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GENERAL ACTEOSIDE OF REHMANNIAE LEAVES IN THE TREATMENT OF PRIMARY CHRONIC GLOMERULONEPHRITIS: A RANDOMIZED CONTROLLED TRIAL

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Abstract

The objective of the study was to investigate the effectiveness and efficacy of the randomized, parallel, and controlled trial of Traditional Chinese Medicine, general acteoside of Rehmanniae leaves, compared with piperazine ferulate in the treatment of primary chronic glomerulonephritis. Rehmanniae leaves and piperazine ferulate can reduce proteinuria and erythrocyturia effectively in the treatment of primary chronic glomerulonephritis. A total of 400 patients diagnosed with primary chronic glomerulonephritis were recruited from outpatient clinics and were randomly assigned to the treatment group (general acteoside of Rehmanniae leaves, two 200mg tablets, bid) or the control group (piperazine ferulate, four 50-mg tablets, bid). The primary outcome was 24-h urinary protein. Secondary outcome measures included estimated glomerular filtration rate (eGFR), erythrocyturia, and electrolytes. After 8 weeks of treatment, the treatment group and the control group showed a mean reduction in 24-h proteinuria of 34.81% and 37.66%. The 95% CI of difference of the mean reduction in 24-h proteinuria between the two groups was [-11.50%, 5.80%]. No significant differences were found between the two groups in the erythrocyturia reduction. Neither group showed obvious changes between baseline and 8 weeks in eGFR or electrolytes. Adverse events occurred at a similarly low rate in the treatment group (1.5%) and control group (2.5%, P = 0.7238). Both general acteoside of Rehmanniae leaves and piperazine ferulate can reduce proteinuria and erythrocyturia effectively in the treatment of primary chronic glomerulonephritis.

Key Words: Chronic glomerulonephritis; General acteoside of Rehmanniae leaves; Piperazine ferulate; Randomized controlled trials

Introduction

Chronic glomerulonephritis is one of the most common causes of end-stage renal disease among chronic kidney disease patients in China (Zhang et al., 2012). Although many approaches have been investigated, no single recommended treatment is established. Risk factors associated with poorer outcomes include urinary protein, and impaired renal function (Holtkamp et al., 2011). Proteinuria has proven to be the most important predictor of renal failure, and therapeutic efforts have been focused on its reduction (Wilmer et al., 2003).

Traditional Chinese Medicine – general acteoside – refers to several substances present in extracts of

Rehmanniae leaves. Studies in animal models of kidney disease have shown that general acteoside Rehmanniae leaf extract is associated with a reduction in urinary protein excretion (Shen et al., 2010b). In addition, clinical trials have demonstrated the ability of general acteoside from Rehmanniae leaf extracts to improve clinical symptoms of patients with chronic glomerulonephritis (Zhou et al., 2005).

Piperazine ferulate is the substrate in the extract of Traditional Chinese Medicine – ligustrazine. Studies in animal models of kidney disease showed that ligustrazine administration has been associated with a reduction in urinary protein excretion and improves the renal function (Liu et al. 2002, Yuan et al. 2012). Clinical trials show that ligustrazine can reduce proteinuria in patients with chronic glomerulonephritis (He et al., 2001; Hu et al., 2001; Liu et al., 2000). It has been widely used in clinical practice in China.

We designed this study to evaluate the efficacy and safety of an 8-week regimen of general acteoside in Rehmanniae leaf extract compared with piperazine ferulate in the treatment of chronic glomerulonephritis.

Materials and Methods

The present research was a prospective, randomized, parallel controlled study. The trial was conducted at West China Hospital, Xian Yang Central Hospital and Military 451 Hospital in China between October 2010 and November 2011. The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The local ethics committee reviewed the protocol and granted approval before the start of the study. The trial was registered with the Chinese Clinical Trial Registry (http://www.chictr.org) (Identifier: ChiCTR-TRC-10000988).

All eligible patients were informed about the purpose of the trial, the operative procedures and their risks, and treatment options. Written informed consent was obtained from all patients participating in the trial. Patients were eligible for enrolment if they were between 18 and 65 years old and had been diagnosed with chronic glomerular nephritis. Additional inclusion criteria were serum creatinine ≤256 μmol/L and systolic/diastolic blood pressure (BP) ≤ 140/90 mmHg. Patients were excluded if they had any of the following: diabetes mellitus; Henoch–Schoenlein purpura; systemic lupus erythematosus; active or recent (<1 year) treatment with immuno-suppressors; dialysis; kidney transplantation; 24-h urinary protein > 3g; pregnancy and lactation; or significant functional impairment of the brain, liver, cardiovascular, or haematopoietic systems.

