Effects of Tripterygium wilfordii Hook F. induction therapy to IgA nephropathy patients with heavy proteinuria

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Medical history and demographic information were obtained from the Department of Nephrology, Yancheng Third People’s Hospital.
Abstract

Although some new drugs have been developed, Tripterygium wilfordii Hook F. (TWHF) has the merits of relatively lower price and fewer side effects. Unfortunately, the efficacy and safety of the TWHF (especially dosage 120mg/day) in the IgA nephropathy (IgAN) are still lacking. A cohort study including 49 IgAN patients with heavy proteinuria who received induction therapy was undertaken. Patients were divided into three groups: Prednisone (PRE), conventional-dose TWHF (CTW) and double-dose TWHF (DTW). The clinical features, laboratory data, histological manifestations and outcomes of the groups were compared. We found that urinary protein excretion and rates of elevated n-acetyl-β-d-glucosaminidase (NAG) and retinol binding protein (RBP) were prominent in all groups. Neither histopathological changes nor the rates of renal insufficiency were significantly different among groups. Patients in the PRE (69.2%) and DTW groups (87.5%) achieved complete remission; none of the CTW group did. Furthermore, the total remission rate of the DTW group was substantially higher than that of the CTW group. The degree of hypoproteinemia, improved considerably in the PRE and DTW groups. Treatment was well tolerated in all patients, and no serious adverse events were observed. Our findings suggested that induction therapy with double dose TWHF significantly improved response rates in IgAN patients with heavy proteinuria, and did not considerably increase side effects.

Key words  Proteinuria; IgA nephropathy; Chinese Traditional
Background:

IgA nephropathy (IgAN) is often progressive, and accounts for 45.26% of primary glomerular diseases in China \(^1, 2\). Patients with increased proteinuria have escalated mortality, enhanced cardiovascular events, and rapid decline in renal function \(^3\). Though corticosteroids are commonly used when there is heavy proteinuria, other immunosuppressive agents are frequently considered because of the side-effects associated with steroid use or their use is contraindicated\(^4\).

Tripterygium wilfordii Hook F. (TWHF), extracts of a perennial vine-like plant, have been used in China for centuries to treat a variety of inflammatory and autoimmune diseases \(^5, 6\). In 1977, Li et al\(^7\) first proposed the treatment of glomerulonephritis with TWHF, numerous Chinese IgAN patients had benefited from TWHF\(^8\). Our previous studies have suggested that oxidative stress was an important effector of tubule cell injury and podocyte injury \(^9, 10\). Recent researches indicated that triptolide, one of the major active components of TWHF, was proved to suppress reactive oxygen species generation and ameliorate podocyte injury \(^11, 12\). However, the application of purified triptolide was hampered by the more often and severe side effects \(^13\). Up to now, TWHF is still widely used in clinical practice. In our study, patients were treated with TWHF, not purified triptolide.

Although some new drugs have been developed, TWHF has the merits of relatively lower price and fewer side effects \(^14-16\). Efficiency and side-effects of TWHF are related to the dose, and the conventional therapeutic dose is 60mg/day, \(^17\). In Crohn’s Disease and diabetic nephropathy, the dose of TWHF was reported to as much as 120mg/day \(^18\). Unfortunately, the efficacy and safety of the TWHF (especially dosage 120mg/day) in the IgAN are still lacking.

To address these issues, a prospective cohort study of 49 IgAN patients with heavy proteinuria was conducted; the efficiency and side-effect profiles of double-dose TWHF used in induction treatment were well evaluated.
Material and methods:

Patients

This study was approved by the ethics committee of Yancheng Third People’s Hospital (No. 201107) and was registered at Chinese Clinical Trial Registry (ChiCTR-OPN-16010028). All patients or their legal representatives provided written informed consent. Forty-nine patients with biopsy proven IgAN and heavy proteinuria (≥3.5g/day), admitted to Yancheng Third People’s Hospital between January 2011 and December 2015, were recruited. Patients with other causes of IgA-positive glomerular staining, such as Henoch-Schönlein nephritis and lupus nephritis, were excluded. In addition, the coincidence of IgAN and minimal change disease confirmed by electron microscopy was also excluded from the study.

