dosing. (E) Urine L-FABP, a marker of proximal tubule disorder, on the last day. (F) Plasma SSAO activity 24 hrs after the last dosing. (D,E,F) *p<0.05, **p<0.01, ***p<0.001.

5. SSAO/VAP-1 expression in STZ-induced diabetic nephropathy model in rats

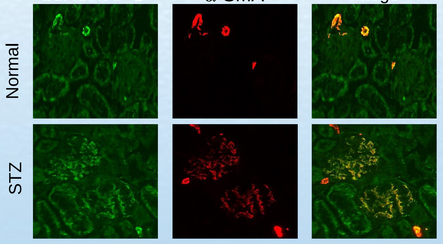
(A) Plasma SSAO/VAP-1 activity from day 1 to day 28 after STZ injection



(B) Expression of SSAO/VAP-1 in the kidney



(C) Identification of SSAO/VAP-1 upregulated cells



▶ **SSAO/VAP-1 activity increases in plasma and SSAO is upregulated in glomeruli in the kidney in STZ rats.**

(A) The time course of plasma SSAO/VAP-1 activity after STZ-injection. Data are presented as the mean±S.D. of 3 animals. (B) Immunofluorescent staining of SSAO in the kidney. The arrowhead indicate the glomeruli, and the arrows show the blood renal vessels. −: negative, ±: minimal, +:mild, ++: moderate staining (C) Double immunofluorescence staining of SSAO and α-SMA. α-SMA is used as a marker of transformed mesangial cells.

Conclusion

1. **UD-014 is a newly identified, orally available, potent, selective, and long-acting SSAO/VAP-1 inhibitor.**
2. **UD-014 improves kidney functional marker (albuminuria and L-FABP, a marker of proximal tubule disorder) at 1 and 3 mg/kg, q.d. without affecting the body weights and blood glucose in the STZ-induced diabetic nephropathy model in rats.**
3. **In the STZ model, SSAO activity increases in plasma and SSAO is upregulated in the glomeruli in the kidney.**

▼

These results suggest that UD-014 has a potential for a therapeutic agent to ameliorate kidney functions in diabetic nephropathy.

Please refer to 499-P for the MOA studies.

COI Disclosures, All authors: UBE Employees

Contact information: *Name: Shigeru Ushiyama* *E mail: 34752u@ube-ind.co.jp*

APPRISOR™