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Randomized control trial for the assessment of the anti-albuminuric effects of topiroxostat in hyperuricemic patients with diabetic nephropathy (the ETUDE study)

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ABSTRACT

Proteinuria is an established risk factor for diabetic nephropathy. Recent studies indicate that some xanthine oxidase inhibitors have a renoprotective effect. The aim of this study was to assess whether topiroxostat reduces albuminuria in hyperuricemic patients with diabetic nephropathy and overt proteinuria. The ETUDE study is an ongoing 24-week, multicenter, open-label, randomized (1:1), parallel group study involving hyperuricemic patients with diabetic nephropathy (estimated glomerular filtration rate [eGFR] ≥ 20 mL/min/1.73 m^2) and overt proteinuria (0.3 ≤ urine protein to creatinine ratio (UPCR) < 3.5 g/g Cr). Patients are randomly assigned to high dose (topiroxostat 160 mg daily) or low dose (topiroxostat 40 mg daily) on top of standard of care. The primary endpoint is the change in albuminuria indicated by urine albumin-to-creatinine ratio after 24 treated weeks relative to the baseline values. This trial was registered at the Japanese University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR: UMIN 000015403). The background, rationale, and study design of this trial are presented here. Seventy-six patients from four registered facilities have already been enrolled and received at least one dose of topiroxostat. This trial will end in 2017. The ETUDE trial is the first randomized controlled study of topiroxostat in hyperuricemic patients with diabetic nephropathy and overt proteinuria. We will clarify the pleiotropic function of topiroxostat including an anti-albumiuric effect as well as its effects on safely decreasing serum uric acid levels.

Key Words: albuminuria, diabetic nephropathy, Topiroxostat, Xanthine oxidase inhibitor

INTRODUCTION

The diabetic population is estimated to continuously increase by 155% from 382 million to 592 million worldwide by the year 2035.[1)] Type 2 diabetes has been the leading cause of end-stage renal disease (ESRD) requiring initiation of dialysis therapy in Japan since 1998.[2)] According to a survey by the Japanese Society for Dialysis Therapy in 2012, the percentage of diabetic nephropathy as a primary cause of ESRD for incident dialysis patients was 44.2%,

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while diabetic nephropathy has also become the most common primary disease among whole maintenance dialysis patients (37.1%).[3)]

While the current golden standard therapy for diabetic nephropathy is still to control blood pressure using renin-angiotensin system antihypertensive (RAS) agents and to optimize hyperglycemia,[4)] the efficacy for prevention against ESRD is still unsatisfactory, hence we should explore promising agents having renoprotective effects. Among these agents, it has been reported that some anti-diabetic drugs have direct renoprotective effects beyond glycemic control and act as antioxidant inflammatory modulators.[5)] Bakris *et al.* recently reported that finerenone, a novel non-steroidal, highly selective mineralocorticoid receptor antagonist demonstrated improvement in albumin-creatinine ratios.[6)] There has been a great deal of evidence that oxidative stress and inflammation may promote renal function deterioration.[7)] We have also recently demonstrated that the addition of the mineralocorticoid receptor antagonist to conventional antihypertensive treatment including a RAS agent resulted in a significant reduction in albuminuria in Japanese patients with diabetic nephropathy in our randomized control study.[8)] In this trial, we also clarified that this anti-albuminuric effect synchronized improvement of tubulointerstitial injuries and decreased local RAS activity in the kidney,[8)] which may be considered to be associated with oxidative stress and inflammation.[9)]

In recent years, xanthine oxidase inhibitors (XOis) have received much attention in the area of diabetic nephropathy in both clinical[10)] and non-clinical research.[11)] Although allopurinol, a representative XOi accepted as the clinical standard for treatment of gout since the 1960s, has the potential to improve endothelial dysfunction and reduce oxidative stress,[12)] it is also associated with severe side effects. Recently, it has been reported that compared to placebo, topiroxostat, a selective XOi, showed statistically significant reduction in albuminuria in patients with hyperuricemia and moderate renal insufficiency in a clinical trial from Japan.[13)] Thus, we aimed to clarify whether topiroxostat shows anti-albuminuric effects in hyperuricemic patients with diabetic nephropathy as well.

In this ongoing randomized controlled study, we will evaluate 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).