Two hundred (200) subjects in each group were required to achieve 80% power at 0.05 significance level. Four hundred patients were randomly allocated with a ratio of 1:1 to either treatment group (general acteoside of Rehmanniae leaves, two 200mg tablets, bid) or control group (piperazine ferulate, four 50-mg tablets, bid). Randomisation was carried out using the Proc Plan Procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, USA).
Medical history, demographic characteristics and physical examination of the patients were recorded at baseline. Follow-up was conducted at 4 and 8 weeks of treatment. At baseline and 8 weeks of treatment, all patients were subjected to a haematology panel and urinalysis, and the following parameters were assessed: renal function, serum alanine aminotransferase (ALT), aspartate transaminase (AST), and electrolytes.

The primary outcome was the percent reduction in proteinuria between baseline and 8 weeks of treatment. Secondary outcome measures included the following: estimated glomerular filtration rate (eGFR), which was calculated using the simplified modification of diet in renal disease (MDRD) equation; erythrocyturia and electrolytes.

All eligible patients were asked whether they were willing to participate in the trial and informed about the purpose of the trial, the operative procedures, as well as their options and risks. Written informed consent was obtained from all patients participating in the trial.

All analyses were performed by using the software package SAS version 9.2. The level of statistical

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significance was set to 0.05. All analyses were based on an intention-to-treat (ITT) approach. Data were expressed as mean ± standard deviation (SD) for continuous variables and total number (percentual frequency) for categorical variables. Group t-test or Wilcoxon rank test was used for continuous variables. Chi-square test and Fisher's exact test were used for categorical variables.

Results

A total of 450 patients were screened and 400 patients were randomised: 200 patients to the treatment group and 200 patients to control group. A total of 6 patients lost to follow-up, of whom 1 was from the treatment group. The randomisation and completion of the study are detailed in Figure 1.

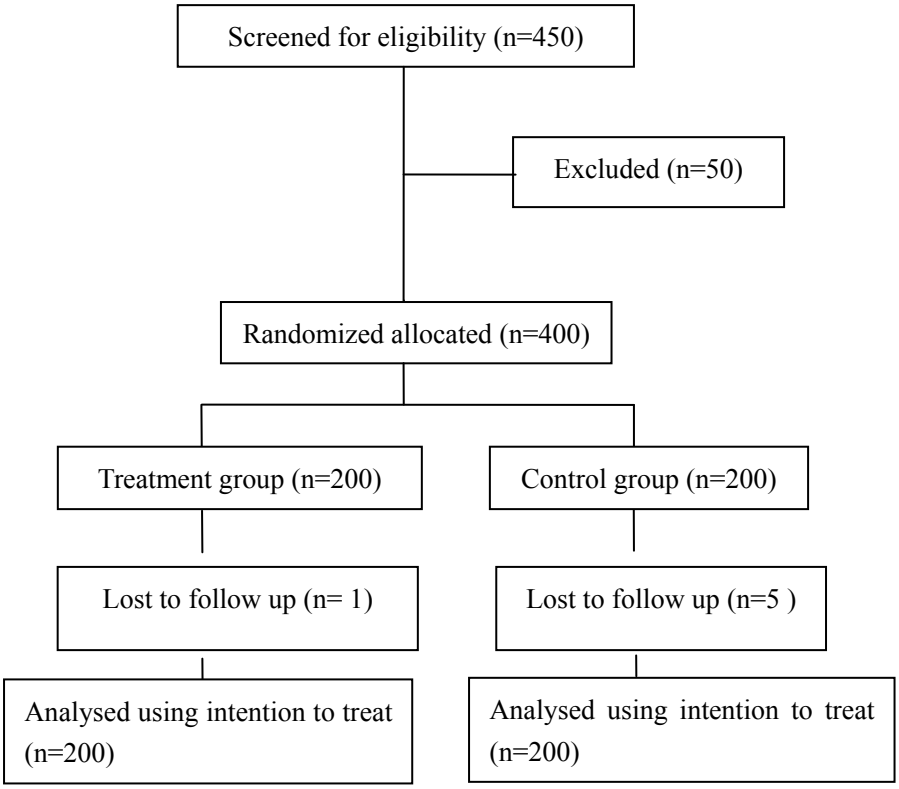

Figure 1: The flowchart showing the randomization and completion of the study

The baseline characteristics of the patients are presented in Table 1. There was no statistically significant difference between the two groups in any characteristics.

