Renoprotective effects of topiroxostat for hyperuricemic patients with overt diabetic nephropathy study (ETUDE Study): A prospective, randomized, multicenter clinical trial

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A Short running title: Topiroxostat for diabetic nephropathy

ABSTRACT

Aim: We aimed to evaluate the anti-albuminuric effects of topiroxostat in Japanese hyperuricemic patients with diabetic nephropathy.

Methods: In this 24-week, multicenter, open-label, randomized (1:1) trial, we assigned hyperuricemic patients with diabetic nephropathy (estimated glomerular filtration rate ≥ 20 mL/min/1.73m^2) and overt proteinuria (0.3 ≤ urine protein to creatinine ratio (UPCR) <3.5 g/g Cr) to either high dose (160 mg daily) or low dose (40 mg daily) topiroxostat. The primary endpoint was the change in albuminuria indicated by urine albumin-to-creatinine ratio (UACR) from the baseline at the final time point.

Results: A total of 80 patients underwent randomization. The changes in UACR after 24 weeks of treatment (or at the final time point if patients failed to reach 24 weeks) relative to the baseline were −122 mg/gCr (95% CI: −5.1 to −240.1, P = 0.041) in patients treated with high dose, while treatment with low dose topiroxostat could not show significant reduction (P = 0.067). In the linear mixed model including baseline albuminuria, eGFR, age, and sex as covariates, the decreases in UACR were still significant from baseline to 12 weeks by 228.7 ± 83.2 mg/gCr (P = 0.0075) in the high

dose group. The adverse-event profile during this study was not different between the groups.

Conclusion: Topiroxostat 160 mg daily reduced albuminuria in patients with diabetic nephropathy. (Funded by Sanwa Kagaku Kenkyusho; Trial registration, UMIN000015403)

Key words: albuminuria, diabetic nephropathy, randomized study, topiroxostat, xanthine oxidoreductase inhibitor

The proportion of patients with diabetes is continuously increasing worldwide[1]. Although proteinuria is a well-known risk factor for end-stage renal disease (ESRD) and cardiovascular disease (CVD) in patients with diabetic nephropathy[2], we have not yet developed a satisfying therapy to stop the progression of renal dysfunction and CVD.

Hyperuricemia is a common complication of metabolic syndrome that may be linked to diabetes and chronic kidney disease (CKD)[3]. There is some evidence demonstrating that uric acid (UA) contributes to the onset of and progression to CKD[4]. However, based on observational studies, it is controversial whether hyperuricemia is a risk factor[5] or just a marker[6] for renal outcomes. Xanthine oxidoreductase, enzyme in change of conversion from hypoxanthine to xanthine to uric acid, plays a physiologic role in generating oxidative stress that may in turn lead to endothelial dysfunction and deterioration of diabetic nephropathy[7]. Indeed, increased oxidative stress was identified in diabetes in both animal models and clinical studies and several studies have demonstrated that xanthine oxidoreductase inhibitors (XORis) have the potential to reduce oxidative stress and improve endothelial dysfunction in these patients group[8].

Moreover, renoprotective effect by lowering serum UA in CKD patients was challenging[9, 10], and although recent reports of interventions using XORis in CKD are promising[11], further study is necessary.

Topiroxostat, a selective XORi, was approved as a treatment for hyperuricemia and gout in Japan in 2013. Its efficacy in lowering uric acid levels has been established[12], and moreover, it has been demonstrated to decrease the urinary albumin-to creatinine ratio (UACR) compared to placebo in hyperuricemic and CKD stage 3 patients[13].

We investigated the anti-albuminuric effects of topiroxostat in Japanese hyperuricemic patients with diabetic nephropathy and overt proteinuria in the ETUDE trial (Effect of Topiroxostat on Urinary albumin in hyperuricemic patients with Diabetic nEphropathy)[14].

METHODS

Trial design and oversight

The trial design and methods of the ETUDE study have been described previously[14]. This trial was registered with the Japanese University Hospital Medical Information Network Clinical Trials Registry (UMIN 000015403, 10/10/2014). The protocol of the study was approved by the following ethical committees: Nagoya University Graduate School of Medicine (No. 2014-0160), Ogaki Municipal Hospital (No. 4), Kasugai Municipal Hospital (No. 189), and Chubu Rosai Hospital (No. 201411-01). This study was consistent with local and national regulations, Good Clinical Practice guidelines and the Declaration of Helsinki. All patients singed informed consent prior to entering the study. Briefly, the ETUDE study is a 24 week, multicenter, open-label, randomized (1:1), parallel group study comparing the effects of topiroxostat 160 mg daily with topiroxostat 40 mg daily, both added to standard care. Changes in type or dose of prior ACEI/ARB antihypertensive drugs and diuretics was restricted throughout the study. The main inclusion criteria were 1) diagnosis of diabetes, 2) hyperuricemia, 3) $0.3 \leq$ urine protein to creatinine ratio (UPCR) <3.5 g/g Cr, 4) estimated glomerular filtration

rate (eGFR) ≥20 mL/min/1.73 m^2. The stratifying factors were proteinuria (>0.5 g/g Cr or ≤0.5 g/g Cr), eGFR (>40 mL/min/1.73 m^2 or ≤40 mL/min/1.73 m^2), and the hospital at which the patients were treated.

