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Benefit of an Extract of *Tripterygium Wilfordii* Hook F in Patients With Rheumatoid Arthritis

A Double-Blind, Placebo-Controlled Study

Xuelian Tao,[1] Jean Younger,[2] Fred Z. Fan,[1] Betty Wang,[3] and Peter E. Lipsky[1]

Objective. **To examine the safety and efficacy of an extract of *Tripterygium wilfordii* Hook F (TWHF) in the treatment of patients with rheumatoid arthritis (RA).**

Methods. **An ethanol/ethyl acetate extract from the roots of TWHF was prepared and used in a prospective, double-blind, placebo-controlled study in patients with longstanding RA in whom conventional therapy had failed. Patients were randomly assigned to receive either placebo or low-dose (180 mg/day) or high-dose (360 mg/day) extract for 20 weeks, followed by an open-label extension period. Clinical responses were defined as 20% improvement in disease activity according to the American College of Rheumatology criteria. Side effects were actively queried and recorded at each visit.**

Results. **A total of 35 patients were enrolled in the trial; 21 patients completed the 20-week study. One patient from each group withdrew because of side effects. Twelve, 10, and 10 patients in the placebo, low-dose, and high-dose groups, respectively, completed at least 4 weeks of treatment. Of these patients, 8 and 4 in the high-dose and low-dose groups, but none in the placebo group, met criteria for clinical response. Four, 4, and 7 patients in the placebo, low-dose, and high-dose groups, respectively, were enrolled in the open-label extension; of these, 2, 4, and 5 patients, respectively, met criteria for clinical response. The most common side effect was diarrhea, which caused 1 patient in the high-dose group to withdraw from the trial. No patients withdrew because of adverse events during the open-label extension.**

Conclusion. **The ethanol/ethyl acetate extract of TWHF shows therapeutic benefit in patients with treatment-refractory RA. At therapeutic dosages, the TWHF extract was well tolerated by most patients in this study.**

Extracts of *Tripterygium wilfordii* Hook F (TWHF) have been widely used in China to treat a broad spectrum of autoimmune and inflammatory diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), ankylosing spondylitis, psoriasis, and idiopathic IgA nephropathy. Results from most of the clinical trials of TWHF have claimed significant therapeutic benefit, although nearly all of the trials were uncontrolled, as mandated by Chinese custom (1).

Initially, a crude water extract (decoction) of TWHF was used, and the dose was adjusted based on the weight of the plant material from which the extract was made (2,3). Patients treated with the decoction of TWHF appeared to experience therapeutic benefit, but frequently developed adverse effects and, occasionally, severe toxicity (4–6). Subsequently, efforts were made to improve the extraction procedure in order to minimize toxicity and maximize therapeutic benefit. Toward this end, a variety of extraction methods were employed, including ethanol (7,8), ethanol/ethyl acetate (9,10), and a proprietary procedure yielding a polyglycoside preparation (11). Among these, the ethanol/ethyl acetate extract and the polyglycoside preparation have been

[1]Xuelian Tao, MD, Fred Z. Fan, MD, Peter E. Lipsky, MD: Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland; [2]Jean Younger, RN: Harold C. Simmons Arthritis Center, The University of Texas Southwestern Medical Center at Dallas; [3]Betty Wang, PhD: Clinical Center, NIH, Bethesda, Maryland.

Address correspondence and reprint requests to Peter E. Lipsky, MD, Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Building 10, Room 9N228, 9000 Rockville Pike, Bethesda, MD 20892. E-mail: lipskyp@mail.nih.gov.

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claimed to exert better therapeutic effects and cause fewer adverse events; these preparations have therefore been most widely used in China recently (12,13).

Experiments in animal models have demonstrated that both extracts inhibit various experimental models of arthritis, as well as nonspecific inflammation, delayed-type hypersensitivity responses, and primary antibody responses (14–17). These results suggested that the extracts exerted both antiinflammatory and immunosuppressive effects. Two uncontrolled clinical trials of the ethanol/ethyl acetate extract of TWHF in the treatment of more than 400 patients with RA have been reported in China (10,12). In these uncontrolled trials, response rates as high as 95% were claimed using various outcome measures. The frequencies of side effects ranged between 4% and 35%. The most common side effects were gastrointestinal tract disturbances and amenorrhea, both of which resolved after the ethanol/ethyl acetate extract of TWHF was tapered or stopped. These results were similar to the results of a double-blind controlled clinical trial of the polyglycoside preparation of TWHF (18).

