Topiroxostat showed the potent inhibition of plasma xanthine oxidoreductase (XOR) activity compared with other XOR inhibitor on type 2 diabetic mice

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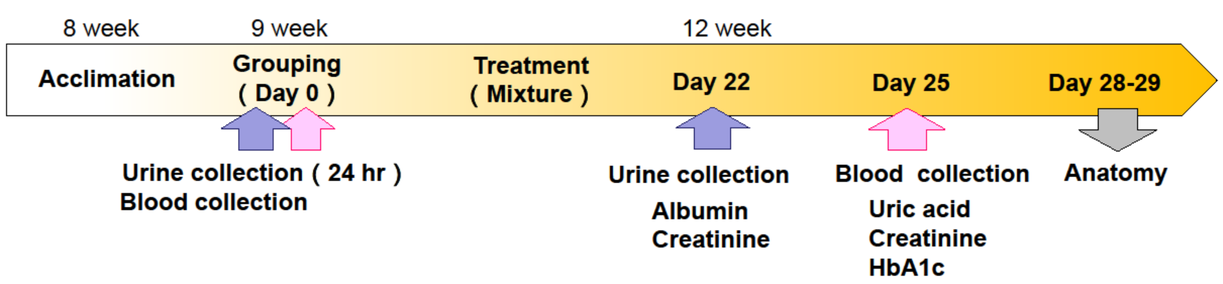
Background・Purpose

Topiroxostat, a xanthine oxidoreductase (XOR) inhibitor, has been approved in Japan as a medicine for hyperuricemia and goat. In the clinical trial, the decrease in urinary albumin to creatinine ratio was observed in hyperuricemic stage 3 chronic kidney disease patients compared with placebo.
The aim of this study is to investigate the involvement of the tissue or plasma XOR activity of type 2 diabetic mice in urinary albumin excretion (UAE) by means of topiroxostat and febuxostat.

Method

Animal Study Male db/db mouse 8 weeks old
(CHARLES RIVER LABORATORIES JAPAN, INC.)



Physiological data at 12-13 weeks of age

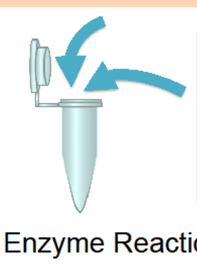
Group	Number	Body weight (g)	Kidney weight (g)	HbA1c (%)	Urine volume (mL)	BUN (mg/dL)	Urinary malondialdehyde (μmol/day)
db/m	4	27.5±1.8	0.34±0.02	3.5±0.1	0.30±0.1	28.7±1.4	–
db/db Control	7	40.7±1.0[a]	0.44±0.01[a]	9.4±0.2[a]	17.0±1.2[a]	29.4±1.4	0.148±0.01
Topiroxostat 0.1mg/kg	8	42.9±1.2	0.44±0.01	9.1±0.2	16.5±2.1	33.6±2.1	0.143±0.01
Topiroxostat 0.3mg/kg	8	41.4±0.5	0.46±0.01	9.2±0.2	16.9±1.5	31.8±2.2	0.137±0.01
Topiroxostat 1mg/kg	8	42.0±0.3	0.43±0.01	9.1±0.2	13.5±1.7	28.1±0.6	0.123±0.01
Topiroxostat 3mg/kg	7	41.0±1.0	0.41±0.01	9.0±0.2	14.4±0.9	29.9±2.7	0.109±0.01[c]
Febuxostat 0.1mg/kg	7	40.5±1.2	0.44±0.01	9.1±0.3	15.7±1.6	25.2±2.2	0.126±0.01
Febuxostat 0.3mg/kg	7	41.1±0.7	0.44±0.01	9.1±0.2	14.6±1.8	37.5±2.6[b]	0.133±0.01
Febuxostat 1mg/kg	7	40.6±0.8	0.45±0.01	9.6±0.3	14.5±0.9	26.0±1.8	0.140±0.01

Data are expressed as mean ± S.E.,
a ; p < 0.001 vs. db / m (t-test), b ; p < 0.05 vs. db / db Control, c ; 0.05 < p < 0.1 vs. db / db Control (Dunnett test)