Primary and secondary end points

The primary endpoint was the change in albuminuria indicated by the UACR after 24 weeks (or the final time point if patients failed to reach 24 weeks) of treatment relative to the baseline values. The secondary endpoints were changes in UACR, eGFR, blood pressure (BP), serum UA, glycosylated hemoglobin (HbA1c), and L-type fatty acid binding protein (L-FABP) at each time point.

Assessments of safety and adverse events

We evaluated any incidence of gouty arthritis to see whether there was a causative relationship to topiroxostat. Gouty arthritis was graded by the primary doctor by the severity of the symptoms. We also evaluated liver injury to detect increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (T-Bil) at

the final time point, and placed in three categories as shown in Table 3. All adverse events were also evaluated and we defined death, life-threatening complications, hospitalization, persistent or significant disability/incapacity, and inducing a congenital anomaly/birth defect as a severe adverse event in this trial.

Statistical analysis

We previously described the statistical analysis procedure used in this trial [14]. Briefly, for assessment of the primary outcome, we used a paired t-test to evaluate the decrease in UACR from baseline at the final time point. Secondary endpoints including changes in UACR, BP, HbA1c, eGFR, serum UA level, and urinary L-FABP from baseline were analyzed using linear-mixed effect models. Decreases in UACR, BP, HbA1c, eGFR, serum UA level, and urinary L-FABP were compared between treatment groups by using a Tukey-Kramer multiple-comparison test.

Results

Eighty Japanese patients were randomized in the trial from December 2014 to May 2016; 79 patients were included in the statistical analysis after the exclusion of one patient who was randomized but not treated because of gastric cancer. The flow diagram of this study is shown in Figure 1. Seventy patients completed the study with 24 weeks of follow-up, and 3, 4, and 2 patients completed 12, 8, and 4 weeks, respectively. The baseline data of the enrolled patients are shown in Table 1. The clinical characteristics and baseline laboratory data were similar in the two groups. The males (77.5%) were over represented as a result of enrolling in a sequence.

Primary end point: the changes in albuminuria from baseline at the final time point

The changes in UACR after 24 weeks (or at the final time point) relative to the baseline were −122 mg/gCr (95% CI: −5.1 to −240.1, $P = 0.041$) in patients treated with high dose, −201.4 mg/gCr (95% CI: 14.5 to −417.3, $P = 0.067$) in patients treated

with low dose, and −162.5 mg/gCr (95% CI: −41.13 to −283.8, $P = 0.009$) in all of the patients. These results show that a high dose of topiroxostat significantly reduces albuminuria.

Secondary end point; the changes in albuminuria and estimated glomerular filtration rate in a linear mixed model

The decreases in UACR in patients treated with high dose topiroxostat were estimated as 81.3 ± 75.9, 90.9 ± 67.7, 228.7 ± 83.2, and 125.7 ± 79.5 mg/gCr after 4, 8, 12, and 24 weeks, respectively, in the linear mixed model that included baseline albuminuria, eGFR, age, and sex as covariates (Figure 2A). A statistically significant difference was detected in UACR only for baseline to 12 weeks ($P = 0.0075$) in the high dose group. Meanwhile, UACR in patients treated with low dose topiroxostat were estimated as -34.0 ± 74.4 (an increase), 121.1 ± 66.0, 136.7 ±81.1, and 203.9 ± 77.1 mg/gCr after 4, 8, 12, and 24 weeks, respectively, in the same mixed model (Figure 2A). A statistically significant difference was detected in UACR from baseline to 24 weeks ($P = 0.001$) in

the low dose group. There was no significant difference in changes in UACR between the two dose groups from baseline to each time point.

During the treatment with topiroxostat, we detected a fluctuation in eGFR in both the high dose and low dose group (Figure 2B). The eGFR in patients treated with high dose topiroxostat decreased at 12 weeks by 2.4 ± 0.8 mL/min/1.73 m^2, but bottomed out at 24 weeks to a decrease of 1.7 ± 0.9 mL/min/1.73 m^2 from baseline (P = 0.078). Meanwhile, eGFR in patients treated with low dose topiroxostat decreased at 8 weeks by 1.8 ± 0.6 mL/min/1.73 m^2, earlier than in the high dose group, and bottomed out at 12 weeks to a decrease of 1.7 ± 0.8 mL/min/1.73 m^2 from baseline, and then recovered at 24 weeks to a decrease of 1.1 ± 0.9 mL/min/1.73 m^2 from baseline. A statistically significant difference was detected in eGFR from baseline to 12 weeks (P = 0.0041) in the high dose group, and at 8 and 12 weeks (P = 0.0078 and P = 0.034, respectively) in the low dose group. There was no significant difference in changes in eGFR between the two dose groups from baseline at any time point.