To evaluate the Chinese experience, we prepared an ethanol/ethyl acetate extract of TWHF and carried out an open-label phase I dose-escalation study in 13 patients with established RA. The results of that trial suggested that the ethanol/ethyl acetate extract of TWHF at dosages of up to 570 mg/day appeared to be tolerated by all patients, except one who developed diastolic hypertension. The patient's blood pressure returned to normal after discontinuing the extract. Dosages higher than 360 mg/day were associated with clinical benefit in 8 of 9 patients in the trial (19).

The active components responsible for the therapeutic and adverse effects of the preparations of TWHF have not been completely delineated. A diterpenoid in TWHF, triptolide, has been shown to be one of the major components responsible for both effects (20,21). Tripdiolide, another diterpenoid, was also found to exert an immunosuppressive and antiinflammatory effect similar in potency to that of triptolide, both in vitro and in vivo (21,22). Our previous studies found that the sum of the content of the two diterpenoid components accounted for most of the in vitro and in vivo activities of the extracts of TWHF (21). We have therefore used the content of the two diterpenoid components as one of the means to adjust the dosage of the ethanol/ethyl acetate extract for clinical trials.

The current double-blind controlled clinical trial was designed to test the hypothesis that the ethanol/ethyl acetate extract suppresses rheumatoid inflammation. We found that RA patients treated with the ethanol/ethyl acetate extract at a dosage of 360 mg/day improved significantly compared with patients receiving placebo, and that this dosage of the extract was safe.

PATIENTS AND METHODS

Patients. All patients in the trial were from the arthritis clinic of Parkland Memorial Hospital (Dallas, TX). Before entry into the trial, the details of the trial were discussed with the patients, and all patients signed an informed consent form. Patient enrollment occurred between March 1998 and March 2000. Eligible patients were 18–75 years of age and had had a diagnosis of RA, according to the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria (23), for at least 1 year.

For entry into the trial, patients were of functional class II, III, or IV and had active disease (24) despite treatment with disease-modifying antirheumatic drugs (DMARDs). Active disease was defined as $\geq$2 swollen joints and 2 of the following 3 features: $\geq$6 painful/tender joints, morning stiffness lasting for $\geq$30 minutes, and an erythrocyte sedimentation rate (ESR) of $\geq$28 mm/hour. Patients were required to have failed an adequate treatment course with at least 1, but no more than 4, DMARDs. The most recent of these must have been discontinued for at least 4 weeks before entering the trial. Those taking glucocorticoids (prednisone $\leq$7.5 mg/day) and/or nonsteroidal antiinflammatory drugs must have been taking a stable dosage for at least 4 weeks before entering the trial and were required to take the same dosage throughout the trial. Women of childbearing age and potential must have had negative findings on a pregnancy test performed at study screening, have agreed to use a medically approved form of contraception, and have agreed to continue that contraception for at least 1 month following the last dose of the study medication.

The study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas, as well as by the Food and Drug Administration (Investigational New Drug no. 41,836).

Preparation of the ethanol/ethyl acetate extract of TWHF. The ethanol/ethyl acetate extract of TWHF was prepared at the University of Texas Southwestern Medical Center at Dallas. The dosage was normalized to previous extracts by assessing the content of triptolide and tripdiolide as described elsewhere (19). The ethanol/ethyl acetate extract was prepared as previously described (25). Briefly, the skinned roots of TWHF were ground to a powder that was sequentially extracted with ethanol and ethyl acetate. The resultant ethanol/ethyl acetate extract was analyzed for the content of triptolide and tripdiolide by high-performance liquid chromatography and bioassay as described previously (21). Batches of the ethanol/ethyl acetate extract used for the current and previous studies were compared, and the in vivo acute toxicity in rodents and the in vitro bioactivity correlated with the level of triptolide and tripdiolide in both batches.