XOR activity

Internal standard (IS) : Synthesized in house
$^{15}N_2$-Xanthine : Cambridge Isotope Lab.
LC / MS : LTQ-Orbitrap (Thermo Scientific)

Enzyme extracts (Liver cytosol, kidney cytosol or plasma of mouse)



- ✓ [$^{15}N_2$] - Xanthine
- ✓ NAD^+
- ✓ Oxonate
- ✓ Tris-HCl Buffer (pH 8.5)

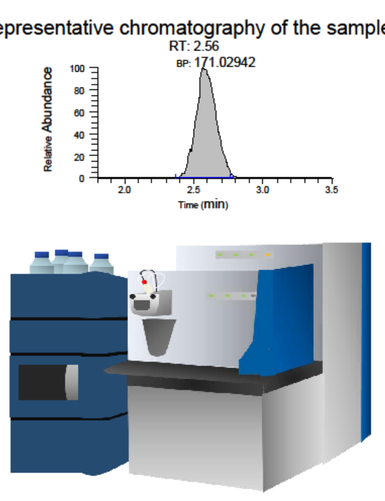
Enzyme Reaction for 30 min at 37℃

- ✓ [$^{13}C_2$,$^{15}N_2$] - Uric acid (IS)

Heated for 5 min at 95℃ to stop the reaction

Centrifuged for 10 min at 15,000 x *g*, 4℃

Supernatants were filtered through an ultrafiltration membrane → Quantification of [$^{15}N_2$] - Uric Acid with LC / MS



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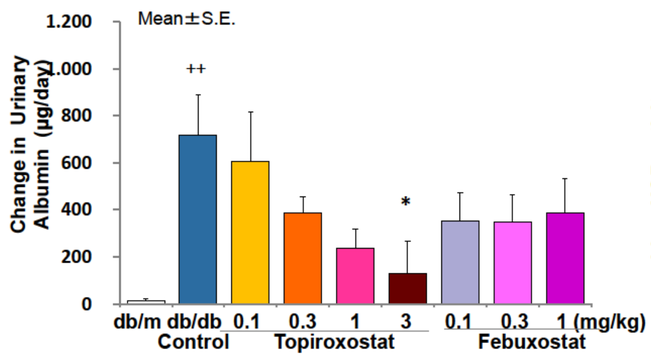
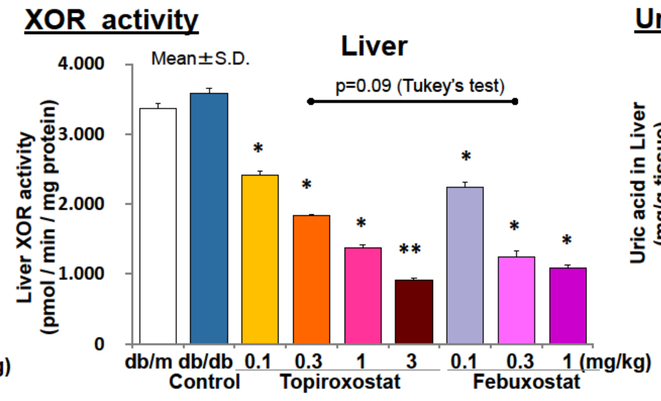
Results