Secondary end point: systolic blood pressure and diastolic blood pressure

Table 2 shows the patients' systolic and diastolic BP during this study. Mild lowering effects on both systolic and diastolic BP were found with treatment with topiroxostat. A statistically significant difference was detected in systolic and diastolic BP from baseline to 12 and 24 weeks ($P = 0.037$ and $P = 0.0024$, respectively) in the high dose group, and to 4 and 12 weeks ($P = 0.0026$ and $P = 0.0002$, respectively) in the low dose group. There was no significant difference in change in systolic and diastolic BP between the two dose groups from baseline to each time point.

Secondary end point: uric acid, L-type fatty acid binding protein and hemoglobin A1c

Topiroxostat showed a steadily UA lowering effect (Figure 3A). The decreases in serum UA levels in patients treated with high dose topiroxostat were estimated to be 1.72 ± 0.18, 2.26 ± 0.31, 2.71 ± 0.62, and 3.3 ± 0.26 mg/dL after 4, 8, 12, and 24 weeks, respectively. A statistically significant difference was detected from baseline to all time points ($P < 0.0001$). The decreases in patients treated with low dose topiroxostat was more moderate than that in the high dose group, but were estimated to

be 1.84 ± 0.18, 1.82 ± 0.30, 1.02 ± 0.60, and 1.50 ± 0.26 mg/dL after 4, 8, 12, and 24 weeks, respectively. A statistically significant difference was detected from baseline to all time points except at 12 weeks ($P < 0.0001$). There was a significant difference in change in serum UA levels between the two dose treatment groups from baseline to 24 weeks ($P = 0.0001$).

We found that urinary L-FABP was reduced by treatment with topiroxostat (Figure 3B). The mean urinary L-FABP was 22.5 ± 23.3 μg/gCr. The decreases in urinary L-FABP in patients treated with high dose topiroxostat were estimated to be 10.13 ± 3.2 and 7.8 ± 1.7 μg/gCr after 12 and 24 weeks, respectively. A statistically significant difference was detected from baseline at both 12 ($P = 0.0021$) and 24 weeks ($P < 0.0001$). Meanwhile, decreases in urinary L-FABP in patients treated with low dose topiroxostat were estimated to be 5.39 ± 3.1 and 9.8 ± 1.6 μg/gCr after 12 and 24 weeks, respectively. A statistically significant difference was detected from baseline to 24 weeks ($P < 0.0001$). There was no significant difference in changes in urinary L-FABP between the two dose groups.

While the levels of HbA1c did not change in patients with low dose topiroxostat, there was a slight, but statistically significant decline in HbA1c levels in patients treated with high dose topiroxostat at 24 weeks (P = 0.050). There were significant differences in change in HbA1c levels between the two dose treatment groups from baseline to 8 weeks (P = 0.037) and 24 weeks (P = 0.023) (Figure 3C).

Safety and adverse events

Adverse events (AE) are listed in Table 3. All severe AE (SAE) were reported by individual attending doctors, and their causal relationship with topiroxostat was evaluated by the Safety Monitoring Committee. Two patients died during the study, and the causes of death were a traffic accident (high dose group) and a cerebral embolism (low dose group). The patient who had a cerebral embolism had suffered from atrial fibrillation before entry into this study. Although a neoplasm was detected in one patient who was allocated to the low dose group 12 weeks after starting the study, his gastric cancer was already advanced at diagnosis. None of the serious adverse events was assessed to be related to the study drugs. One case each of mild gouty arthritis, a

gastrointestinal disorder, and a cutaneous disorder occurred in the high dose group. There was the same rate of liver dysfunction in both groups.

Discussion

Topiroxostat is a selective XORi that approved in Japan in 2013, while it has not yet been available in the US or European countries. This is the first prospective, randomized, multicenter, and open-label clinical trial aiming to confirm the efficacy of topiroxostat to overt albuminuria in diabetic nephropathy. In this trial, we demonstrated that treatment with topiroxostat resulted in a reduction in albuminuria and in L-FABP. This reduction of albuminuria was independent from baseline UACR, age, sex, and baseline eGFR. We also found that topiroxostat treatment reduced HbA1c value and contributed to decrease of BP. Based on these findings, topiroxostat was expected to suppress UA and to prevent development of diabetic nephropathy.

There have been some clinical trials reporting the effects on renal outcomes of other XORi on renal outcomes[15-17]. Allopurinol treatment significantly lowered proteinuria with no differences in BP and serum creatinine [15] and slowed the progression of renal disease with no effect on proteinuria and BP[16]. Febuxostat decreased albuminuria along with L-FABP while lowering PB and eGFR[17]. Although further study is necessary, XORi may have a renoprotective effect on diabetic

nephropathy patients and our results now added the evidence of renoprotective effect by XORi.