After adding an appropriate amount of starch, the ethanol/ethyl acetate extract was formulated into capsules. Capsule formulation of the ethanol/ethyl acetate extract of

Table 1. Characteristics of patients at baseline*

	Placebo (n = 12)	TWHF extract	
		Low-dose (n = 12)	High-dose (n = 11)
No. of women	12	8	8
Age, mean ± SD years	51 ± 15	54 ± 12	57 ± 8
Race, no. of patients			
Caucasian	6	9	7
Hispanic	3	0	1
African American	3	2	2
Disease duration, mean ± SD years	14 ± 15	14 ± 11	20 ± 10†
Functional class, mean	2.5	2.1	2.2
Previous use of DMARDs, no. of patients	12	12	11
Concomitant therapy at baseline			
NSAIDs	10	12	8
Glucocorticoid	7	4	10
Prednisone dose, mean ± SD mg/day	6 ± 2	5 ± 1	6 ± 1
No. of tender joints, mean ± SD	27 ± 15	19 ± 14	19 ± 8
No. of swollen joints, mean ± SD	15 ± 8	13 ± 6	14 ± 5
ESR, mean ± SD mm/hour	47 ± 29	39 ± 25	40 ± 13
CRP, mean ± SD μg/ml	2.8 ± 2.6	1.4 ± 0.8	2.3 ± 1.7

* TWHF = *Tripterygium wilfordii* Hook F (ethanol/ethyl acetate extract); DMARDs = disease-modifying antirheumatic drugs; NSAIDs = nonsteroidal antiinflammatory drugs; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.
† $P < 0.01$ versus the low-dose extract group and versus the placebo group.

TWHF and the placebo was done by Analytical Research Laboratories (Edmond, OK). Each capsule contained 60 mg of the extract, which included 30 μg of the sum of triptolide and tripdiolide. Except for the replacement of the ethanol/ethyl acetate extract with cocoa powder, the placebo capsules were made in the same manner as the experimental drug and were identical in appearance.

Treatment plan. Two doses were used. The high dose (360 mg/day) was equivalent to the highest dose that was effective and tolerated by 88% of RA patients in the previous phase I study (19). The low dose was half of the high dose (180 mg/day). According to a randomization block provided by the office of Investigational Drug Service at Parkland Memorial Hospital, patients were randomly assigned to 1 of the following 3 treatment groups: 2 capsules of placebo 3 times a day (placebo group), 1 capsule of placebo and 1 capsule of the ethanol/ethyl acetate extract 3 times a day (low-dose group), or 2 capsules of the ethanol/ethyl acetate extract 3 times a day (high-dose group). The treatment course was 20 weeks.

Patients who completed the full course of the double-blind trial were given the option of receiving treatment with the ethanol/ethyl acetate extract of TWHF during an open-label extension. During the open-label period, the extract was given at a dosage of 180 mg/day or 360 mg/day (1 or 2 capsules, respectively, 3 times a day).

Based upon the assumptions that there would be a 20% dropout rate by week 20, that the placebo response would be ≤20%, and that the response to the TWHF extract would be ≥60%, and using an alpha level of 0.05, a total of 90 patients would be necessary to generate a meaningful result with a power of 0.80. However, because of relocation of the investigators, the trial was terminated after 35 patients were enrolled.

Assessment of clinical response. Clinical and laboratory variables were examined at screening, baseline, and every 4 weeks thereafter throughout the treatment course, including the open-label extension period. Disease activity was evaluated by an independent assessor, who was blinded to the treatment plan and had no knowledge of the study medication. Disease activity and treatment response were evaluated according to the ACR criteria (23). In addition, the duration of morning stiffness and serum titers of rheumatoid factor (RF; measured by nephelometry) were assessed.

Assessment of adverse reactions. In addition to followup every 4 weeks, adverse effects were assessed beginning at 2 weeks after the treatment was started. Patients were actively queried for adverse events at each visit. When an adverse event was reported, followup questions about the severity and timing relative to the administration of the treatment were asked. Blood pressure, complete blood cell counts, liver enzyme levels, urinalysis, and serum creatinine levels were evaluated before study entry and every 4 weeks after the beginning of the treatment.

Statistical analysis. The statistical analyses were carried out using a modified intent-to-treat approach that included all patients who received at least 4 weeks of the study drug. All hypotheses were tested against a 2-sided $\alpha = 0.05$ significance level. *P* values were reported without adjustment for multiple comparisons. In all measures of disease activity, a last observation carried forward approach was used for values that were missing because of missed visits or early dropout. Between-group differences in the percentages of patients achieving an ACR 20% response were analyzed by the

Table 2. Withdrawals and treatment completions during each phase of the trial

	Placebo (n = 12)	TWHF extract	
		Low-dose (n = 12)	High-dose (n = 11)
Double-blind phase			
No. of withdrawals	6	5	3
Reason for withdrawal			
Lack of efficacy	4	2	0
Side effects	1	1	1
Unrelated	1	1	2
Unknown	0	1	0
Treatment period completed			
4 weeks	12	10	10
20 weeks (full trial period)	6	7	8
Open-label phase			
No. of patients entered	4	4	7
Duration of treatment received			
One year	1	0	0
≥20 weeks	3	3	6
≥8 weeks	0	1	1
Reason for early termination			
Lack of efficacy	0	0	1
Adverse effects	0	0	0
Drug shortage	3	4	6

* TWHF = *Tripterygium wilfordii* Hook F (ethanol/ethyl acetate extract).