METHODS

Trial design

The ETUDE study is a multicenter, open-label, randomized (1:1), parallel group study comparing the effects of topiroxostat 160 mg daily with topiroxostat 40 mg daily on top of standard of care in hyperuricemic patients with diabetic nephropathy and overt proteinuria. This trial was registered at Japanese University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR: UMIN 000015403). 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). All patients provide written informed consent to participate in this study after they have received information of the purpose of this study as well as the potential risks and benefits.

Patients

We have been recruiting study subjects since September 2014. Inclusion criteria are 1) diag-

nosis of diabetes, 2) hyperuricemia, 3) 0.3 ≤urine protein to creatinine ratio (UPCR) <3.5 g/g Cr, 4) estimated glomerular filtration rate (eGFR) ≥20 mL/min/1.73 m^2, 5) on diet and exercise therapies for more than 8 weeks prior to providing informed consent, 6) age 20 years and older, and 7) outpatient status (not planning to be hospitalized).

Patients are excluded from this study if they have 1) poorly-controlled glycemia, 2) taken oral or intravenous steroid agents, 3) other kidney diseases except diabetic nephropathy (exception: a patient with findings suggestive of nephrosclerosis), 4) cancer (exception: a patient who is fully recovered from cancer), 5) systemic diseases except diabetes which induce proteinuria (for example; connective tissue disease, vasculitis, or amyloidosis), 6) a history of gouty arthritis during the 6 months prior to providing informed consent, 7) double the normal level (defined by the upper limit of normal of each testing facility) of alanine aminotransferase (ALT) or aspartate aminotransferase (AST), or 8) active chronic hepatitis C or B, and 9) cirrhosis. Those patients judged to be inadequate to participate in this study based on the primary doctor's judgment are excluded.

Registration and randomization

Patients are enrolled via a web-based registration and follow-up system developed by the Center for Advanced Medical and Clinical Research of the Nagoya University Hospital, Aichi, Japan. Once a primary doctor who is a registered member of this research project obtains a patient's consent, he has access to the registration system of this study and enters required information at enrollment. The system automatically evaluates the eligibility of each patient and randomly assigns patients to either the high dose group (Group G; 160 mg daily) or the low dose group (Group L; 40 mg daily) of topiroxostat. The allocation ratio is 1:1 and a dynamic allocation strategy using a minimization method is used. The stratifying factors for randomization are proteinuria (>0.5 g/g Cr or ≤0.5 g/g Cr), abnormal eGFR (>40 mL/min/1.73 m^2 or ≤40 mL/min/1.73 m^2), and the hospital to which the patients belong.

Study medication

Eligible participants are randomly assigned (1:1) to Group G (160 mg daily) or Group L (40 mg daily) of topiroxostat. After a run-in period of up to 8 weeks, we administer topiroxostat 20 mg orally twice daily for 4 weeks to Group G patients, and we dose-up topiroxostat 40 mg orally twice daily for the next 4 weeks, then further dose-up topiroxostat 40 mg orally twice daily for 16 weeks. In contrast, for Group L patients, we maintain topiroxostat treatment at 20 mg orally twice daily for 24 weeks. The dosage and administration for topiroxostat in the high-dose group are determined on the basis of the Japanese Ministry of Health, Labor, and Welfare approved dosage provided on the package leaflet for topiroxostat and in accordance with a previous report.[13] The administration of topiroxostat is in the morning and in the evening. A change in type or dose of prior antihyperuricemic/anti-gout drug; antituberculosis drugs, which affect the level of serum urate; immunosuppressive drugs, which affect the level of serum uric acid; and any drug with a potential interaction with topiroxostat is prohibited throughout the study. Changes in type or dose of prior RAS antihypertensive drugs and diuretics is restricted throughout the study. The study flowchart is shown in Figure 1.

Outcome

The primary endpoint of the protocol is the change in albuminuria indicated by the urine albumin-to-creatinine ratio (UACR) after 24 weeks of treatment relative to the baseline values. The secondary endpoints are change in UACR after 24 weeks of treatment relative to each visit (4, 8 and 12 weeks), and change in UPCR after 24 treated weeks relative to each visit (0, 4,