To study the mechanism of improvement in albuminuria by XORi, Kosugi et al. demonstrated a significant decrease in albuminuria by allopurinol treatment concomitantly with suppression of tubulointerstitial injury and inhibition of infiltrate inflammatory cells in an animal model using type 2 diabetic *db/db* mice[18]. In another diabetic rat model, febuxostat treatment significantly decreased albuminuria and ameliorated infiltration of macrophages with reduced oxidative stress, transcript levels of inflammatory genes, inflammation-induced enzymes, and inflammatory mediators [19]. Interestingly, Nakamura et al. demonstrated that although both topiroxostat and febuxostat reduced UACR in *db/db* mice, only topiroxostat showed a dose-dependent and significant decrease[20]. They speculated that this superiority of topiroxostat to febuxostat was related to serum XOR levels[20]. The circulated XOR is trapped with proteoglycan in vascular endothelial cells, and then superoxide is generated locally. The superoxide is reacted with NO in intervascular and generated cytotoxic peroxinitrate. Circulating blood volume is reduced due to decrease in vascular endothelial dilatation

reaction through the elimination of NO. It is possible to evaluate that topiroxostat improved vascular endothelial function and affected systolic BP and renal blood flow as a result of inhibiting circulation XOR, moored XOR, and vascular endothelial XOR. In this study, we demonstrated that topiroxostat treatment was effective in both lowering blood pressure and decreasing albuminuria due to recovery of endothelial function. However, decreasing albuminuria was not always synchronous with lowering blood pressure. We speculated that the anti-albuminuric effect of topiroxostat may be in part independent of alteration of hemodynamics

In our trial, the eGFR showed statistically significant decrease with topiroxostat at 12 weeks and were recovered at 24 weeks. Araki et al. followed kidney function up to 12 years in type 2 diabetic patients with microalbunuria and normoalbuminuria [21]. In the result, the annual rate of decline in eGFR was −1.80 mL/min/1.73 m^2/year (−1.05 to −3.21). Moreover, the annual number of patient per 1,000 person for a 50% decline in eGFR from baseline and for the progression to CKD stage 4 were 18.3 and 11.1, respectively. In our trial, decline in eGFR from baseline were −1.7 ± 0.9 mL/min/1.73 m^2/24weeks in the low dose group and −1.1 ± 0.9

mL/min/1.73 m^2/24weeks in the high dose group in patients who were almost equivalent of CKD stage 4 with overt albuminuria. Makino et al. reported that the eGFR decreases dramatically when eGFR falls below CKD stage3 for the natural course of diabetic nephropathy[22]. These results suggested the possibility that the decrease in eGFR was delayed with topiroxostat.

Since urinary L-FABP is expressed due to tubulointerstitial damage and oxidative stress on proximal tubule, L-FABP plays an important role of monitoring progression of diabetic nephropathy. Ikemori et al. followed kidney function up to 4 years for type 2 diabetic patients with micro- to normoalbuminuria[23]. Urinary L-FABP accurately reflects severity of type 2 diabetic nephropathy, and level is high in the patients with normoalbuminuria[23]. In diabetic nephropathy, chronic ischemic condition near the proximal renal tubule is considered as one of the mechanisms of production of reactive oxygen species (ROS). It was speculated that urinary L-FABP increased with ROS which was generated with activation of XO in renal tubule due to a decrease in mitochondrial ATP production function, promotion of ATP catabolism and increase hypoxanthine. In adenine-induced renal injury model mice, topiroxostat treatment

attenuated tubulointerstitial damage while decreasing albuminuria and avoiding increases in serum creatinine level [24]. They also showed that mice treated with high doses of topiroxostat (3 mg/kg) had lower serum creatinine, urinary L-FABP, and renal XOR than the mice group treated with febuxostat (3 mg/kg) [24]. In this trial, topiroxostat treatment showed a significant reduction in albuminuria synchronized with a reduction in L-FABP. We believe that this anti-albuminuric effect is, in part, due to decreased tubulointerstitial injury from diabetic nephropathy in our patients. Moreover, since both the high and low dose groups showed a sufficient albuminuric lowering effect in linear models, the anti-albuminuric effect of topiroxostat may be independent of serum UA levels.

Some limitations of this trial should be noted. First, this trial had no placebo arm; thus, the efficacy/tolerability of this drug as compared to no treatment cannot be adequately assessed. Moreover, this was an open-label trial; therefore, patients and physicians might be influenced by the allocation. However, CKD patients are prone to hyperuricemia, gout, and nephrolithiasis[25] and hyperuricemia itself can induce renal damage[26]. Moreover, in Japan, therapy for asymptomatic hyperuricemia is approved and

generally accepted. Thus, we designed the trial as the present style in consideration of safety reasons. Second, to evaluate true renoprotective effect, an observation period of several years, at least, may be needed. However, because albuminuria predicts renal outcomes and temporary interventions for albuminuria contribute to preventing end-stage renal disease[27], it is worthwhile to reduce albuminuria in patients with diabetic nephropathy even for a short term.