Cochran-Armitage exact test. For other categorical variables (e.g., sex), between-group differences were analyzed using Fisher's exact test. For continuous variables (e.g., each component of the ACR 20% improvement criteria), between-group differences were analyzed by the Kruskal-Wallis test or the Wilcoxon rank sum exact test, and within-group differences were analyzed by the Wilcoxon signed rank test.

RESULTS

Characteristics of the patients. Thirty-five patients were enrolled in the trial, 12 in the placebo group, 12 in the low-dose extract group, and 11 in the high-dose extract group. The initial characteristics of the patients and concomitant medications at baseline are shown in Table 1. There were no significant differences among the 3 treatment groups in the variables at study entry, except that there were more males in the 2 groups taking the extract than the placebo group ($P = 0.0146$) and patients in the high-dose extract group had longer disease duration than the patients in either the low-dose extract or the placebo group ($P < 0.01$).

Six (50%), 7 (58.3%), and 8 (72.7%) patients in the placebo, low-dose extract, and high-dose extract groups, respectively, completed the 20-week treatment course (Table 2). Four patients in the placebo group withdrew because of lack of therapeutic effect, 1 withdrew because of side effects, and 1 withdrew for reasons unrelated to the study medication. In contrast, of the 3 patients in the high-dose group who withdrew, 1 was because of side effects and 2 were for reasons unrelated to the study medication. In the low-dose group, side effects and worsening disease caused 1 and 2 patients to discontinue the study; 2 other patients in this group discontinued the treatment for unknown or unrelated reasons.

Efficacy. Twelve, 10, and 10 patients in the placebo, low-dose extract, and high-dose extract groups, respectively, completed 4 weeks of treatment. Of these patients, 8 in the high-dose group (80%) and 4 in the low-dose group (40%) experienced disease improvement, fulfilling the ACR 20% improvement criteria. In contrast, none of the patients in the placebo group attained these criteria (Figure 1). The difference between the high-dose and placebo groups was statistically significant ($P = 0.0001$). Five patients in the high-dose group (50%) and 1 patient in the low-dose group (10%) met the ACR 50% improvement criteria. One patient in the high-dose group (10%), but none in either the low-dose or the placebo group met the ACR 70% improvement criteria.

In the high-dose group, patients achieved an ACR 20% response rapidly, with 50% of them improving during the first 4 weeks of treatment. Of the 4 patients in the low-dose group who achieved an ACR 20% response, 1 occurred within 4 weeks, 2 within 12 weeks, and the fourth after 20 weeks of treatment. The mean duration of treatment to reach an ACR 20% response was 7 weeks and 12 weeks for the high-dose and the low-dose groups, respectively.

Similar results were obtained when the individual components of the ACR 20% improvement criteria were analyzed for each group. As shown in Figure 2, the

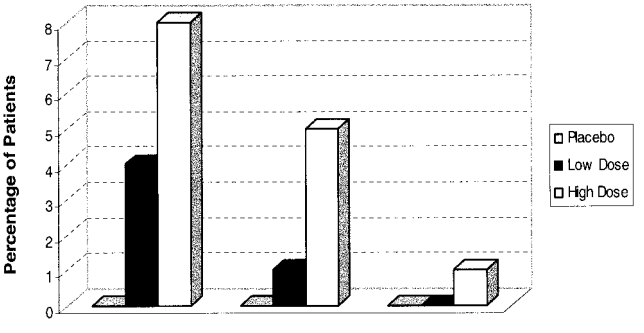

Figure 1. Response to treatment, as evaluated by the American College of Rheumatology 20%, 50%, and 70% improvement criteria, respectively, in the 3 treatment groups.