Treatment protocol

The cohort studied consisted of 49 IgAN patients with heavy proteinuria, and were divided into three groups according to the treatment regime they received: Prednisone (PRE) group (1mg/kg prednisone once daily, up to a maximum of 60 mg/day), conventional-dose TWHF (CTW) group (1mg/kg TWHF daily, up to a maximum of 60 mg/day, two 10-mg capsules three times daily) and double-dose TWHF (DTW) group (2mg/kg TWHF daily, up to a maximum of 120 mg/day, four 10-mg capsules three times daily). There was no intervention for research for all subjects. Usually, the oral prednisone was given at a dose of 1 mg/kg once daily for 6~8 weeks, and then the dose was tapered by 5–10 mg every two weeks. To avoid the possible side-effects, after induction therapy with TWHF for 6~8 weeks in DTW group, the dose of the TWHF was reduced to conventional-dose (1mg/kg TWHF daily, up to a maximum of 60 mg/day) in the maintenance phase. Nevertheless, the dose in CTW group remained unchanged. TWHF was provided as a 10-mg triptolide in a piece form by Mei-Tong Pharmaceutical Ltd. (Tai-Zhou, China). Typically, TWHF is used when steroids have failed. Low protein diets were generally prescribed in all patients. Participants were not required to restrict activities of daily living.

Data collection and measurements

After completion of the first follow-up, clinical features and laboratory data as well as the remission
rates were carefully investigated. Patient information was collected from their medical records and follow-up data. Urine and blood samples from individual patients were obtained from routine test results. Steroid-related factors were defined as steroid-dependence and steroid-resistance as well as contraindications or side effects of steroid. Total remission included complete remission and partial remission. Urinary protein excretion of $<0.4\text{g/day}$ was defined as complete remission, while urinary protein excretion of $>0.4\text{g/day}$ and a decrease of at least 50% was considered a partial remission. Microscopic hematuria was defined as the RBC of urinary sediment $>10 \times 10^4/\text{ml}$. Urinary n-acetyl-$\beta$-d-glucosaminidase (NAG) and retinol binding protein (RBP) were measured as markers of tubular injury.

**Renal pathology**

The biopsy specimens were processed for light microscopy, immunofluorescence study and electron microscopy. All renal biopsy specimens were reviewed by a single pathologist unaware of the patients’ clinical condition. Though the Oxford classification scheme is useful for evaluating chronic graft dysfunction in patients with posttransplantation IgAN, however, Hass and Oxford classifications were comparable in predicting progression of IgAN $^{19, 20}$. In the present study, glomerular pathological phenotypes were analyzed using the Haas' histological grading system for IgAN. Patients with subclasses I or II were considered mild cases; subclasses III, IV or V were considered severe cases.

**Statistical analysis**

Descriptive statistics for continuous variables were expressed as mean ± standard deviation (SD). Statistical evaluation of the continuous variables was made with one-way analysis of variance (ANOVA) with post-hoc Bonferroni's test for multiple comparisons. Categorical variables were expressed as proportions (percentage). A Kruskal-Wallis or Mann–Whitney nonparametric test was used for binomial variables. A p-value of $<0.05$ was considered significant. All data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA.).
Results:

General conditions

The baseline clinical features of the different treatment groups are presented in table 1. Disease duration of the DTW group was significantly longer than the PRE and CTW groups. Compared with the PRE group a higher proportion of both the CTW and DTW groups had previously received steroid and therefore had higher rates of steroid-related factors. The rate of renal insufficiency was lowest in the PRE group, although this difference was not statistically significant. Renin-angiotensin system (RAS) blocker, angiotensin-converting enzyme inhibitor (ACEI) or an angiotensin II receptor blocker (ARB), was commonly in combination for IgAN patients (Table 1). RAS blockers were generally titrated to be sure that the patients have the maximal tolerated dose. High rates of combined use of ACEI or ARB were observed in each group. There were no significant differences in the gender, age, rate of hypertension and liver function between the three groups.

Laboratory examinations

Results of urinary analysis and blood examinations are presented in table 2. Urinary protein excretion, NAG and RBP were significantly elevated in each group, with no intergroup differences. However, the percentage of patients with urinary sediment RBC was significantly lower in the PRE group than the CTW and DTW groups. The frequencies of anemia and elevated serum creatinine were non-significantly higher in both the CTW and DTW groups compared with the PRE group. Other blood examinations, including serum albumin, were not significantly different between the groups.

Histological features

Severe IgAN subclasses (subclass III, IV, and V) were the most frequent type of histological renal lesion in the population studied (Table 3). The percentage of patients with severe renal lesions was non-significantly higher in the CTW and DTW groups compared with the PRE group.