In conclusion, in this interventional study, we demonstrated that topiroxostat had a steady anti-albuminuric effect among patients with type 2 diabetes and overt proteinuria who were at high risk for progressive diabetic nephropathy. Despite every active intervention for overt diabetic nephropathy had been performed, there is an issue of quick progression to end stage renal failure and dialysis. It is suggested that the selective XORi topiroxostat could inhibit the progression of overt diabetic nephropathy.

Acknowledgments

This study was supported by Sanwa Kagaku Kenkyusho, Japan. The authors are grateful to all members of the Safety Monitoring Committee: Enyu Imai, Yukio Yuzawa, and Ishii Hideki; the Clinical Research Coordinator: Keiko Aoyama and Mitsuyo Ito; and the Data Quality Management Group in the Center for Advanced Medicine and Clinical Research. We would like to thank Editage (www.editage.jp) for English language editing.

Conflict of Interest Statement

The Department of Nephrology, Nagoya University Graduate School of Medicine, report receiving research promotion grants from Astellas, Alexion, Otsuka, Kyowa Hakko Kirin, Takeda, Torii, Pfizer, and MSD. However, the research topics of these donation grants are not restricted. The Center for Advanced Medicine and Clinical Research, Nagoya University Hospital received a donation from Pfizer; however, the donation is not directly associated with this study.

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Figure Legends

Figure 1

Flow of the patients who enrolled in the ETUDE study.

Figure 2

Changes of urinary albumin creatinine ratio (A) and eGFR (B) from baseline to each time point. The *diamonds* represent the high dose topiroxostat group (160 mg/daily), and the *circles* represent the low dose topiroxostat group (40 mg/daily). †P <0.05, ††P <0.01

Figure 3

Changes of serum uric acid levels from baseline to each time point (A), urinary L-type fatty acid binding protein (L-FABP) from baseline to 12 and 24 weeks, (B) and HbA1c from baseline to each time point (C). The *diamonds* represent the high dose topiroxostat group (160 mg/daily), and the *circles* represent the low dose topiroxostat group (40 mg/daily).

*; P <0.05 between high dose and low dose topiroxostat group, **; P < 0.01. †; P < 0.05 from baseline to each time point. ††; P < 0.01

Table 1. Characteristics of the Patients at Baseline

	Topiroxostat high dose (160 mg daily)	Topiroxostat low dose (40 mg daily)
Number	40 (50.0)	40 (50.0)
Age (years)	69.8 ± 9.4	68.6 ± 12.0
Sex (male; %)	30 (75.0)	32 (80.0)
Body mass index	26.7 ± 4.2	26.3 ± 3.9
Blood pressure (mmHg)		
systolic blood pressure	135.5 ± 19.1	137.3 ± 18.1
diastolic blood pressure	74.0 ± 12.1	74.5 ± 13.0
Medications (N; %)		
ACE/ARBs	34 (85.0)	36 (90.0)
Diuretics	19 (47.5)	24 (60.0)
Antidiabetic drugs	39 (97.5)	38 (95.0)
Hemoglobin (g/dL)	12.8 ± 2.2	12.7 ± 1.9
BUN (mg/dL)	25.5 ± 8.7	26.9 ± 9.4
Serum creatinine (mg/dL)	1.42 ± 0.5	1.49 ± 0.5

eGFR (mL/min/1.73 m^2)	41.1 ± 15.5	39.7 ± 14.9
UA (mg/dL)	7.8 ± 1.6	8.4 ± 1.5
Total cholesterol (mg/dl)	168.6 ± 29.1	167.4 ± 38.1
HDL cholesterol (mg/dL)	47.8 ± 12.6	44.8 ± 14.3
LDL cholesterol (mg/dL)	87.8 ± 25.2	85.4 ± 31.4
Triglyceride (mg/dL)	179.3 ± 99.4	175.7 ± 117.2
Glucose (mg/dL)	145.5 ± 64.3	147.6 ± 45.5
HbA1c (%)	6.98 ± 0.9	6.88 ± 0.9
Albuminuria (mg/gCr)	757.9 ± 757.1	800.7 ± 923.8

Values are expressed as mean ± SD or numbers (%).

Abbreviations: ACE-I/ARBs; angiotensin converting enzyme inhibitor/angiotensin II receptor blockers, BUN; blood urea nitrogen, UA; uric acid, HDL, high-density lipoprotein; LDL, low-density lipoprotein

Table 2. Changes in Blood Pressure Levels

	SBP		DBP	
	Topiroxostat high dose	Topiroxostat low dose	Topiroxostat high dose	Topiroxostat low dose
Baseline	135.5 ± 19.1	137.3 ± 18.1	74.0 ± 12.1	74.5 ± 13.0
week 4	134.1 ± 17.3	130.7 ± 17.1*	72.3 ± 13.5	71.6 ± 12.7*
week 8	133.9 ± 19.0	133.1 ± 20.1	72.9 ± 13.5	72.9 ± 13.4
week 12	131.3 ± 15.2*	129.0 ± 17.6*	71.3 ± 12.7*	71.5 ± 13.5*
week 24	127.5 ± 15.5*	132.5 ± 20.2	71.0 ± 10.8*	73.4 ± 13.5

Values are expressed as mean ± SD. *; $P < 0.05$ from baseline to each time point.