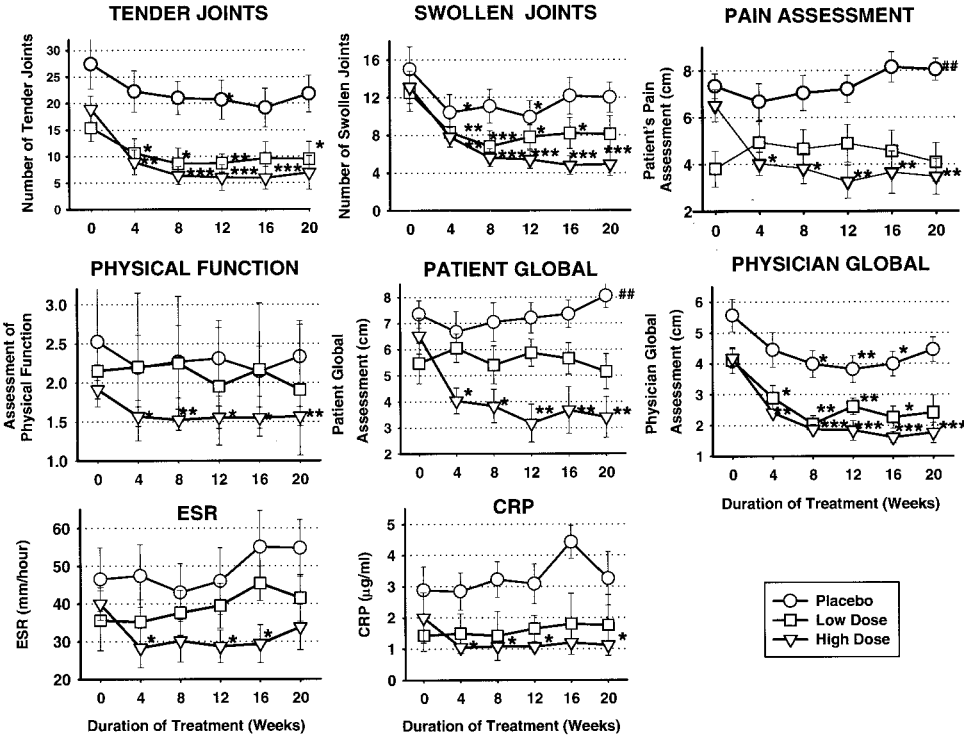

Figure 2. Changes in individual measures of disease activity in the 3 treatment groups. Student's *t*-test was employed to evaluate differences in individual measures at each visit versus corresponding baseline of the same group of patients. Values are the mean ± SEM. $* = P < 0.05$, $** = P < 0.01$, and $*** = P < 0.005$ versus baseline for the variables that decreased and $\#\# = P < 0.01$ versus baseline for the variables that increased, by Student's *t*-test. ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.

number of tender joints, number of swollen joints, patient's assessment of pain, patient's and physician's global assessments of disease activity, and patient's assessment of physical function improved significantly in the high-dose group from the first visit at 4 weeks and throughout the trial. Significant decreases were also found in the number of tender joints, number of swollen joints, and the physician's global assessment in the low-dose group. In the placebo group, decreases were occasionally seen in the number of tender joints, the number of swollen joints, and physician's global assessment. However, the changes were not consistent and none of them reached statistical significance at the end of the trial. In contrast, patient's assessment of pain and patient's global assessment of disease activity were significantly increased compared with baseline in the placebo group ($P < 0.01$ for both variables).

Decreases in the ESR and C-reactive protein (CRP) levels were observed in the high-dose group. Decreases in CRP levels at all time points except 16 weeks and decreases in ESR at 4, 12, and 16 weeks were significant ($P < 0.05$) compared with baseline (Figure 2). In contrast, ESR and CRP values tended to increase in the patients treated with placebo.

Between-group comparisons of the differences between the values at baseline and at each visit for the individual variables of the ACR improvement criteria were performed. Significant decreases in all 8 variables were observed in the high-dose group compared with the placebo group.

Along with the variables included in the ACR 20% improvement criteria, morning stiffness and RF titers were also evaluated (Figure 3). As with the other clinical parameters, morning stiffness significantly decreased from a mean of 145 minutes at baseline to 37 minutes at the 4-week visit and further decreased to 26 minutes at the 20-week visit in the high-dose group. Significant decreases in RF titers were observed after 20 weeks in the high-dose group ($P < 0.05$). In contrast, RF titers in the placebo group fluctuated and increased significantly at the 20-week followup compared with baseline ($P < 0.01$).