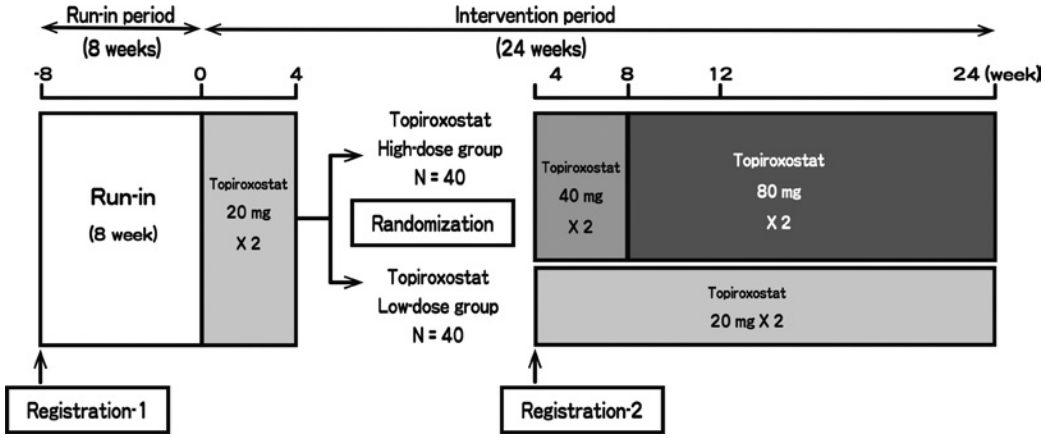

Fig. 1 Flowchart of the current trial.

8 and 12 weeks). Changes in systolic blood pressure and diastolic blood pressure, glycosylated hemoglobin (HbA1c), eGFR, serum urate, and L-type fatty acid binding protein (L-FABP) are assessed after 24 weeks of treatment relative to the baseline values as the secondary endpoints. Moreover, rate of adverse drug reaction is also set as the secondary endpoint.

Follow-up

After an 8-week run-in period, follow-up visits are conducted at 4 weeks, 8 weeks, 12 weeks, and at 24 weeks as a final visit after study commencement, and data about adverse events, drug adherence, physical examination, blood pressure, and serum uric acid (sUA), creatinine, AST, ALT, alkaline phosphatase (ALP), total bilirubin, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, HbA1c, glucose, blood urea nitrogen, hemoglobin, UPCR, UACR, and urinary L-FABP levels are obtained. As a precaution, a decision was made prior to study commencement to discontinue the study for any patient whose 1) AST and/or ALT level is ≥ 200 IU/L, or 2) sUA level is ≤ 2.0 mg/dL or ≥ 10.0 mg/dL at more than one sequential visit. These criteria applied to both male and female subjects.

Laboratory measurements

The measurement of urinary albumin, urinary creatinine, and urinary L-FABP is entrusted to the laboratory of SRL Inc., Aichi, Japan. Serum uric acid, creatinine, AST, ALT, ALP, total bilirubin, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, HbA1c, glucose, blood urea nitrogen and hemoglobin levels are determined by routine procedures at the clinical chemistry facilities of each hospital. The eGFR from creatinine levels was calculated as follows: eGFRcreat (mL/min/1.73 m^2) = 194 × $SCr^{-1.094}$ × $Age^{-0.287}$ × 0.739 (if female).[14)]

Sample size

Based on the results of a previous clinical trial,[13)] the geometric mean ratio and geometric standard deviation of UACR in topiroxostat group at the last observation period was 0.665 and 1.981, respectively. We estimated that at least 32 patients were required per treatment group in order to have 90% power to detect a significant difference between high and low dose topiroxostat groups in the change of log 10-transformed UACR from baseline to the end of treatment

with an effect size of 0.81 SD. Thus, we set 40 subjects per group (80 subjects in total) with consideration for potential discontinuation or dropout of enrolled patients in this study period.

Statistical analysis

This randomized trial is designed to demonstrate that high-dose topiroxostat treatment is superior to low dose topiroxostat in terms of the decrease of UACR from baseline. In this group of patients, after confirmation of a significant decrease in the UACR from baseline at the final visit in the topiroxostat high-dose group by paired t-test, the levels of decrease in UACR were compared between treatment groups by analysis of covariance including age, sex, baseline UACR, and baseline eGFR as covariates. Secondary endpoints included the change in UACR, UPCR, blood pressure level, HbA1c, eGFR, serum urate level, and L-FABP from the baseline analyzed using linear-mixed effect models, and the proportion of patients suffering from gouty arthritis and liver dysfunction were compared using chi-squared test or Fisher's exact test. The level of statistical significance is set at two-sided $p < 0.05$ for all statistical analyses.

RESULTS

The objective of the ETUDE study protocol is to confirm the anti-albuminuric effect of topiroxostat in hyperuricemic patients with diabetic nephropathy and overt proteinuria in a randomized controlled trial.

