A Phase I Study of Ethyl Acetate Extract of the Chinese Antirheumatic Herb *Tripterygium wilfordii Hook F* in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To explore the efficacy and safety of ethyl acetate (EA) extracts of the Chinese herbal remedy *Tripterygium wilfordii Hook F* (TWHF) for treatment of patients with a variety of inflammatory and autoimmune diseases including rheumatoid arthritis (RA).

Methods. The roots of TWHF were extracted sequentially by ethyl alcohol and ethyl acetate and the content of the extract documented. An open label, dose escalation Phase I study was performed in 1993 in 13 patients with established RA. Clinical manifestations and laboratory findings were examined before and every 4 weeks after starting treatment with the EA extract.

Results. Three patients withdrew from the trial during the first 16 weeks of the dose escalation. These patients received a maximum dosage of 180 mg/day. There were no adverse effects or disease improvement observed in these patients. Nine of the remaining 10 patients tolerated the EA extract up to a dosage of 570 mg/day. There were no withdrawals related to adverse events in the trial except for one patient who developed diastolic hypertension at a dose of 180 mg/day of EA extract. Six of 10 patients treated with 180 mg/day of EA extract showed disease improvement. Eight of the 9 patients who received EA extract at doses > 360 mg/day experienced improvement in both clinical manifestations and laboratory findings. One patient met American College of Rheumatology criteria for remission.

Conclusion. The EA extract of TWHF at dosages up to 570 mg/day appeared to be safe, and doses > 360 mg/day were associated with clinical benefit in patients with RA. (J Rheumatol 2001; 28:2160–7)

Key Indexing Terms:
ANTIRHEUMATIC THERAPY DISEASE MODIFYING ANTIRHEUMATIC DRUG
ETHYL ACETATE EXTRACT HERBAL REMEDIES *TRIPTERYGIUM WILFORDII HOOK F*

Tripterygium wilfordii Hook F (TWHF) has been used as a remedy for the treatment of joint pain for hundreds of years[1]. It has become extensively used in China since the 1970s for treatment of a wide spectrum of autoimmune and inflammatory diseases, including rheumatoid arthritis (RA) and ankylosing spondylitis, as well as various skin and kidney diseases including psoriasis and idiopathic IgA nephropathy. Clinical trials of TWHF have reported significant therapeutic benefit in treated patients, although nearly all of the trials were uncontrolled.

Various extraction methods have been employed to minimize toxicity and maximize therapeutic benefit. The doses of the extracts initially were adjusted based upon the weight of plant material from which the extract was prepared. Patients treated with these crude preparations appeared to experience therapeutic benefit, but frequently developed adverse effects and occasionally severe toxicity[2,3]. Phytochemical and pharmacological studies on the active components of TWHF suggested that specific hydrophobic diterpenoid compounds, especially triptolide and tripdiolide, accounted for the therapeutic efficacy of TWHF[4]. As a result, a variety of extracts of the plant material, including an ethanol extract[5,6], an ethyl acetate (EA) extract[7,8], and a preparation of polyglycosides[9], have been produced and monitored by the content of active diterpenoids. Since the EA extract and the polyglycoside preparation have been claimed to exert better therapeutic effects but cause less adverse events than other crude preparations, these 2 preparations have been used most widely in China. However, there is minimal experience with these extracts outside China.

Both extracts have been shown to inhibit joint swelling induced by a number of nonspecific irritants and also suppress adjuvant arthritis[10,11]. These extracts also reduced delayed type hypersensitivity responses and primary anti-

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body responses in mice[10,11]. These results suggest that the extracts exerted antiinflammatory and immunosuppressive effects comparable to the effect of prednisone[10,11]. Two uncontrolled clinical trials of the EA extract of TWHF in the treatment of 155 and 270 patients with RA, respectively, have been reported[12,13]. Similar results were obtained from these studies with a total response rate claimed to be 95%, with a significant improvement rate of 74%. Incidences of side effects were reported to be 35 and 44% in the 2 trials. The most common side effects were gastrointestinal tract disturbances and amenorrhea, both of which resolved after the EA extract of TWHF was tapered or stopped. These results were similar to the findings from a double blind, controlled clinical trial of the polyglycoside preparation[14].