Follow-up and efficacy

Patients were followed up for a mean of 7.11±0.45 weeks. The follow-up time of the DTW group was significantly shorter than those of the PRE and the CTW groups (Table 4). Urinary protein
excretion decreased in all groups following treatment (Figure 1A). As shown in Figure 1B, serum albumin concentrations improved in all groups. Nevertheless, the degree of improvement was only significant in the PRE and DTW groups (86.7\% vs. 16.7\%, 88.9\% vs. 55.6\%, both p<0.05). As shown in Figure 1C, the percentage of patients with renal impairment in the PRE group decreases, while it increases in the other two groups. However, there was no significant difference between the percentage before and after induction therapy. The majority of the PRE (69.2\%) and DTW (87.5\%) groups achieved complete remission. None of the CTW group did. Further, total remission of the DTW group was significantly greater than that of the CTW group (100\% vs. 60\%, p<0.05) (Figure 1D).

**Safety**

During the induction therapy period, one patient in the PRE group developed Cushing’s syndrome. One patient in the DTW group experienced a temporary liver injury, alanine aminotransferase (123U/L) which recovered to normal within three weeks spontaneously (<40U/L). Overall, no serious adverse event was observed in any group.

**Discussion:**

IgAN has a variable clinical course, and the ultimate outcome can be unpredictable\(^21, 22\). Consistent with previous reports, this study demonstrated that IgAN occurs more common in the second and third decades of life\(^23\). However, the study did not have the expected male predominance; this may be related to the race of the study population or the limited sample size\(^24\). All patients included in this study had heavy proteinuria, a risk factor for the progression of CKD in IgAN\(^25\). Hematuria is also a marker which identifies IgAN patients at high risk of progression\(^26, 27\). In our study, the rates of hematuria in the CTW and DTW groups were higher than that of the PRE group.

Renal biopsy can provide critical information about the severity of the disease, the prognosis and guide treatment. Though the Oxford classification scheme is more useful for evaluating chronic graft dysfunction in patients with posttransplantation IgAN, however, Hass and Oxford classifications were comparable in predicting progression of IgAN\(^19, 20\). In our study, neither the histopathologic changes
nor the rates of renal insufficiency were significantly different among the three groups. Excessive amounts of proteinuria induced the expression of proinflammatory factors, including growth factors, cytokines, and chemokine, which lead to tubular damage\textsuperscript{28, 29}. In agreement with previous studies\textsuperscript{30}, the majority of the study patients had significant degrees of tubulointerstitial injury, increased NAG and RBP.

Though the best treatment of IgAN remains to be defined, the commonly used treatments include tonsillectomy, anti-platelet drugs, anticoagulants, prednisolone, immunosuppressants, fish oil, ACEI, and ARB\textsuperscript{31, 32}. ACEI and ARB provide marked antiproteinuric and long-term renoprotective effects in patients with IgAN\textsuperscript{33, 34}. In our study, most of the patients were commonly combined application of AECI/ARB, and this could partially account for the relatively higher remission rates than the previous reports\textsuperscript{31}.

In 1977, Li et al\textsuperscript{7} first proposed TWHF to treat glomerulonephritis. Cumulative evidence suggests that some Chinese herbal medicines, including triptolide and rhubarb, have a beneficial role in slowing the progression of CKD\textsuperscript{35, 36}. Due to the merits of resource abundance and cheap price, TWHF has been widely used in glomerulonephritis and kidney transplantation\textsuperscript{11, 37-39}. Triptolide is a major active ingredient of TWHF and has been shown to possess multiple biological activities. Triptolide was proved to be a potent inhibitor of VEGF expression and production in endothelial cells, which could contribute to its anti-proteinuria effect in Thy1.1 glomerulonephritis and puromycin-induced nephrosis\textsuperscript{11, 40, 41}. In cultured mouse podocytes, triptolide pretreatment suppressed reactive oxygen species generation and p38 mitogen-activated protein kinase activation while restoring RhoA signaling activity\textsuperscript{42}. Our previous studies have confirmed that increased oxidative stress led to prominent tubule cell injury and podocyte damage\textsuperscript{9, 10}.

Efficiency and side-effects of TWHF are related to the dose, and the conventional therapeutic dose is 60mg/day,\textsuperscript{17}. TWHF at dosages up to 570 mg/day has been reported to be well tolerated in patients with rheumatoid arthritis\textsuperscript{43}. To the best of our knowledge, the present study was a first step toward TWHF (120mg/day) used in IgAN. In our study, both the total remission and the complete remission rates were similar between the PRE and the DTW groups. Both groups had significantly higher
remission rates than the CTW group. Hypoproteinemia was corrected equivalently in the PRE and DTW groups. The serum albumin improvement of the PRE group was significantly better than the CTW group. Outcome after induction therapy showed the DTW groups had higher frequency of nephritic dysfunction than the PRE group, this might due to its relatively elevated frequencies of nephritic dysfunction and more severe type of histological renal lesion before induction therapy. Results obtained in this study indicate that double dose TWHF is more efficacious than the conventional therapeutic dose in IgAN patients with significant proteinuria.