Eighty-two patients received renal biopsies. The morphological changes are shown in Table 2. This study excluded the glomerulonephritis patients with proliferative lesions at renal biopsy treated with glucocorticoids and immunosuppressive agents.

Table 3 shows the 24 hour protein results after 8 weeks. The treatment group and the control group showed a mean reduction in 24-h proteinuria of 34.81% and 37.66%. The 95% CI of difference of the mean reduction in 24-h proteinuria between the two groups was (-11.50%, 5.80%), which indicated that there was no statistical difference in the 24 h urine protein reduction between general acteoside of Rehmanniae leaves and piperazine ferulate in the treatment of patients with primary chronic glomerulonephritis.

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Table 4 shows the improvement of reducing the urinary erythrocyte excretion in both groups. No significant differences were found between the two groups in the erythrocyturia reduction.

Analysis of eGFR variation showed no significant differences in the two groups. There was no statistical difference between the two groups with regard to serum albumin, potassium, calcium and phosphate, see table 5. Overall, adverse events occurred at a similarly low rate in the treatment group (1.5%) and control group (2.5%, P = 0.7238). The adverse events experienced by patients in both groups were mild and transient. In the treatment group, one (0.5%) patient reported dizziness, one (0.5%) patient,fatigue; and one (0.5%) patient had a cold. In the control group, two (1%) patients reported mild stomach discomfort; one (0.5%) patient, thirsty; one (0.5%) patient, the stool is not forming; and one (0.5%) patient, joint pain. All these symptoms disappeared without treatment.

Table 1: Baseline characteristics of the treatment and control groups

Item	Treatment group	Control group	P
Age (years)	38.31±11.17	38.70±9.72	0.7096
Male sex, no. (%)	90(45.00)	98(49.00)	0.4229
Body weight (kg)	59.29±9.08	60.77±9.48	0.1122
Body mass index (kg/m^2)	22.25±2.74	22.68±2.91	0.0624
Length of disease (year)	5.35±5.33	4.93±5.05	0.6711
Urinary protein excretion (g/24h)	1.27±0.62	1.32±0.62	0.3032
eGFR (mL/min/1.73 m^2)	99.28±28.73	98.93±29.60	0.9058
Hemoglobin(g/L)	134.47±16.28	136.91±16.51	0.1369
Serum phosphate (mmol/L)	1.28±0.18	1.30±0.18	0.2635
Serum Calcium(mmol/L)	2.49±0.15	2.50±0.14	0.7696
Serum potasium(mmol/L)	4.03±0.33	4.02±0.37	0.7468
ALT(u/L)	24.08±8.60	24.27±9.45	0.8418
AST(u/L)	25.46±12.01	26.14±13.51	0.5986

*Values are written as mean (SD), unless otherwise indicated.

Abbreviations: HP, high power mirror field of vision; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate transaminase.

Table 2: Pathology changes of patients in the treatment and control groups

Pathology types	Treatment group (n=44)	Control group (n=38)
Mild Change	6 (13.6%)	4 (10.5%)
IgA Nephropathy (Lee's stage 1)	10 (22.7%)	9 (23.7%)
IgA Nephropathy (Lee's stage 2)	13 (29.5%)	12 (31.6%)
Mesangial Proliferative Glomerulonephritis (mild)	7 (15.9%)	8 (21.1%)
Membranous Nephropathy	3 (6.8%)	2 (5.3%)
Focal Segmental Glomerulosclerosis	5 (11.4%)	3 (7.9%)

Table 3: Changes in 24-h urinary protein after 4 and 8 weeks of treatment

Measurement*	**Treatment group**	**Control group**	**P-value****
Urinary protein (g/24 h)			
At baseline	1.27±0.62	1.32±0.62	0.3032
At week 4	0.93±0.45	0.93±0.50	—
P^1	<0.0001	<0.0001	—
At week 8	0.66±0.33	0.69±0.45	—
P^2	<0.0001	<0.0001	—
Percent change in urinary protein from baseline			
At week 4	14.21±42.00	21.70±41.83	0.1263
At week 8	34.81±44.56	37.66±43.40	0.5781

*****Values are written as mean ± SD.

**comparisons between groups; P^1, comparisons within groups from baseline to week 4; P^2, comparisons within

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groups from baseline to week 8; Abbreviations: HP, high power mirror field of vision