The study has currently enrolled 79 patients from the participating four facilities and is still recruiting patients (December 2015). We planned to enroll 80 patients. Two patients refused to participate in the study, and two patients were not able to continue in the study because of sudden death by traffic accident and terminal gastric cancer, respectively. One additional patient dropped out because of compliance with stop criteria as his sUA level was ≥ 10.0 mg/dL on two consecutive follow-up visits. There has been no severe adverse event due to topiroxostat. Initially, the enrollment period was scheduled to end in August 2015, but this period was extended due to shortage of enrolled patients. The study will end in 2017.

DISCUSSION

Topiroxostat is one of the newest xanthine oxidase inhibitors (XOis) available and was recently approved for patients with hyperuricemia and/or gout in Japan. This current study will be the first trial to evaluate the efficacy of topiroxostat on albuminuria in patients with diabetic nephropathy in a randomized control study. These results will be helpful for practical use of the treatment of hyperuricemic patients with diabetic nephropathy and proteinuria in the clinical setting.

Hyperuricemia is a common complication in chronic kidney disease. Although it is controversial whether increasing uric acid levels can be considered a surrogate marker for decreased renal function or not, substantial evidence has shown that hyperuricemia may be a inducer and accelerator of hypertension and chronic kidney disease (CKD).[15, 16] In animal models, raising uric acid induced oxidative stress and endothelial dysfunction, and resulted in systemic and glomerular hypertension.[17, 18] Interestingly, reduced albuminuria with amelioration of tubulointerstitial injury, as well as lowered uric acid levels was observed by treatment with allopurinol in diabetic mice with experimentally elevated uric acid.[11] Salvatore *et al.* reported that serum uric acid was an independent risk factor of progressive renal dysfunction and in part was associated with onset of albuminuria in a 4-year follow-up cohort of about 14000 patients with type 2 diabetes.[19]

A XOi is any substance that inhibits the activity of xanthine oxidase, an enzyme involved in purine metabolism. Inhibition of xanthine oxidase reduces the production of uric acid in humans, and several XOis have been applied in clinical practice for the treatment of gout and hyperuricemia all over the world.[20] XOis are of two kinds: purine analogues, including allopurinol, and other non-purine agents. Although allopurinol has been a standard drug for the treatment of these diseases in CKD patients with decreased excretion of uric acid for a few decades, it is sometimes associated with adverse effects[21] and difficulty of achievement of target uric acid levels under recommended doses.[22] Febuxostat and topiroxostat are both non-purine selective inhibitors of xanthine oxidase, and topiroxostat has just become clinically available in Japan in 2013.[23] Febuxostat was already reported to be more powerful than allopurinol,[24] and may be more beneficial and safer for CKD patients since it is metabolized mainly by glucuronide formation and oxidation in the liver.

Several interventional studies using XOis have been reported in CKD. Siu *et al.* conducted a 12-month, prospective, randomized controlled trial comparing allopurinol to current therapy in 54 hyperuremic patients with mild and moderate CKD.[25] In the allopurinol group, 16% reached the combined endpoints of dialysis and significant deterioration in renal function compared with 46% in the control group ($p = 0.015$). Monemi *et al.* conducted a 4-month, double-blind randomized control trial comparing allopurinol to placebo in 40 patients with diabetic nephropathy and proteinuria.[10] Serum uric acid levels and proteinuria were significantly lower in the patients taking allopurinol compared with the control group. In 2015, Sircar *et al.* reported that febuxostat slowed a reduction in the eGFR in a 6-month, double-blind, randomized, control study involving 45 patients with CKD stage 3 and 4.[26] In 2015, Tanaka *et al.* reported that febuxostat decreased albuminuria as well as other urinary tubulointerstitial markers with significantly greater reduction in serum uric acid levels in a 12-week, randomized, open-label, control study in 40 hyperuricemic patients with stage 3 CKD.[27] Hosoya *et al.* reported that compared to placebo, topiroxostat significantly reduced albuminuria in a 22-week, double-blind, randomized, controlled study in hyperuricemic patients with stage 3 CKD.[13]

In conclusion, the ETUDE trial is the first randomized controlled study of topiroxostat in hyperuricemic patients with diabetic nephropathy and overt proteinuria. Recruitment of subjects is ongoing and the trial will end in 2017. Although further studies are needed, topiroxostat could be recommended as an alternative drug to control albuminuria in patients with diabetic nephropathy based on the results of this trial.

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CONFLICT OF INTEREST

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