Outcomes of Tacrolimus Therapy in Adults With Refractory Membranous Nephrotic Syndrome: A Prospective, Multicenter Clinical Trial

Wei Chen, MD, PhD, Qinghua Liu, MD, PhD, Yunhua Liao, MD, Zhenhua Yang, MD, Jian Chen, MD, PhD, Junzhou Fu, MD, PhD, Jinli Zhang, MD, Yaozhong Kong, MD, Ping Fu, MD, PhD, Tanqi Lou, MD, PhD, Zhengrong Liu, MD, PhD, Yulian Ji, MD, Zhibin Li, PhD and Xueqing Yu, MD, PhD

Abstract: *Introduction:* The treatment of adult refractory idiopathic membranous nephropathy with steroid and other immunosuppressant-resistant nephrotic syndrome can be a significant challenge. The authors investigated the efficacy and safety of tacrolimus (TAC) as a promising regimen. *Methods:* A prospective, multicenter trial was conducted in 9 nephrology centers from 2006 to 2008. Fourteen patients were enrolled. In conjunction with prednisone, TAC was started at 0.05 mg/kg/d, titrated to achieve a trough blood level of 5 to 10 ng/mL for the first 6 months, then reduced to 4 to 6 ng/mL for the subsequent 6 months. The primary endpoints included complete or partial remission. Secondary endpoints included relapse, change of clinical parameters and adverse events. *Results:* After 12 months, complete remission was achieved in 35.7% of patients and partial remission in 42.9%, yielding a response rate of 78.6%. Proteinuria, serum albumin, cholesterol, triglyceride and low-density lipoprotein were improved significantly ($P < 0.001$, $P < 0.001$, $P = 0.002$, $P = 0.01$, $P = 0.004$, respectively). Proteinuria and serum albumin were significantly improved (42.0% ± 13.2%, $P = 0.02$; 15.2% ± 4.5%, $P = 0.01$, respectively) even after the first month of treatment. One patient relapsed during the subsequent 6 months of follow-up. Adverse events included 2 cases of infection and 1 case each of hyperglycemia, hand tremor, sudden death (nondrug related) and diarrhea. *Conclusions:* TAC plus prednisone may be an alternative therapeutic option for steroid and general immunosuppressant-resistant membranous nephrotic syndrome patients, with a favorable safety profile. However, given the limitation of a small number of patients in this trial, further study with a larger number and longer follow-up is needed.

Key Indexing Terms: Tacrolimus; Membranous nephropathy; Nephrotic syndrome; Refractory. **[Am J Med Sci 2013;345(2):81–87.]**

Idiopathic membranous nephropathy (IMN) is one of the most common causes of nephrotic syndrome in adults.[1,2] Untreated, a considerable part of patients with IMN will progress to end-stage renal disease (ESRD).[2–4] Over the past few decades, alkylating agents have been shown to increase the remission rate and improve renal survival in IMN compared with conservative therapy.[5–8] Nevertheless, serious toxicity of these aggressive therapeutic regimens is an important concern. On the other hand, patients failing previous immunosuppression account for approximately 30% of the whole population of IMN patients with persistent nephrotic syndrome, and these patients are with the highest risk of progression to ESRD or of complications related to the nephrotic syndrome.[4,9] Therefore, in the refractory cases of steroid and general immunosuppressant resistance, alternative therapeutic agents are needed.

As a calcineurin inhibitor, tacrolimus (TAC) is a macrolide antibiotic and has a relatively selective inhibitory action on CD4 T-helper lymphocyte activation and proliferation. Both *in vivo* and *in vitro* studies have shown that TAC is more potent than cyclosporine A (CsA) in its actions and with a lower incidence of nephrotoxicity, hypertension and dyslipidemia.[10,11] Praga et al conducted a randomized trial evaluating TAC monotherapy in IMN with nephrotic syndrome. In comparison with no immunosuppressant therapy in subjects, TAC monotherapy showed a favorable effect on the outcome of patients.[12] Our recent study also showed that combined with steroid, TAC might be an effective regimen for lupus membranous nephropathy patients.[13] However, the role of TAC in the management of refractory nephrotic IMN patients is not clear. In particular, for the group of patients who show resistance to steroid and conventional immunosuppressive regents, the possible benefit from TAC with prednisone remains undetermined.