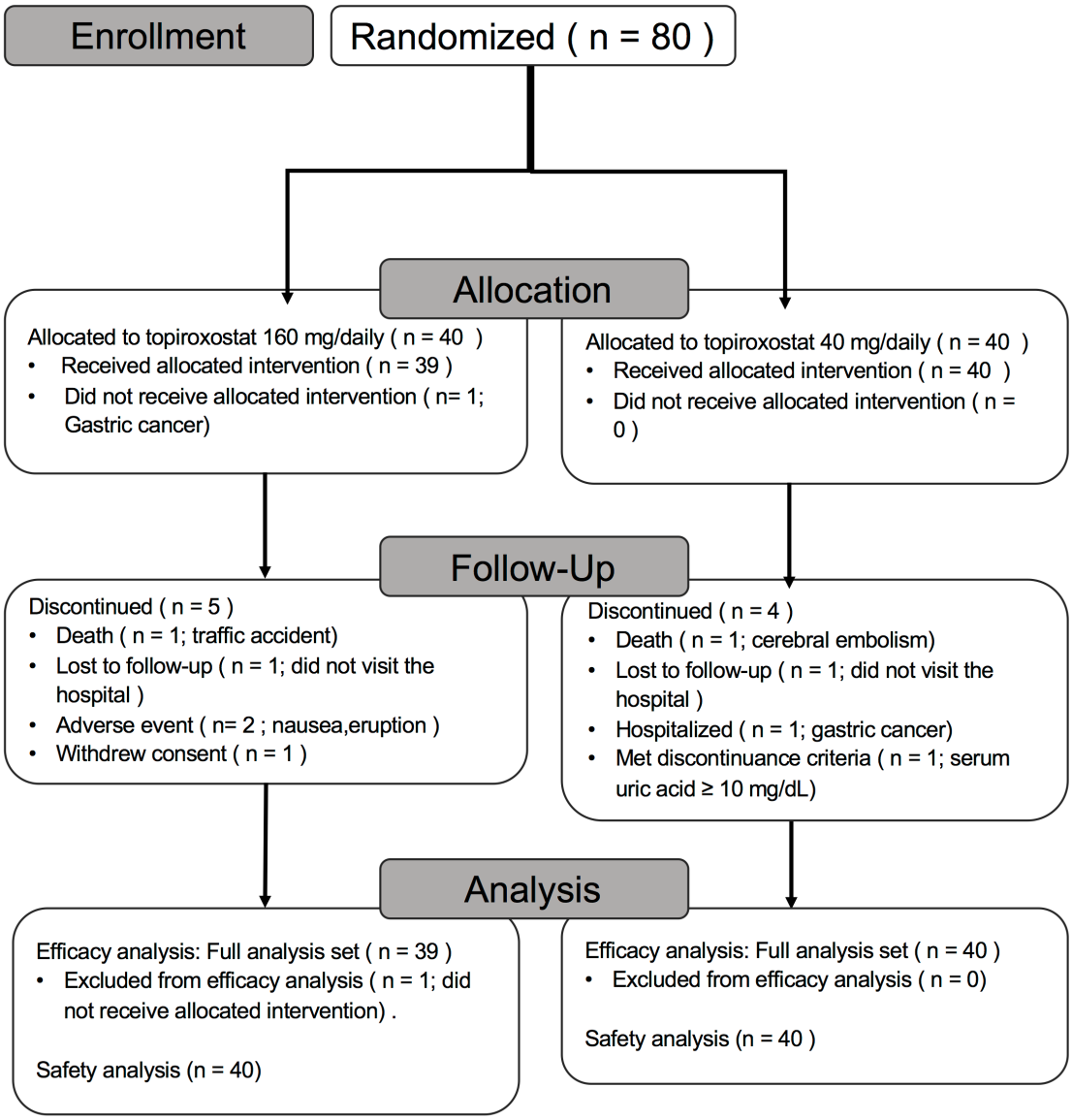
Table 3. Summary of the Adverse Events

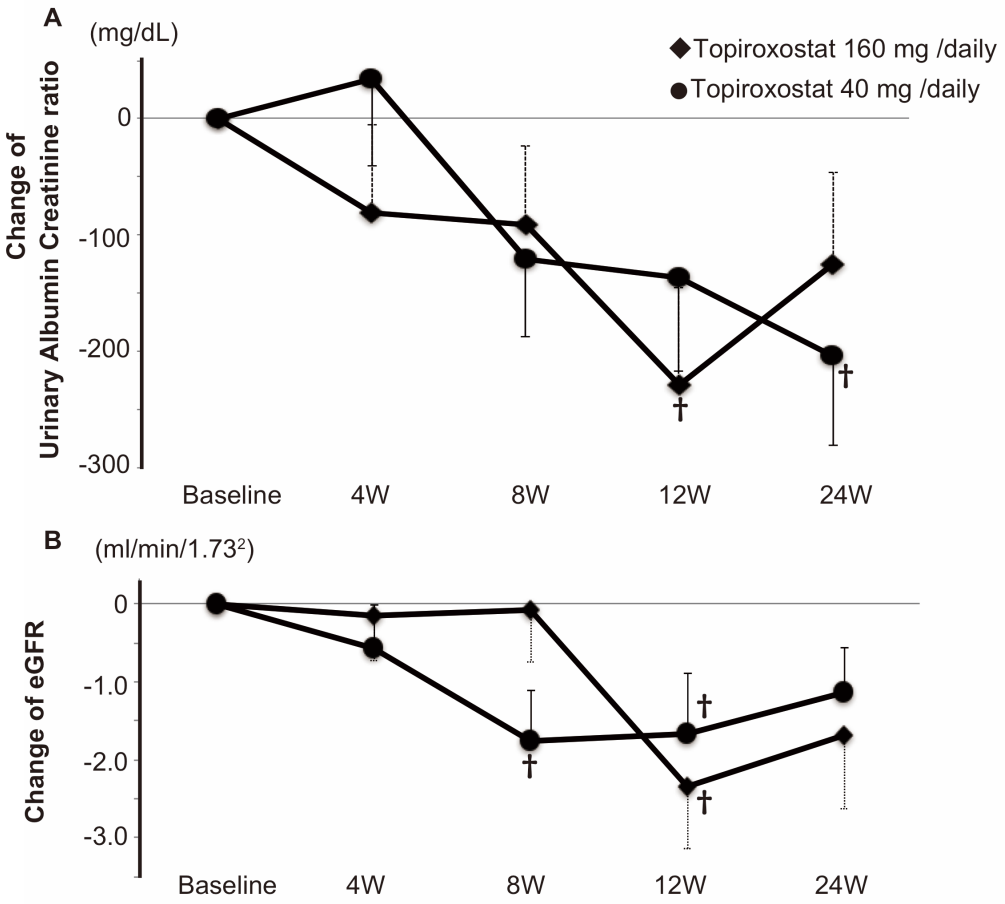
	Topiroxostat high dose (160mg daily)	Topiroxostat low dose (40mg daily)
Any adverse event, n (%)	8 (20.0)	5 (12.5)
Gouty arthritis	1 (2.5)	0 (0.0)
mild	1 (2.5)	0 (0.0)
moderate & severe	0 (0.0)	0 (0.0)
Gastrointestinal disorders	1 (2.5)	0 (0.0)
nausea	1 (2.5)	0 (0.0)
Cutaneous disorders	1 (2.5)	0 (0.0)
eruption	1 (2.5)	0 (0.0)
ALT increased (≧50 IU/L)	3 (7.5)	3 (7.5)
mild (50 – 99 IU/L)	2 (5.0)	3 (7.5)
moderate (100 - 499 IU/L)	1 (2.5)	0 (0.0)
severe (≧500 IU/L)	0 (0.0)	0 (0.0)
AST increased (≧50 IU/L)	3 (7.5)	3 (7.5)
mild (50 - 99 IU/L)	2 (5.0)	3 (7.5)
moderate (100 - 499 IU/L)	1 (2.5)	0 (0.0)

