Study Protocol

Study Design

In this prospective open-label, multicenter, randomized controlled trial, we compared a regimen of tacrolimus (TAC) monotherapy following short-term intravenous methylprednisolone with conventional glucocorticoid (GC) treatment for adult-onset minimal change nephritic syndrome (MCNS). The study protocol was registered at the Chinese Clinical Trial Registration of WHO International Clinical Trials Registry Platform (number ChiCTR-TRC-11001454). Detailed information is available on the website http://www.chictr.org.cn/. The trial was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the research ethics committees of all participating hospitals. All patients provided informed consent.

Participants

In this trial, patients were recruited from eight nephrology centers across China. The inclusion criteria were as follows: age 18–65 years; renal biopsy-verified diagnosis of minimal change nephropathy (examined using light microscopy, immunofluorescence, and electron microscopy); new-onset nephrotic syndrome (proteinuria>3.5 g/day, serum albumin<30 g/L); initial serum creatinine (SCr) level of <133 μmol/L, and urine volume of >600 mL/day (or>1000 mL/day after the use of diuretic drugs). Patients with the following conditions were excluded: secondary minimal change disease, acute kidney injury (AKI), hepatitis B or C infection, diabetes mellitus, a history of pancreatitis or gastrointestinal ulcer, a history of congenital or acquired immunodeficiency, or previous treatment with corticosteroids or other immunosuppressants (e.g., cyclophosphamide, calcineurin inhibitors, or mycophenolate mofetil).

Randomization and Treatment Procedures

Following the confirmation of eligibility, the patients were randomly assigned (1:1) to one of two groups (according to a random number label): 1) GC Group, short-term intravenous methylprednisolone followed by a conventional tapering oral prednisone regimen; or 2) TAC Group, short-term intravenous methylprednisolone followed by TAC monotherapy. The

minimization method was applied using a computer-generated sequence.

All patients received an intravenous methylprednisolone (0.8 mg/kg/day) infusion for 10 consecutive days at hospital admission. In the GC group, patients subsequently received oral prednisone at 1 mg/kg/day (maximum 80 mg/day) for 6–8 weeks according to the treatment response. This dose was subsequently reduced by 5 mg every week to 30 mg on alternate days and was maintained for 8 weeks, followed by tapering of the dose over approximately 12 weeks until complete withdrawal. In the TAC group, TAC therapy was initiated on the 8th day, with an initial oral dose of 0.05 mg/kg/day (that was divided into 2 doses, administered over a 12h interval). The TAC dosage was adjusted to a target trough whole-blood level of 4 to 8 ng/mL and was maintained for 16–20 weeks according to the treatment response. Subsequently, the dose was tapered to achieve a target trough level of 2–5 ng/mL over approximately 18 weeks until complete withdrawal. The treatment course for both groups was 36 weeks. The treatment was prolonged by 8 weeks for patients who underwent partial remission after 12 weeks of therapy. Patients who showed no remission after 12-weeks therapy of GC or TAC were recommended to withdraw from the trial, and undertake the other treatment trial (TAC monotherapy or conventional GC therapy, respectively). Patients who relapsed during the therapy were treated with a second cycle of GC or TAC therapy. Patients who relapsed twice were recommended to withdraw from the study.

The dose of angiotensin-converting enzyme inhibitors or angiotensin-II receptor blockers used by the respective patients was maintained for the duration of therapy. If required, additional antihypertensive drugs were administered to achieve adequate blood pressure control. Statins were administered to those patients who were still dyslipidemia after 12 weeks of therapy.

Definitions and Outcome Measures

Complete remission was defined as a decrease in proteinuria to 0.3 g/day or less. Partial remission was defined as a decrease in proteinuria to less than 3.5 g/day but greater than 0.3 g/day. The persistence of nephrotic proteinuria after 12 weeks of treatment with prednisone (1.0 mg/kg/day) or oral TAC (with a trough whole-blood level of 4–8 ng/mL) was considered to be no remission (treatment resistance). The time to remission was defined as the time from the

initiation of the therapy to the day when remission (complete or partial remission) was observed. Relapse was defined as an increase in proteinuria to 3.5 g/day or more in patients who underwent partial or complete remission. Time to relapse was defined as the time from initiation of remission to the day when the first relapse occurred. Frequent relapse was defined as two or more relapses within 6 months of the initial response, or four or more relapses in any 12-month period and two consecutive relapses during steroid (or TAC) therapy, or within 14 days of ceasing therapy were considered to be drug (steroid or TAC) dependence. Late nonresponder was defined as no response to the second period of steroid or TAC therapy after relapsing during tapering or discontinuation of steroid or TAC therapy. AKI was defined as an increase in SCr to more than 50% above baseline or more than 26.4 μmol/L during 48 hours according to KDIGO guidelines.