Table 4: Erythrocyturia changes after 4 and 8 weeks of treatment

Measurement*	Treatment group	Control group	P-value**
Erythrocyturia (/HP)			
At baseline	13.80±6.46	14.77±8.58	0.6656
At week 4	11.01±6.61	11.29±5.93	—
P^1	<0.0001	<0.0001	—
At week 8	8.28±8.49	8.60±8.45	—
P^2	<0.0001	<0.0001	—
Percent change in erythrocyturia from baseline			
At week 4	14.64±47.61	11.29±48.39	0.4859
At week 8	40.89±59.51	38.57±46.20	0.2600

*****Values are written as mean ± SD.

**comparisons between groups; P^1, comparisons within groups from baseline to week 4

P^2, comparisons within groups from baseline to week 8; Abbreviations: HP, high power mirror field of vision.

Table 5: Changes in eGFR, ALT, AST and electrolytes after 8 weeks of treatment

Parameter*	Treatment group			Control group		
	Baseline	Week 8	Difference**	Baseline	Week 8	Difference**
eGFR (mL/min/1.73 m^2)	99.28±28.73	107.95±29.23	8.67±37.39	98.93±29.60	108.62±32.42	9.68±37.52
ALT (IU/L)	24.08±8.60	23.11±9.55	-0.84±12.09	24.27±9.45	23.44±9.43	-0.87±13.23
AST (IU/L)	25.46±12.01	24.87±6.75	-0.59±13.78	26.14±13.51	25.27±7.29	-0.75±15.02
Serum potassium (mmol/L)	4.03±0.33	4.05±0.37	0.02±0.49	4.02±0.37	4.09±0.39	0.07±0.51
Serum calcium (mmol/L)	2.49±0.15	2.48±0.14	-0.00±0.17	2.50±0.14	2.48±0.12	-0.02±0.15
Serum phosphonium (mmol/L)	1.28±0.18	1.28±0.20	0.02±0.26	1.30±0.18	1.32±0.18	0.02±0.26

*****Values are written as mean ± SD.

**Differences between baseline and 8 weeks of treatment were similar for the two groups ($P > 0.05$).

Discussion

The pathogenesis of primary chronic glomerulonephritis is still unknown, and proteinuria is one of the most important risk factors for renal failure in chronic kidney disease patients. No specific treatment is established, although many approaches have been investigated.

General acteoside in extract of Rehmanniae leaves has therapeutic effect with a dose-response manner on accelerated nephrotoxic nephritis in rat (Shen et al., 2010a). However, very few clinical studies were reported (Zhou et al., 2005).

A significant reduction in proteinuria was obtained after two months of general acteoside. How acteoside in Rehmanniae leaf extract reduces proteinuria is still not well understood. Given that acteoside, an active phenylethanoid glycoside of Rehmanniae, displays anti-inflammatory properties in vitro (Yokozawa et al., 2004),

we speculate that the proteinuria reduction is due to the compound's anti-inflammatory and immunomodulatory effects. Erythrocyturia is a known marker of inflammation in chronic glomerulonephritis patients. Both general acteoside in extract of Rehmanniae leaves and piperazine ferulate medicines reduce erythrocyturia. Acteoside and similar compounds may inhibit the expression of cell adhesion molecules (CAMs) (Hwang et al., 2011), which may partially explain the role of glycoside of Rehmanniae leaves in chronic glomerulonephritis patients.

This study demonstrates that general acteoside in extract of Rehmanniae leaves can reduce proteinuria effectively in treating chronic glomerulonephritis patients for 8 weeks. The frequency of adverse events was similarly low with general acteoside (1.5%) as with piperazine ferulate (2.5%, P = 0.7238). This suggests that an 8-week regime of general acteoside is well tolerated.

However, there are some limitations. First, this is an open trial. Second, we excluded the glomerulonephritis patients with proliferative lesions at renal biopsy treated with glucocorticoids and immunosuppressive agents; hence, the choice of a selected group of chronic glomerulonephritis patients with mild or moderate histologic lesions and proteinuria, which may limit the applicability of this therapeutic intervention to certain patients. Therefore, further clinical studies with longer follow-up or with immunosuppressive agents-treated patients are necessary to evaluate the effectiveness of this treatment.

Conclusion

Both general acteoside of Rehmanniae leaves and piperazine ferulate can reduce proteinuria effectively in the treatment of primary chronic glomerulonephritis. Both medicines reduce erythrocyturia. Studies with proliferative nephritis and longer follow-up are necessary to confirm the effectiveness and the benefits of general acteoside of Rehmanniae leaves and piperazine ferulate in clinical practice.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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