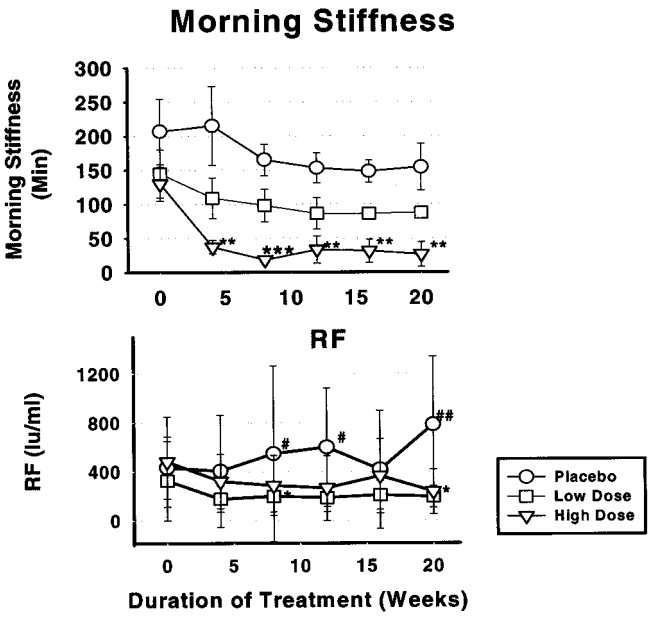

Figure 3. Changes in morning stiffness and titers of rheumatoid factor (RF) in the 3 treatment groups. Student's *t*-test was employed to evaluate differences in individual variables measured at each visit versus corresponding baseline of the same group of patients. Values are the mean ± SEM. $* = P < 0.05$ and $** = P < 0.01$ versus baseline for the variables that decreased and $\# = P < 0.05$ and $\#\# = P < 0.01$ versus baseline for the variables that increased, by Student's *t*-test.

Open-label extension. Four, 4, and 7 patients in the placebo, low-dose extract, and high-dose extract groups were enrolled in the open-label extension. During the extension period, all patients received 360 mg/day, except for 2 patients who completed 20 weeks of treatment with high-dose extract during the double-blind trial and then were changed to the low-dose extract (180 mg/day) during the open-label extension (Table 2). Two, 4, and 5 patients in the placebo, low-dose, and high-dose groups achieved an ACR 20% response. For the 6 patients who were initially in the placebo and low-dose groups, the mean treatment time to achieve 20% improvement was 7 weeks (Figure 4).

The two patients who were changed from the high-dose to the low-dose group after entering the open-label trial continued to fulfill ACR criteria for 20% improvement. One patient in the initial high-dose group who was taking high-dose extract during the open-label extension also maintained a response until he underwent lithotripsy for a kidney stone; his arthritis flared after the procedure, although he continued taking the study medication.

Adverse events. All study patients were evaluated for adverse events. The frequencies of patients who developed ≥1 adverse event were 4 of 12, 6 of 12, and 5