Therefore, we performed a multicentre prospective study aimed to evaluate the efficacy and safety of TAC plus prednisone regimen for adult Chinese patients with refractory membranous nephrotic syndrome, who were resistant to the steroid and other usual immunosuppressive drugs.

From the Department of Nephrology (WC, QL, YJ, ZL, XY), The First Affiliated Hospital, Sun Yat-sen University, Key Laboratory of Nephrology, Ministry of Health, Guangzhou; Department of Nephrology (YL, ZY), The First Affiliated Hospital of Guangxi Medical University, Nanning; Department of Nephrology (JC), Fuzhou General Hospital, Fuzhou; Department of Nephrology (JF), The First Municipal People's Hospital of Guangzhou, Guangzhou; Department of Nephrology (JZ), The People's Hospital of Yunnan Province, Kunming; Department of Nephrology (YK), The First Municipal People's Hospital of Foshan, Foshan; Department of Nephrology (PF), The West China Hospital of Sichuan University, Chengdu; Department of Nephrology (TL), The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou; Department of Nephrology (ZL), The Nanfang Hospital, Southern Medical University, Guangzhou; and Epidemiology Research Unit, Translational Research Center (ZL), The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China.

Submitted September 28, 2011; accepted in revised form January 24, 2012.

Astellas Pharmaceuticals supplied the tacrolimus used in this study, but it played no role in the design and conduct of this study as well as in the analysis and the interpretation of results. Writing and publication of the article were not contingent on the approval of Astellas Pharmaceuticals.

This work was supported by grants from National Natural Science Foundation of China (No. 30971378 and No. 30871166), Scientific and Technologic Committee of Guangdong province (No. 2009B030801129 and 2005B30701002), Guangdong province Health office (A2005189), Guangdong Natural Science Foundation (06021368) and by the Sun Yat-sen University Clinical Research 5010 Program (to XY), the grant (No. 2010-76) from Guangdong province university academic and discipline development (to XY) and the 973 project (No. 2011CB50400050) (to XY). This work has also been made possible through Dr. Wei Chen's ISN Fellowship.

Presented in part in poster form at the APCN 2010, June 5–8, Seoul, Korea.

Correspondence: Wei Chen, MD, PhD, Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, 58# Zhongshan Road II, Guangzhou 510080, China (E-mail: vvchen66@gmail.com).

MATERIALS AND METHODS

Patient Selection

This study was a multicenter prospective trial conducted in 9 Nephrology centers in China from September 2006 to December 2008. These 9 centers were the primary regional tertiary renal clinics with adequate facility and skills. The participating physicians were specially trained on the subject of clinical trial before the trial began to minimize variations in patient care and clinical laboratory determination among different centers. The eligibility criteria for this trial included age 14 to 65 years, renal biopsy-proven membranous nephropathy based on light microscopy, immunofluorescence and electronic microscopy according to standard classifications, failure to respond (either complete remission [CR] or partial remission [PR]) after a standard course of 8 weeks of 1.0 mg/kg/d prednisone and at least 1 standard therapy of other immunosuppressant regimens (the protocol for cyclophosphamide [CTX] was oral or intravenous administration of 200 mg every other day with a total accumulation dose of at least 150 mg/kg or treated as Ponticelli's regimen[14]; the protocol for CsA was administration of a dosage of 3–5 mg/kg/d for at least 3 months and the protocol of mycophenolate mofetil [MMF] was administration of at least 2 g/d for more than 3 months). In addition, laboratory tests documented the presence of nephrotic syndrome, defined as proteinuria (>3.5 g/24 hr) along with hypoalbuminemia (serum albumin <30 g/L). Patients with the following conditions were excluded: serum creatinine >3 mg/dL, secondary membranous nephropathy, acute severe infection, active hepatitis, diabetes mellitus, women who were pregnant or unwilling to use contraception, existing tumor or transplanted organ and those who had received treatment with MMF, CTX, CsA, methotrexate or other immunosuppressive agents within 1 month before enrollment. Written informed consent was obtained from all patients and/or guardians. The study was approved by The Human Ethics Committees in all participating hospitals and was conducted according to the Declaration of Helsinki Principles and registered at website www.ClinicalTrials.gov (registry number NCT00615667).