The primary outcome measures were the cumulative number of patients who experienced complete remission or partial remission. Secondary outcome measures were determined using the following variables: relapse, time to remission, time to relapse, changes in the SCr and estimated glomerular filtration rate (eGFR) levels, AKI, metabolic disorders (being overweight, glucose intolerance, diabetes mellitus, dyslipidemia, and hyperuricemia), AEs, and serious AEs.

Follow-up

In this trial, the follow-up duration was 64 weeks after therapy. Follow-ups were performed weekly for the first 4 weeks, every 4 weeks for the subsequent 36 weeks, and every 8 weeks thereafter. During each visit, the patients underwent anthropometric measurements (body weight, and standing height), and blood pressure measurements. Complete blood count, proteinuria, blood glucose, SCr, serum uric acid levels, lipid profile, albumin, alanine aminotransferase, and aspartate aminotransferase levels were determined. The trough TAC level was measured every week until stable, followed by measurements every four weeks. The body mass index (BMI, weight in kilograms divided by the square of the height in meters) was measured, and the short form 36-item health survey and bone mineral density measurement (with dual energy X-ray absorptiometry) were performed at the baseline and every 12 weeks.

Sample Size

TAC therapy was hypothesized to be non-inferior to GC therapy in achieving remission. On the basis of the results of previous studies and our observations that the remission rate of GC and TAC therapy in adult-onset MCNS was 80% and 90%, respectively, a sample size of 54 patients in each group (total 108 patients) was required to determine that TAC therapy was not more than 9.5% inferior to GC therapy with a type I error probability of 2.5% and a power of 80%, according to the Gart and Nam formulas. Allowing for an expected dropout rate of 10%, we aimed at enrolling 120 patients.

Statistical Analyses

All efficacy analyses were based on the intention-to-treat principle, comparing groups according to the randomly assigned treatment. For the primary efficacy analysis for non-inferiority, we carried out a per-protocol analysis, which included patients who received protocol treatments as scheduled. Non-inferiority was assessed by estimating the two-sided 95% confidence interval (CI) for the difference in remission rates between the GC and TAC group using the Gart and Nam method, and verifying that the lower limit of the CI was not lower than -9.5%. The 9.5% margin was based on clinical adjustment.

All data were expressed as the mean ± standard deviation (SD) or median (interquartile range) for continuous variables, and as percentage for categorical variables. The differences in normally distributed continuous variables between two groups were compared using the two-tailed independent sample Student's *t*-test. A paired *t*-test was performed to compare two means obtained at different times during the therapy. Nonparametric variables obtained at different times were compared using the Wilcoxon signed-rank test, and categorical variables were compared by Fisher's exact or a chi-squared test. The probability of the first relapse between two groups was estimated by the Kaplan-Meier method, and survival curves were compared with log-rank tests. A P value <0.05 was considered statistically significant. All statistical analyses were performed using the SAS software, version 9.2.

Supplemental Table 1. Changes of metabolic indices during therapy with GC or TAC

Metabolic indices	Group	Baseline	Week 12	Week 24	Week 36
BMI (kg/m^2)	GC group	23.4±2.9	23.1±3.0	23.2±3.0	23.2±3.0
	TAC group	23.2±3.6	22.1±3.1*	22.1±3.3*	22.0±3.4*
Weight（kg）	GC group	64.4±11.0	62.7±9.5	63.4±10.6	63.4±10.9
	TAC group	62.9±10.4	59.9±9.5	59.4±9.5*	59.1±10.5*
FBS (mmol/L)	GC group	4.7±0.7	4.5±0.8	4.7±1.0	4.7±0.6
	TAC group	4.7±0.5	5.1±0.5	5.0±0.5	5.0±0.6
CH (mmol/L)	GC group	9.8±2.9	5.7±2.1	5.2±2.1	4.9±1.3
	TAC group	10.4±3.8	5.0±2.3*	4.4±1.2*	4.7±1.4
TG (mmol/L)	GC group	2.4±1.2	1.8±0.8	2.0±1.7	1.6±0.8
	TAC group	2.3±1.3	1.6±1.4	1.3±0.6	1.3±0.7
LDL (mmol/L)	GC group	6.6±2.6	3.2±1.6	2.8±1.1	2.6±0.9
	TAC group	6.9±2.9	2.8±1.2	2.5±0.9	2.6±1.1
UA (μmol/L)	GC group	358.9±101.4	313.6±85.1	335.0±97.6	320.2±80.0
	TAC group	359.2±100.5	364.3±111.9*	340.0±98.2	347.0±104.0

BMI=body mass index; FBS=fasting blood sugar; CH=serum cholesterol; TG=serum triglyceride; LDL=serum low density lipoprotein; UA=serum uric acid.

* Significant differences between the two groups, $P<0.05$.