Efficacy of leflunomide combined with prednisone in the treatment of refractory nephrotic syndrome

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ABSTRACT

Objective: To assess the safety and clinical efficacy of leflunomide (LEF) and prednisone on refractory nephrotic syndrome (RNS).

Methods: A total of 52 patients with RNS were treated for 24 weeks between 2010 and 2014 in our hospital. In the treated group, 26 patients were treated with LEF and prednisone, and, in the control group, 26 patients were treated with cyclophosphamide (CTX) and prednisone. During the treatment, 24 h urinary protein excretion and the serum levels of albumin and cholesterol, and kidney function were assayed before and after the therapy. Adverse reactions during treatment were recorded.

Results: In the LEF group, the medication was markedly effective in eight cases and effective in nine cases; the total efficacy rate was 65.30%. In the CTX group, the treatment was markedly effective in six cases and effective in nine cases; the total efficacy rate was 57%. There were no significant differences between the results of the total efficacy rate (p > 0.05). The 24 h urinary protein excretion and the serum levels of albumin and cholesterol, and kidney function were assayed before and after the therapy. Adverse reactions during treatment were recorded.

Conclusion: LEF combined with prednisone has a certain efficacy on the RNS and displays few adverse reactions. A large-sample, randomized double-blind controlled study and long-term follow-up are needed to verify the efficacy of LEF combined with prednisone.

Introduction

Nephrotic syndrome is usually diagnosed when patients’ laboratory tests present with severe proteinuria (>3.5 g/day), edema, hypoalbuminemia (<30 g/L), and hyperlipidemia. For differing reasons, many patients have normal or impaired renal function at the time of diagnosis. Until now, there has been no unified definition of refractory nephrotic syndrome (RNS). However, RNS is generally characterized as a steroid-dependent, steroid-resistant, and subject to frequent relapse. Steroid-dependence is defined as the occurrence of relapse during treatment with alternate-day steroids or no more than 14 days after gradually cessation of the use steroids. Steroid-resistant refers to persistent proteinuria after an 8–12 week course of oral prednisolone 1 mg/kg/d. Frequently relapsing nephrotic syndrome refers to at least two relapses during treatment within six months or more than three relapses within 12 months of the initial presentation. It is a common nephropathic disease. It has different pathological types. These can present with different clinical features, and prognosis can vary. The main pathological types of RNS can be classified into membranous nephropathy, focal segmental lesions, membranous proliferative glomerulonephritis, mesangial proliferative glomerulonephritis (MPGN), and some minor glomerular abnormalities, in which the focal segmental glomerulosclerosis, and membranous proliferative glomerulonephritis types cause the most serious impairments of renal function. They can even cause renal failure. There is no unified, international opinion regarding treatment of RNS. Recent research has focused on the use of immunosuppressant agents containing alkylating agents, on calcineurin inhibitors including mycophenolate mofetil, tacrolimus, cyclophosphamide (CTX), cyclosporine, and adrenocorticotropic hormone, and on some targeted medicines.
However, the treatment outcomes and the medical prognosis are poor. Leflunomide (LEF) is an inhibitor of dihydroorotate dehydrogenase (DHODH) and has been used to treat patients with rheumatoid arthritis, membranous nephropathy, psoriatic arthritis, and ankylosing spondylitis.\(^4\) To do this, the efficacy of combined effect of prednisone and LEF was assessed, and prednisone combined with CTX was administered as the control.

**Materials and methods**

From July 2010 to September 2014, a total of 52 patients (20 male and 32 female; age range 14–62 years; average age 26.79 ± 13.32 years). All the cases were diagnosed as refractory NS in the First Affiliated Hospital of Bengbu Medical College. Renal histopathological findings included membranous nephropathy in 20 patients, membranoproliferative nephritis in three patients, IgA nephropathy in 12 patients, mesangial proliferative nephritis in five patients, lupus nephritis in five patients, focal segmental lesions in four patients, minor glomerular abnormalities in three patients. This study was approved by Institutional Review Board of the First Affiliated Hospital of Bengbu Medical College and all participants provided informed consent. The patients were selected and divided randomly into an LEF treatment group and CTX control group. The exclusion criteria included abnormal liver function or severe infection, poor compliance, leucopenia disease, patients who had HIV, hepatitis B or C, or malignancy, and LEF allergies.

**Methods**

The patients in the LEF treatment group were orally administered prednisone combined with LEF. The initial prednisone dose was 1 mg/kg/d; the dose of prednisone was slowly reduced eight weeks later (10% of the total dose was reduced once every two weeks when the urinary protein excretion dropped. The LEF dose for the first three days was 40 mg/d and for 20 mg/d for subsequent days. The patients in the CTX control group were given prednisone administered orally and intravenous CTX administered CTX intravenously. The CTX dose was 8–12 mg/kg/per administration, given once every four weeks. The dose of prednisone was the same as that administered to the LEF treatment group. No other cytotoxic drugs or immunosuppressants were used during the treatment period; 24 h urinary protein excretion (24 h-UP) and the serum levels of albumin and cholesterol and kidney function were tested before and after the six-month mark.