It is noteworthy that the side-effects of TWHF are related to both the dose used and the processing methods used. The major side effects of the different preparations of TWHF include gastrointestinal tract disturbances, particularly diarrhea, skin rashes and pigmentation, decrease in blood cell counts, and malfunction of the male and female reproductive system\(^ {44}\). It should be pointed out that most of these adverse reactions cease either spontaneously or after dose adjustment. Importantly, treatment-related death has rarely been seen\(^ {37}\). In kidney transplant recipients, Ji et al\(^ {45}\) reported that the prevalence of elevated liver enzymes secondary to TWHF was similar with both conventional and double dose. In the present study, one patient developed liver dysfunction secondary to TWHF; this recovered spontaneously. The lower overall toxicity of TWHF seen in this study could be related to the method of TWHF preparation.

Although this study was a retrospective analysis, a novel therapeutic regimen (120mg/day) with the aim of testing its effect was introduced on IgAN patients. However, further prospective, randomized controlled trials are now needed to clarify the efficacy and the safety of DTW induction therapy in patients with IgAN and significant proteinuria.

Conclusions:

Induction therapy with double dose TWHF significantly improved response rates in IgAN patients with heavy proteinuria, and did not significantly increase side effects. A randomized controlled trial is now required to further evaluate the optimal dose of TWHF in IgAN.

Acknowledgements:

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**Conflict of interest:** TWHF was purchased form Mei-Tong Pharmaceutical Ltd. (Tai-Zhou, China) as a 10 mg triptolide in a piece.
References:

20) Lim BJ, Joo DJ, Kim MS, Kim YS, Kim SI, Kim Y, Huh KH, Koh MJ, Jeong HJ: Usefulness of Oxford...


### Tables

#### Table 1 Clinical features of the study populations before induction therapy

<table>
<thead>
<tr>
<th></th>
<th>PRE group (n=15)</th>
<th>CTW group (n=16)</th>
<th>DTW group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>6 (40)</td>
<td>8 (50)</td>
<td>10 (55.6)</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>9 (60)</td>
<td>8 (50)</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>33.5 ± 10.6</td>
<td>32.5 ± 11.6</td>
<td>33.6 ± 11.8</td>
</tr>
<tr>
<td><strong>Disease duration (months)</strong></td>
<td>4.3 ± 4.5</td>
<td>12.1 ± 8.4</td>
<td>23.6 ± 18.2*†</td>
</tr>
<tr>
<td><strong>Hypertension(^a)</strong></td>
<td>5 (33.3)</td>
<td>2 (12.5)</td>
<td>5 (27.8)</td>
</tr>
<tr>
<td><strong>Renal insufficiency(^b)</strong></td>
<td>2 (13.3)</td>
<td>6 (37.5)</td>
<td>7 (38.9)</td>
</tr>
<tr>
<td><strong>Liver dysfunction</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Steroid-related factors(^c)</strong></td>
<td>0 (0)</td>
<td>13 (81.3) *</td>
<td>17 (94.4) *</td>
</tr>
<tr>
<td><strong>RAS blocker(^d)</strong></td>
<td>12 (80)</td>
<td>16 (100)</td>
<td>18 (100)</td>
</tr>
</tbody>
</table>

Data are expressed as n (%) or means ± SD. *significantly different versus PRE group; †significantly different versus CTW group.

Hypertension\(^a\): systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg. Renal insufficiency\(^b\): SCr > 1.24 mg/dl. Steroid-related factors\(^c\): steroid-dependence and steroid-resistance as well as contraindications or side effects of steroid. RAS blocker\(^d\): combined use of an angiotensin-converting enzyme inhibitor (ACEI) or an angiotensin II receptor blocker (ARB).
Table 2  Laboratory examinations before induction therapy