Treatment Protocol and Follow-up Schedule

All previous immunosuppressant agents were discontinued for at least 1 month before initiating the study. TAC was started at a dose of 0.05 mg/kg/d, divided into 2-time doses at 12-hour intervals, and the dose was titrated to achieve target trough blood concentrations of 5 to 10 ng/mL for the first 6 months and then reduced to target trough levels of 4 to 6 ng/mL for the subsequent 6 months.

All subjects received concomitant corticosteroid therapy according to the protocol which consisting of oral prednisone (or equivalent) at an initial dose of 1.0 mg/kg/d (maximum 60 mg/d). Prednisone was gradually tapered by 10 mg every 2 weeks to 40 mg/d, followed by a decrease of 5 mg/d every 2 weeks until a daily dose of 10 mg/d was achieved, which was then maintained to the end of 12 months. The dose of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers remained unchanged during the period of this study. Target blood pressure was 130/80 mm Hg. Hyperlipidemia was treated with statins and/or fibric acid derivatives as required, who were then on a stable statin and/or fibric acid derivatives dose once enrolled into the study. Pulse intravenous corticosteroids were prohibited 2 weeks before enrollment and throughout the study. Nonsteroid antiinflammatory drugs were prohibited during the study period.

Patients were requested to come back to the hospital for weekly follow-up for the first 4 weeks, then every other week for 8 weeks and monthly thereafter. On each follow-up visit, patients were evaluated for clinical manifestations and laboratory investigations of nephrotic syndrome and any adverse effects of therapy. Blood pressure and laboratory assessments including a complete blood count, urinalysis, 24-hour proteinuria, liver function and renal function (estimated glomerular filtration rate [eGFR], calculated using the estimating equation which was developed by modifying Modification of Diet in Renal Disease equation based on data from Chinese chronic kidney disease patients),[15] glucose and electrolytes were performed at each visit. A fasting lipid profile was also measured every month.

TAC trough blood levels were routinely checked for all patients weekly for 4 weeks, then every 2 weeks for 8 weeks and then monthly. If the serum creatinine level increased by more than 20% compared with the baseline values or the fasting plasma glucose level >6.1 mmol/L and/or a 2-hour postprandial plasma glucose level >7.8 mmol/L, the dosage of TAC was adjusted to the half of previous dosage followed by repeat testing of the TAC trough blood level and renal function or plasma glucose level. At the discretion of the physicians, TAC was given at a lower dosage if the increment of serum creatinine reversed. Reasons for early withdrawal from this study included severe adverse effects, intolerance to therapy or whether the patient received prohibited treatment regimens.

Outcomes

The primary endpoint of this study was the response rate (defined as CR or PR) of patients. Predefined secondary endpoints included relapse rate, changes of clinical parameters (including proteinuria, serum albumin, serum creatinine and lipid profile) and adverse events (including infections, hyperglycemia, hand tremor, new-onset hypertension and nephrotoxicity).

CR was defined as urinary protein excretion less than 0.3 g per 24 hours, with normal serum albumin concentration (serum albumin ≥35 g/L) and a stable renal function (normal range of serum creatinine or no more than 15% above the baseline values). PR was defined as a value for urinary protein excretion that was between 0.3 and 3.5 g per 24 hours and a decrease in the value of at least 50% of the baseline level, with a serum albumin concentration of at least 30 g/L and a stable renal function. Treatment failure was defined as a value for urinary protein excretion that remained at or above 3.5 g per 24 hours or a value of 0.3 to 3.5 g per 24 hours, but with a serum albumin concentration of less than 30 g/L, an increase in the serum creatinine concentration greater than 50% above the baseline value. Time of response was defined as the number of days from the start of treatment to the first day of CR or PR.[16] Renal relapse was defined as an increase in urinary protein excretion >3.5 g/d in consecutive analyses in patients with CR or PR. Acute reversible nephrotoxicity was defined as an increase of serum creatinine level greater than 25% compared with baseline, which improved after 50% reduction of TAC daily dosage for 15 days. Persistent nephrotoxicity was defined as an increase of serum creatinine level greater than 50% compared with baseline, which persisted despite 50% reduction of TAC dose after 15 days.[17] Patients with diastolic blood pressure higher than 90 mm Hg or systolic blood pressure higher than 140 mm Hg were defined as having hypertension.

Statistical Analysis

For descriptive statistics, results were present as mean ± standard deviation, median (interquartile range) or n (%). Proteinuria was log transformed to obtain a better approximation of the normal distribution for analysis and then transformed back for reporting with median (interquartile range). To compare