### Evaluation of therapeutic effects

(ii) Complete remission, which was here defined as the absence of symptoms, 24 h urine protein <0.3 g and serum albumin ≥35 g/L; (ii) partial remission, which was defined as proteinuria of 0.3 g/24 h or more and less than 3.5 g/24 h, or more than 50% reduction of the initial proteinuria level with stable renal function; (iii) no remission. Inefficacy was defined as no improvements in laboratory results or symptoms.

### Statistical analysis

Data were recorded as the mean ± SD and analyzed using t-tests. All data were recorded as mean [95% confidence interval (CI)]. All the measurement data were analyzed using the t-tests. \(p < .05\) was considered statistically significant. Results were analyzed using SPSS for Windows version 12.0 software (Bengbu, Anhui, China).

### Results

#### Basic data

The before-treatment data of the two groups were analyzed using \(t\)-tests. There were no significant differences with respect to age, 24 h-UP levels, serum albumin, serum cholesterol, or renal function (\(p > .05\)) (Table 1).

#### Efficacy analysis and laboratory examination

In the LEF treatment group, the medication was effective in 17 cases, and the total efficacy rate was 65.3%; it was not effective in nine cases. In the CTX control group, the medication was effective in 15 cases and not effective in 11 cases, and the total efficacy rate was 57.0% (Table 2).

The patients in the CTX treatment group (26 cases), Renal histopathological findings included membranous
nephropathy in 12 patients (which effective cases are 6), membranoproliferative nephritis in two patients (which effective cases are 1), IgA nephropathy in eight patients (which effective cases are 6), mesangial proliferative nephritis in one patient (which effective case is 1), lupus nephritis in two patients (which effective cases is 1), focal segmental lesions in one patients (which effective case is 0). The patients in the LEF treatment group (26 cases), Renal histopathological findings included membranous nephropathy in eight patients (which effective cases are 4), membranoproliferative nephritis in one patients (which effective case is 0), IgA nephropathy in four patients (which effective cases are 4), mesangial proliferative nephritis in four patients (which effective cases are 3), lupus nephritis in three patients (which effective cases are 2), focal segmental lesions in three patients (which effective case is 1), minor glomerular abnormalities in three patients (which effective cases are 3). According to the results of observation, after six months treatment, both two groups, the various pathological types all have some degrees of ease, minor glomerular abnormalities, MPGN and IgA nephropathy (IgAN) total effective rate was higher than that of membranous glomerulonephritis (MGN) and focal segmental glomerular sclerosis (FSGS) (Table 3).

During the first six months, the 24 h urinary-protein excretion levels in both groups decreased significantly

### Table 3. Patients pathological types of NS in each group.

| Group                                | CTX Effective cases | LEF Effective cases |
|--------------------------------------|---------------------|---------------------|
| Membranous nephropathy               | 12                  | 8                   |
| Membranoproliferative nephritis      | 2                   | 1                   |
| IgA nephropathy                      | 8                   | 6                   |
| Mesangial proliferative nephritis    | 1                   | 1                   |
| Lupus nephritis                      | 2                   | 1                   |
| Focal segmental lesions              | 1                   | 0                   |
| Minor glomerular abnormalities       | 3                   | 3                   |

### Table 4. Datas before and after intervention for two groups.
after medication, and the serum ALB levels in both groups increased significantly after medication ($p < .05$). In the LEF treatment group, proteinuria decreased from 4.9 g/24 h at baseline to 0.92 g/24 h, serum cholesterol levels decreased from 9.4 mmol/L to 6.25 mmol/L, and serum albumin levels increased from 23.3 g/L to 37.4 g/L. In the CTX control group, proteinuria decreased from 4.9 g/24 h at baseline to 1.02 g/24 h, serum cholesterol levels decreased from 8.8 mmol/L to 6.32 mmol/L, and serum albumin levels increased from 22.2 g/L to 37.08 g/L. All of these data were statistically significant different from pretherapy figures ($p < .05$). However, there were no significant differences between the serum creatinine of the two groups compared with data from before intervention (Tables 3 and 4). The mean eGFR of the cases, as assessed using the MDRD equation, in the LEF treatment group, before the therapy was 96.6 ± 3.8 mL/min/1.73 m$^2$, and after therapy was 97.2 ± 4.2 mL/min/1.73 m$^2$. In the CTX treatment group, the mean eGFR was 97.8 ± 2.4 mL/min/1.73 m$^2$, and after therapy was 97.6 ± 3.2 mL/min/1.73 m$^2$, respectively. There were no significant differences.