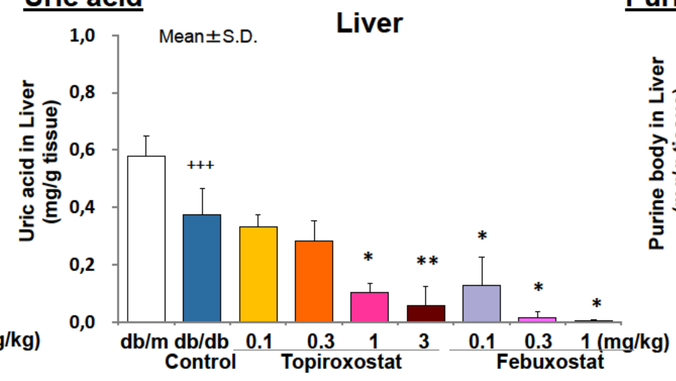
Change in albuminuria



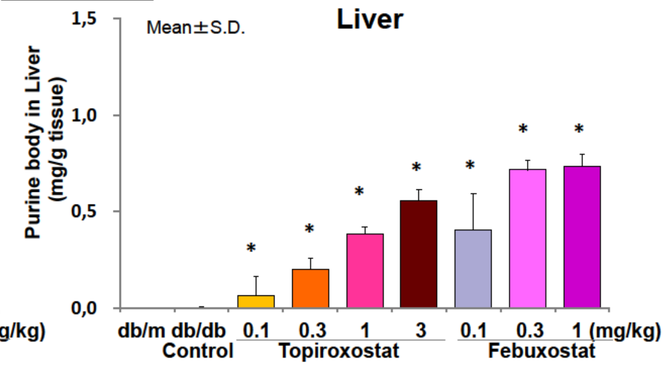
XOR activity



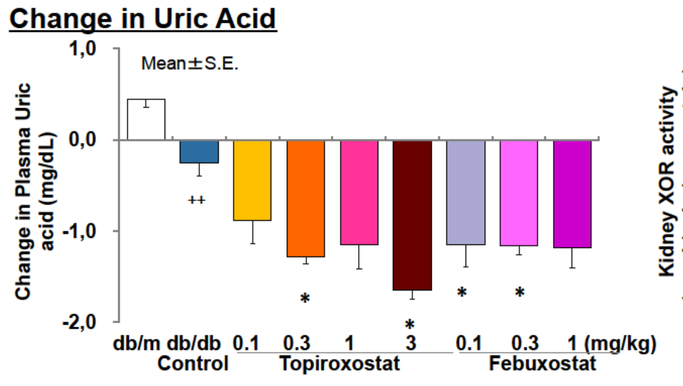
Uric acid

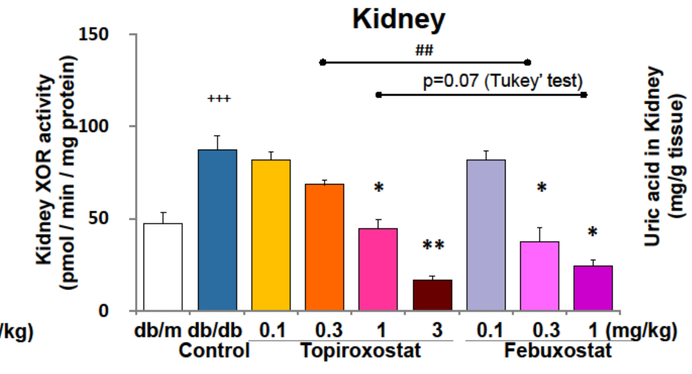


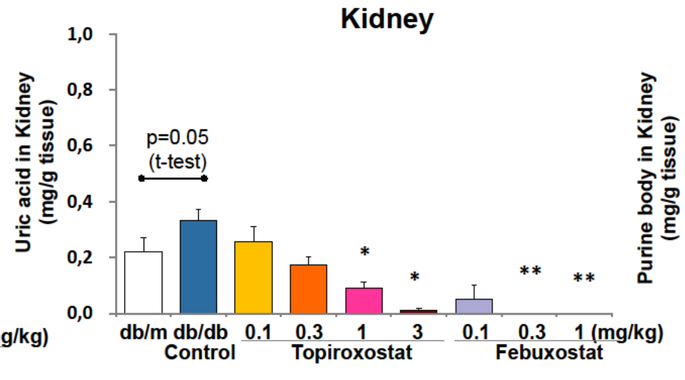
Purine body

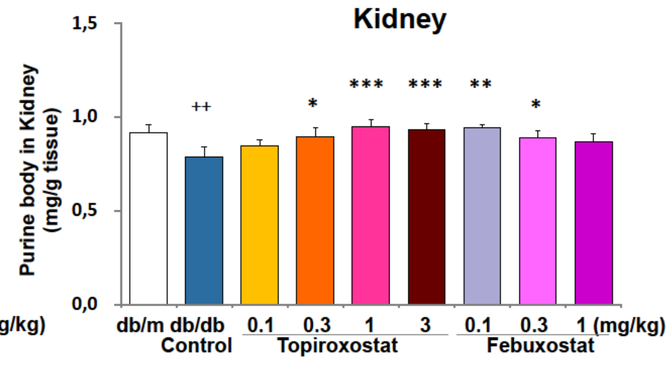


Change in Uric Acid

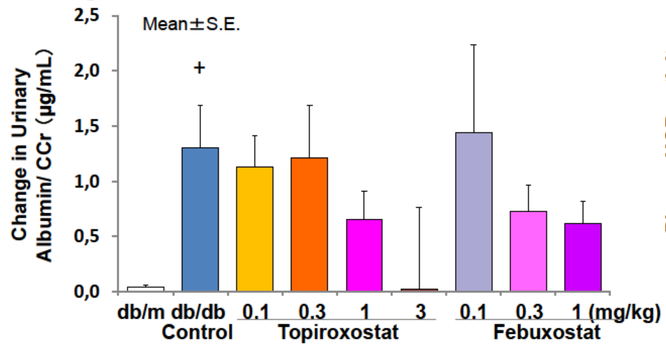


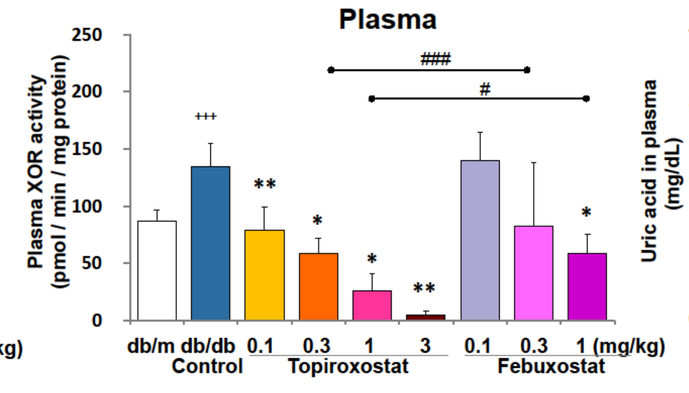


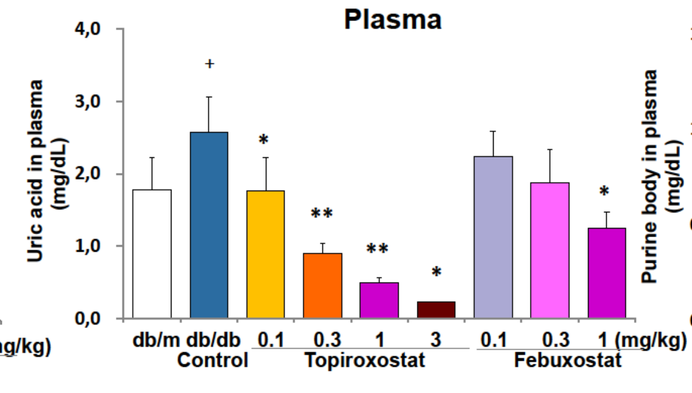


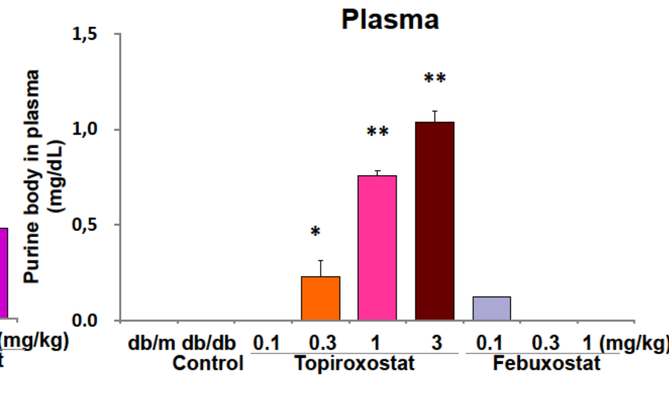


Change in albumin / GFR



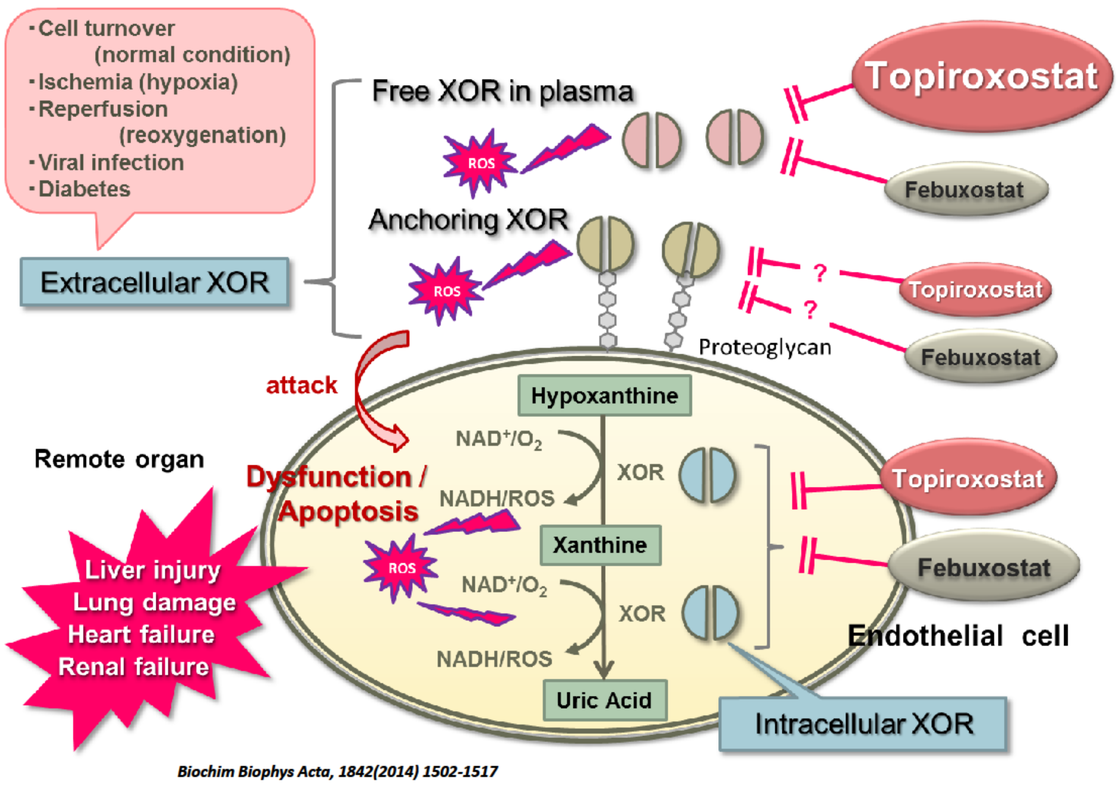






*+ ; p < 0.05, ++ ; p < 0.01, +++ ; p < 0.001 vs. db / m (t-test), * ; p < 0.05, **; p < 0.01,*** p ; < 0.001 vs. db / db control (Dunnett or steel test) , # ; p < 0.05, ## ; p < 0.01, ### ; p < 0.001 (Tukey's test)*

Discussion



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Summary

1. The elevated plasma and kidney XOR activity in db/db mice might contribute to the increase in UAE compared with db/m mice.
2. Topiroxostat, but not febuxostat, dose-dependently suppressed the UAE without depending on the GFR.
3. Topiroxostat more potently decreased uric acid level in plasma than febuxostat, while topiroxostat-induced XOR inhibition in the liver and kidney was less potent than that by febuxostat.
4. Topiroxostat dose-dependently increased the purine level in plasma, but febuxostat did not.
5. The results indicate that topiroxostat strongly inhibited the activity of the extracellular XOR, while febuxostat did against intracellular XOR.

Conclusion

In diabetic mice, topiroxostat showed reduced albuminuria, which might be mediated by plasma XOR inhibition.

The author has no conflict of interest to disclose with respect to this presentation.
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