<table>
<thead>
<tr>
<th></th>
<th>PRE group (n=15)</th>
<th>CTW group (n=16)</th>
<th>DTW group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine protein (g/day)</td>
<td>7.19±3.65</td>
<td>5.02±1.40</td>
<td>5.29±2.35</td>
</tr>
<tr>
<td>RBC of Urinary sediment (&gt;10×10⁴/ml)</td>
<td>3(20.0)</td>
<td>12(75.0) *</td>
<td>15(83.3) *</td>
</tr>
<tr>
<td>NAG (&gt;16.5u/g.cr)</td>
<td>13(86.7)</td>
<td>15(93.8)</td>
<td>16(88.9)</td>
</tr>
<tr>
<td>RBP (&gt;0.5mg/L)</td>
<td>8(53.3)</td>
<td>8(50.0)</td>
<td>10(55.6)</td>
</tr>
<tr>
<td>Hb(&lt;11g/dl)</td>
<td>0(0)</td>
<td>5(31.3)</td>
<td>3(16.7)</td>
</tr>
<tr>
<td>Alb(&lt;35g/L)</td>
<td>13(86.7)</td>
<td>10(62.5)</td>
<td>16(88.9)</td>
</tr>
<tr>
<td>SCr(&gt;1.24mg/dl)</td>
<td>2(13.3)</td>
<td>6(37.5)</td>
<td>7(38.9)</td>
</tr>
</tbody>
</table>

Data are expressed as n (%) or means ± SD. *significantly different versus PRE group.
Table 3 Hanss’s Histologic Subclassification of IgA Nephropathy before induction therapy

<table>
<thead>
<tr>
<th></th>
<th>PRE group (n=15)</th>
<th>CTW group (n=16)</th>
<th>DTW group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Subclasses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subclass I</td>
<td>4 (26.7)</td>
<td>0 (0)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>Subclass II</td>
<td>6 (40)</td>
<td>5 (31.3)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>Subclass III</td>
<td>2 (13.3)</td>
<td>6 (37.5)</td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>Subclass IV</td>
<td>3 (20)</td>
<td>5 (31.3)</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>Severe subclasses</td>
<td>11 (73.3)</td>
<td>16 (100)</td>
<td>15 (83.3)</td>
</tr>
</tbody>
</table>

Data are expressed as n (%). Mild subclasses: Subclass I and Subclass II. Severe subclasses: subclass III, subclass IV and subclass V.
<table>
<thead>
<tr>
<th></th>
<th>PRE group (n=15)</th>
<th>CTW group (n=16)</th>
<th>DTW group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up time(weeks)</td>
<td>7.03±1.43</td>
<td>8.18±2.11</td>
<td>6.76±1.88*†</td>
</tr>
<tr>
<td>Urine protein a (g/day)</td>
<td>0.87±1.32</td>
<td>2.47±1.68*</td>
<td>0.99±.055†</td>
</tr>
<tr>
<td>Partial remission</td>
<td>3(23.1)</td>
<td>9(60) *</td>
<td>2(12.5) †</td>
</tr>
<tr>
<td>Complete remission</td>
<td>9(69.2)</td>
<td>0(0) *</td>
<td>14(87.5) †</td>
</tr>
<tr>
<td>Total remission</td>
<td>12(92.3)</td>
<td>9(60)</td>
<td>16(100) †</td>
</tr>
<tr>
<td>Alb (&lt;35g/L)</td>
<td>2(16.7)</td>
<td>9(60) *</td>
<td>10(55.6)</td>
</tr>
<tr>
<td>SCr a (&gt;1.24mg/dl)</td>
<td>1(7.7)</td>
<td>6(40)</td>
<td>8(44.4) *</td>
</tr>
<tr>
<td>Liver dysfunction</td>
<td>0(0)</td>
<td>0(0)</td>
<td>1(5.6)</td>
</tr>
</tbody>
</table>

Data are expressed as n (%) or means ± SD. *Significantly different versus PRE group; †significantly different versus CTW group.

*: Because laboratory examinations (including urine protein, Alb and SCr) were not performed for all patients at the first follow-up, the actual numbers in the PRE/CTW/DTW group were: 13/15/16(urinary protein); 12/15/18(Alb); 13/15/18(SCr) respectively.
Figure 1. Results of induction therapy. (A) The urinary protein decreased significantly in all groups. (B) The serum albumin concentrations improved in all groups, while the degree of improvement was only significant in the PRE and DTW groups (86.7% vs. 16.7%, 88.9% vs. 55.6%, respectively; both p<0.05). (C) The percentage of patients with renal insufficiency decreased in the PRE group and increased in the other two groups, however, there was no significant difference. (D) The total remission of the DTW group was significantly greater than that of the CTW group (100% vs. 60%, p<0.05). Since measurement of urinary protein was not performed for all patients, the actual numbers were: 13 (PRE group), 15 (CTW group), 16 (DTW group).