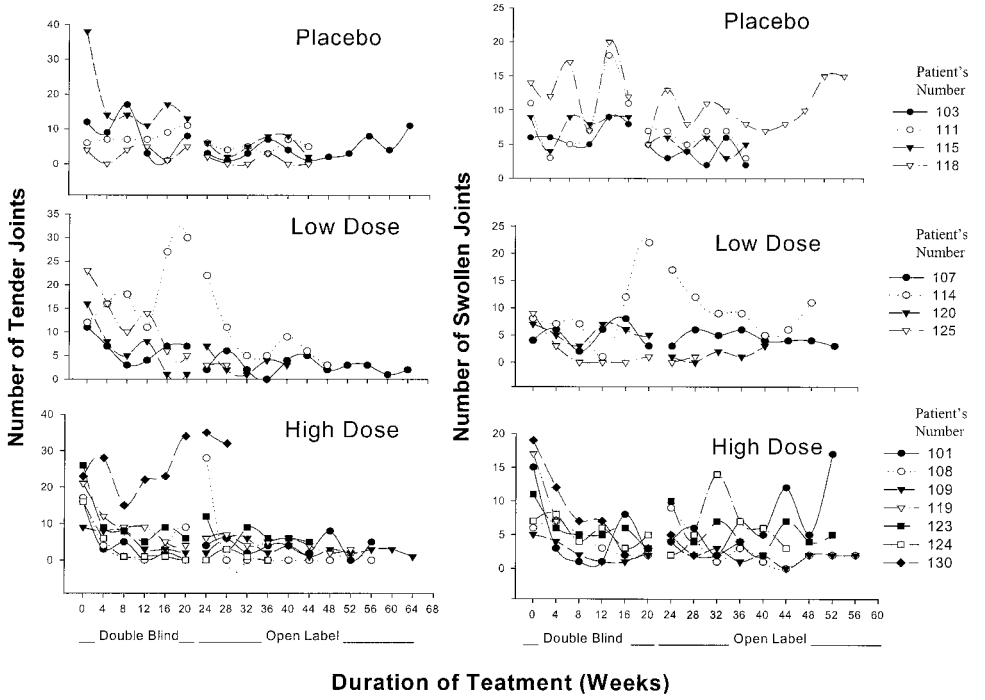

Figure 4. Changes in the number of tender joints and the number of swollen joints in individual patients who completed the double-blind trial (weeks 0–20) and then entered the open-label extension (after 20 weeks). Numbers shown in the key to the right are the individual patient numbers.

Table 3. Adverse events occurring during treatment

Adverse event	Placebo (n = 12)	TWHF extract	
		Low-dose (n = 12)	High-dose (n = 11)
Diarrhea	–	4	3
Headache	2	1	1
Hair loss	–	2	1
Blisters	1	–	2
Nausea	–	2	1
Flatulence	1	–	1
Constipation	1	1	1
Heartburn	–	2	–
Facial rash	–	–	1
Tinnitus	–	–	1
Abdominal pain	–	–	1
Vaginal spotting	–	–	1
Indigestion	1	–	1
Total no. of patients with ≥1 adverse event	4	6	5

* TWHF = *Tripterygium wilfordii* Hook F (ethanol/ethyl acetate extract).

of 11, respectively, for the placebo, low-dose extract, and high-dose extract groups (Table 3). One patient from each of the 3 groups discontinued treatment during the double-blind trial because of side effects.

The most common side effect was diarrhea, which developed in 4 patients in the low-dose group (33.3%) and 3 patients in the high-dose group (27.2%) but none in the placebo group. Diarrhea usually consisted of 1–3 loose stools per day, lasted for 1–3 days, and stopped without cessation of the study treatment. The next most common side effects in the groups taking TWHF extract were hair loss and nausea, each of which developed in 2 patients in the low-dose group and 1 patient in the high-dose group. Headache developed in 2 patients in the placebo group and in 1 patient each in the low-dose and high-dose groups. This side effect caused 1 patient in the placebo group to withdraw from the trial. One patient, a 50-year-old woman receiving high-dose extract, developed headache as well as moderate diarrhea and vaginal spotting. She completely recovered 1 week after discontinuing treatment.

Similar adverse effects were observed during the open-label extension. However, there were no withdrawals related to the side effects during this period. One patient from the low-dose group, a 29-year-old woman, developed amenorrhea 20 weeks after starting high-dose extract during the extension phase of the trial. A year after treatment was stopped, menstruation resumed.

DISCUSSION

Results from the present study indicate that the difference in 20% ACR improvement rates was statistically significant in patients treated with the high dose (360 mg daily) of the ethanol/ethyl acetate extract of TWHF compared with those treated with placebo (P = 0.0001). The effectiveness of the low-dose extract was less than that of the high-dose extract (P = 0.027), but was still greater than that of placebo (P = 0.0287). Adverse effects of the extract were mild and were tolerated by most patients. The incidence of withdrawal because of adverse effects in patients receiving either the low dose or the high dose of the extract was similar to that experienced by the patients receiving placebo, which suggests the safety and tolerability of the ethanol/ethyl acetate extract of TWHF, as was previously suggested in an uncontrolled clinical trial (19).

A number of clinical trials of various preparations of TWHF have been performed in China (1). However, only two clinical trials of an extract of TWHF in RA patients were controlled, and they involved use of the polyglycoside-enriched preparation (18,26). Although results from several trials of an ethanol/ethyl acetate extract of TWHF in the treatment of RA have been reported in China, none of these was placebo controlled (10,12). Moreover, no trials have been conducted outside of China.

We examined the safety and efficacy of the ethanol/ethyl acetate extract of TWHF in the current studies for a number of reasons. First, we have noted that there are many similarities between the ethanol/ethyl acetate extract and the polyglycoside-enriched extract in terms of the major active components and the in vitro and in vivo bioactivities (21,27). Second, the method for extracting the polyglycoside preparation is proprietary and not available.