**Adverse reactions**

In the LEF treatment group, the alanine aminotransferase (ALT) levels increased slightly in one patient, while another two patients showed fatigue and mild poor appetite. In the control group, the ALT level increased slightly in two patients, leukocytopenia was observed in two patients, and hair loss and irregular menstruation were observed in three patients. After supportive treatment, these patients all returned to normal. No impaired renal function, no remarkable hematological abnormalities, and no other serious adverse reactions were observed over the course of treatment. No patient in either group ended drug treatment early.

**Discussion**

At present, several therapies, including glucocorticoid and immunosuppressive agents have been shown to be effective and safe in the treatment of RNS. CTX is a classical drug that has been used to treat RNS in clinical settings since the 1970s. The therapeutic effects of CTX in the treatment of refractory NS have been unanimously accepted. However, recent U.S. guidelines, published in 2009, no longer strongly recommend using CTX for refractory nephritic syndrome because of its relatively low efficacy, gonadal toxicity, myelosuppression, and carcinogenicity. Currently, CTX is defined as the third-line drug for steroid-dependent nephritic syndrome in U.S. guidelines. Among the newly therapeutic targeted agents, rituximab can effectively anti-inflammatory and protect kidney function from decline and can decrease protein excretion retard relapse. However, most patients cannot afford targeted therapeutic agents because of their high cost. Although the efficacy of LEF for the treatment of refractory NS has only rarely been reported, the present survey showed LEF to be a suitable immunomodulatory medicine that has been safely and effectively used in the therapy of rheumatoid arthritis in psoriatic arthritis, ankylosing spondylitis, secondary Sjögren’s syndrome polyoma BK virus nephropathy, and systemic lupus erythematosus (SLE). LEF is also as a rescue treatment in ganciclovir-resistant infection in kidney transplant recipients, and it is effective in the treatment of adult Henoch–Schönlein nephritis with nephrotic protein-urea. Many clinical trials have shown that some immunosuppressants combined with glucocorticoid have a good efficacy on membranous nephropathy (IMN) patients. LEF plus oral prednisone decreased proteinuria significantly. It may be a suitable alternative treatment option for Chinese patients with nephrotic IMN or with steroid-resistant or steroid-dependent MCD. It is also associated with a lower amount of prednisone required to maintain remission and with a lower rate of relapse than with CTX therapy. LEF is excreted in the urine via the kidney (43%) and in the feces via bile (48%). The chemical name of LEF is 5-methyl-N-[4-trifluoro-methylphenyl]-5-methyl-isoxazole-4-carboxamide. After oral administration, it was converted to an active metabolite (A77 1726) and to many minor metabolites. The active metabolite A77 1726 is responsible for all the in vivo activity of LEF. This metabolite is a potent non-cytotoxic inhibitor of the enzyme DHODH, a key enzyme in the de novo synthesis of uridine monophosphate (UMP), which is essential to provide precursors for new RNA and DNA synthesis. In vitro studies have indicated that cytochromes P450 (CYPs), including CYP1A2, CYP2C19, and CYP3A4, are taking part in LEF metabolite activation. LEF plays a role in immunoregulation via A77 1726. LEF is a selective inhibitor of de novo pyrimidine synthesis. It acts by inhibiting T-cell proliferation and by inhibiting tyrosine protein kinase activity and the formation of autoantibodies. It not only has antiproliferative action but also anti-inflammatory actions. These prevent activation and gene expression of nuclear factor (NF) kB, increase the production of immunosuppressive TGF-β protein, and inhibit the production of proinflammatory TNF and interleukin 1β. However, its use has also been associated with some adverse reactions involving diarrhea, liver toxicity, nephrotoxicity, myelosuppression, toxic
In this report, 52 patients who had been diagnosed with refractory NS were given different drugs. The patients in the LEF treatment group (26 cases) received therapy with glucocorticoid and LEF, while another 26 patients in the CTX control group received therapy with hormone treatment and CTX. Both therapies showed obvious ameliorative effects. Both groups can stop patients with RNS from undergoing glomerulosclerosis by protecting the kidneys. The current study shows the total effective rate of LEF combined with prednisone on the treatment of RNS was found to be 65.3%, which was higher than that of CTX combined with prednisone. The results were consistent with those of previous studies. The total efficacy rates of two groups showed no significant differences (p > .05). However, after treatment, the levels of 24 h urine protein and the levels of serum cholesterol decreased and the levels of serum albumin increased markedly in both groups, relative to pretreatment data. The values were statistically significant (p < .05). In the current study, none of the 26 patients in the LEF group ended drug treatment early. LEF may be both safe and more effective than CTX. It also showed a lower incidence of leukocytopenia and gonad-inhibition. This makes it a good choice for patients with fertility requirements. LEF was here administered orally, making it more convenient and conducive to patient compliance than other drugs that are responsible for the statistical analyses, and Dr. Yan Zhang, Lei Liu and Xiaolong Qu for assisting in the writing of the manuscript.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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