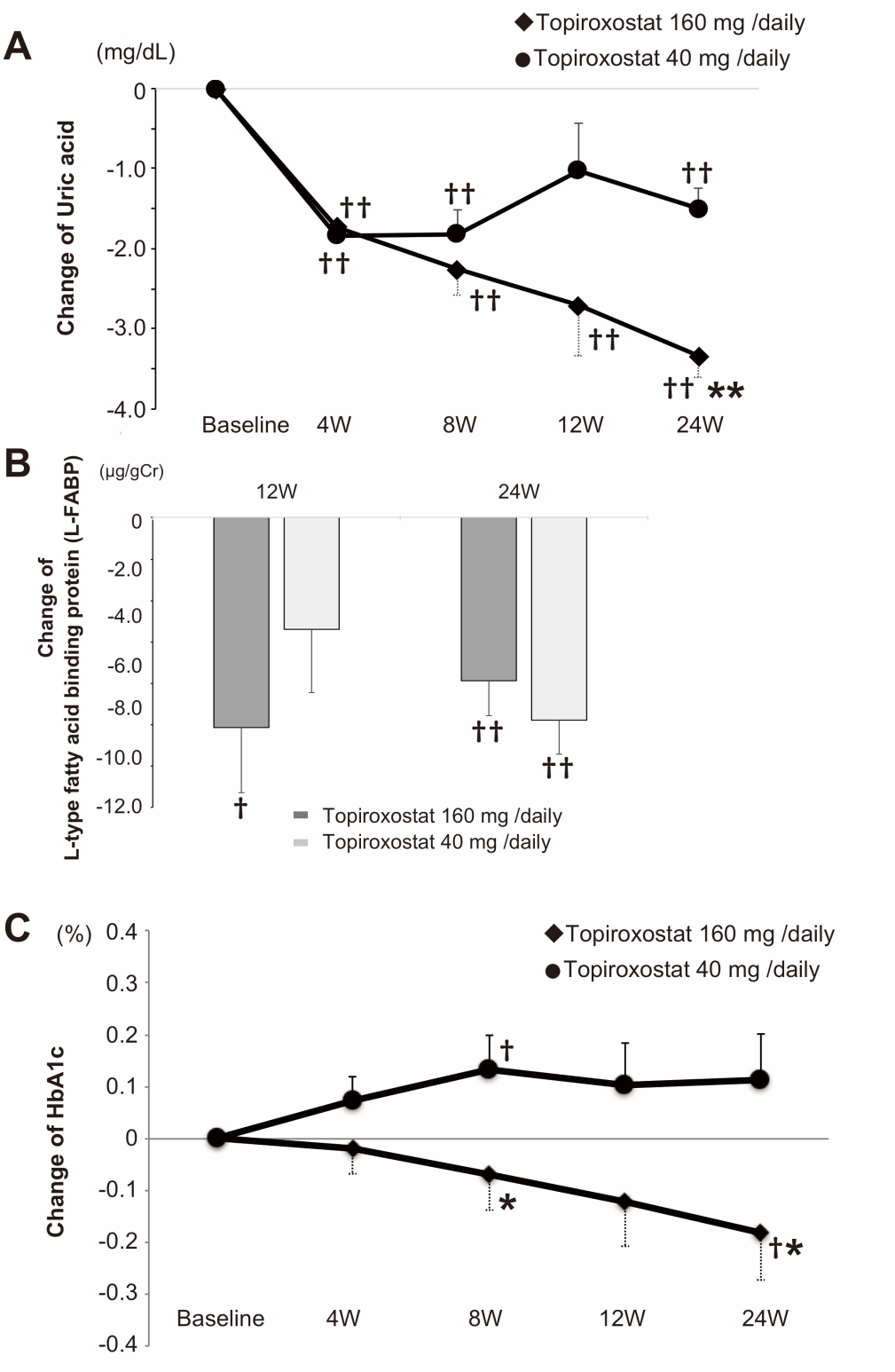
severe (≧500 IU/L)	0 (0.0)	0 (0.0)
T-Bil increased (≧1.6 mg/dL)	0 (0.0)	0 (0.0)
Serious adverse event, n (%)	1 (2.5)	2 (5.0)
Death	1 (2.5)	1 (2.5)
Any malignant neoplasm	1 (2.5)	1 (2.5)
gastric cancer	1 (2.5)	1 (2.5)

Values are expressed in number of patients (%).

Abbreviations: ALT; alanine aminotransferase, AST; aspartate aminotransferase, T-Bil; total bilirubin


Enrollment
Randomized (n = 80)
Allocation
Allocated to topiroxostat 160 mg/daily (n = 40)
• Received allocated intervention (n = 39)
• Did not receive allocated intervention (n= 1; Gastric cancer)
Allocated to topiroxostat 40 mg/daily (n = 40)
• Received allocated intervention (n = 40)
• Did not receive allocated intervention (n = 0)
Follow-Up
Discontinued (n = 5)
• Death (n = 1; traffic accident)
• Lost to follow-up (n = 1; did not visit the hospital)
• Adverse event (n= 2 ; nausea,eruption)
• Withdrew consent (n = 1)
Discontinued (n = 4)
• Death (n = 1; cerebral embolism)
• Lost to follow-up (n = 1; did not visit the hospital)
• Hospitalized (n = 1; gastric cancer)
• Met discontinuance criteria (n = 1; serum uric acid ≥ 10 mg/dL)
Analysis
Efficacy analysis: Full analysis set (n = 39)
• Excluded from efficacy analysis (n = 1; did not receive allocated intervention) .
Safety analysis (n = 40)
Efficacy analysis: Full analysis set (n = 40)
• Excluded from efficacy analysis (n = 0)
Safety analysis (n = 40)


A
(mg/dL)
Topiroxostat 160 mg /daily
Topiroxostat 40 mg /daily
Change of
Urinary Albumin Creatinine ratio
0
-100
-200
-300
†
†
Baseline
4W
8W
12W
24W
B
(ml/min/1.73²)
Change of eGFR
0
-1.0
-2.0
-3.0
†
†
†
Baseline
4W
8W
12W
24W


A
(mg/dL)
Topiroxostat 160 mg /daily
Topiroxostat 40 mg /daily
Change of Uric acid
0
-1.0
-2.0
-3.0
-4.0
Baseline
4W
8W
12W
24W
B
(μg/gCr)
12W
24W
Change of
L-type fatty acid binding protein (L-FABP)
0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
Topiroxostat 160 mg /daily
Topiroxostat 40 mg /daily
C
(%)
Topiroxostat 160 mg /daily
Topiroxostat 40 mg /daily
Change of HbA1c
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
Baseline
4W
8W
12W
24W