Therapeutic benefits of the ethanol/ethyl acetate extract were seen in both clinical and laboratory outcomes. It is obvious that improvements in individual variables occurred in parallel for most patients in the high-dose group. In contrast, some patients treated with placebo showed improvements only in a few variables, and there was no correlation between clinical outcome measures. As a result, 80% of patients in the high-dose group but none in the placebo group were identified as clinical responders according to the ACR 20% improvement criteria. These results are similar to those reported previously in the mostly uncontrolled studies reported in the Chinese literature (12,13), although the ACR 20% improvement criteria were not used for evaluations in the Chinese trials because most of them had been conducted before the ACR criteria were established.

Some decreases in the mean ESR and CRP values were shown in the high-dose group within the first

4 weeks of treatment and throughout the 20 weeks of treatment course, although the decreases in ESR did not reach statistical significance compared with baseline. However, 5 and 6 patients in the high-dose group (total of 10 patients) had a >20% decrease in the ESR and CRP, respectively, compared with the baseline. In contrast, 9 and 10 patients in the placebo group (total of 12 patients) experienced increased ESR and CRP values, resulting in a statistically significant difference when compared between the two groups, as analyzed by either Wilcoxon's 2-sample test or the Kruskal-Wallis test ($P < 0.0249$ for ESR, $P < 0.0423$ for CRP) at the end of the treatment course.

The pattern of early responses to the ethanol/ethyl acetate extract is consistent with that observed in our previous uncontrolled study (19) and most of the clinical trials performed in China (12,13,18). The short time needed for the ethanol/ethyl acetate extract to become effective may be explained by the findings that this extract inhibits the production of cytokines and other mediators from mononuclear phagocytes by inhibiting the up-regulation of a number of proinflammatory genes, including tumor necrosis factor α (28), cyclooxygenase 2 (27,29), and inducible nitric oxide synthase (30). In addition, extracts of TWHF inhibit proliferation and cytokine production by T cells and immunoglobulin production by B cells (31,32). These results suggest that the ethanol/ethyl acetate extract of TWHF exerts not only immunosuppressive effects, but also direct antiinflammatory effects. This may explain the rapid onset of action and the sustained suppression of rheumatoid inflammation.

Although the incidence of adverse events in the patients receiving the ethanol/ethyl acetate extract was slightly higher than that in patients receiving placebo, the frequency of the treatment-related withdrawals was the same across all 3 groups (1 withdrawal per treatment group). Many of the side effects were noted in patients treated with placebo as well as in those treated with the extract, suggesting that the side effects may not be specifically associated with administration of the drug. Diarrhea developed in 4 patients in the low-dose group and 3 patients in the high-dose group, suggesting a relationship between this side effect and the extract. The severity of adverse events seemed milder and the drug-related withdrawal rate was less in the current study than in most of the trials conducted in China (11–13,18).

At baseline, there were differences in sex and duration of disease among the 3 groups. To exclude the possibility that the differences in outcomes were related to the between-group differences in sex distribution, we analyzed the number of female patients who achieved an ACR 20% response. Eight of the 14 female patients in the drug-treated groups who finished at least 4 weeks of therapy, but none of the 12 patients in the placebo group, reached ACR 20% response criteria ($P = 0.0004$). Therefore, disease improvement in the female patients was clearly associated with the treatment. Statistical analysis of the male patients could not be done because of the small number of patients. The impact of disease duration on outcome was also analyzed, since the mean duration of disease was somewhat longer in the high-dose extract group. However, the likelihood of improvement was not related to the duration of disease, since there is no significant difference in disease duration in patients who achieved an ACR 20% response and those who did not ($P = 0.26$). Therefore, imbalances in the groups at baseline did not appear to contribute to the therapeutic outcome.

It should be noted that the number of patients enrolled in the current trial was less than that originally planned. Despite this, significant differences in outcome were noted between patients treated with the TWHF extract and those treated with placebo. However, additional evaluation of the efficacy and adverse effects of the ethanol/ethyl acetate extract of TWHF in larger studies is warranted.

In conclusion, the ethanol/ethyl acetate extract of TWHF, at a dosage of 360 mg/day, appears to be safe in RA patients. The symptoms and signs of inflammation and the physical functioning of most of the patients in the trial improved.

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