This study was undertaken to examine the safety of extracts of TWHF. Because the method for the extraction of the polyglycoside preparation is proprietary and not available, studies with the EA extract were undertaken. An EA extract from the woody portion of the roots of TWHF was produced and examined for its content of diterpenoids, and its safety and efficacy were tested in a Phase I study in patients with RA.

MATERIALS AND METHODS

Patients. To be eligible for this trial, it was necessary for patients to fulfill the American College of Rheumatology (ACR) 1987 revised criteria for the classification of RA[15]. Patients had to be age 18 years or older and have had the diagnosis of RA for at least one year and have an ACR functional class of II, III, or IV[15]. Patients had to have active disease defined as having 2 or more swollen joints and 2 of the following 3: 6 or more painful/tender joints, morning stiffness for 30 min or longer, and erythrocyte sedimentation rate (ESR) ≥ 28 mm/h despite conventional therapy. Moreover, patients needed to have failed an adequate treatment course with at least one disease modifying antirheumatic drug (DMARD) and the most recent DMARD must have been discontinued for at least 4 weeks. The exclusion criteria included patients with serious illness, including any chronic viral infection, uncontrolled hypertension, active duodenal ulcer or colitis, congestive heart failure, life threatening pulmonary dysfunction, abnormal blood cell count or liver enzymes or kidney function. In addition, a negative urinary test for pregnancy was required for female patients of childbearing age.

Preparation of the EA extract. The EA extract was prepared as described[16]. Initially, plant material for extraction was identified by its content of diterpenoids using sequential ethanol and EA extraction and analysis by thin layer chromatography as described[16]. Subsequently, selected preparations of peeled roots of TWHF were ground to a powder that was then sequentially extracted with ethanol and ethyl acetate. The ethyl acetate extract was concentrated to dryness and ground to a fine powder. The resultant EA extract was examined for the content of active diterpenoids, including triptolide and tripdiolide, the inhibitory activities on *in vitro* T cell proliferation and interleukin 2 (IL-2) production and acute toxicity in mice as described[17]. Notably, the content of triptolide and tripdiolide correlated well with the bioactivities and the acute toxicity of the EA extract used for the clinical trial. Afterwards, the extract powder was standardized by comparison of the sum of the content of triptolide and tripdiolide with a reference extract prepared in China[8,18]. After adding an appropriate amount of starch, the EA extract was formulated into capsules. Each capsule contained 30 mg of the extract and a total of 9.9 μg of triptolide and tripdiolide.

Treatment plan. This was a single center, open label, Phase I dose escalation study. The patients discontinued their DMARD for at least 4 weeks before receiving treatment with the EA extract. Patients who were taking nonsteroidal antiiflammatory drugs (NSAID) and prednisone ≤ 7.5 mg/day before entry into the trial were allowed to continue the same dose of the medication. The EA extract was administered from the second visit, starting with 30 mg/day. According to the sum of triptolide and tripdiolide content, 30 mg of the EA extract was equivalent to 1/6 to 1/20 of the daily dosage of similar products of TWHF reported to be clinically effective by Chinese investigators[14,19]. Moreover, this dose was roughly 1/40 of the LD50 dose of the EA extract in mice. In comparison, the therapeutic doses of the polyglycoside preparation and the EA extract made in China were 1/2 and 1/6 of the corresponding LD50 dose in mice, respectively[19,20]. Therefore, this starting dosage was considered to be safe.

The dose escalation plan is shown in Table 1. Briefly, the dose was increased at 4–8 week intervals. An additional 60–90 mg/day were given at the time of dose escalation to the patients who had not developed significant adverse effects until a maximum effective dose or a dose causing significant side effects was reached.

Assessment of clinical response. To evaluate the efficacy of the EA extract, the effect on a number of measures of disease activity was assessed. The following clinical, laboratory, and functional variables were assessed: tender joint count, swollen joint count, patient's global assessment [using a visual analog scale (VAS) of 10 cm], physician's global assessment (MD 10-cm VAS), duration of morning stiffness, C-reactive protein (CRP) level, and ESR. Rheumatoid factor (RF) titers were also measured. A baseline assessment was carried out twice with an interval of 2 weeks in between. The average of the measured values was recorded as the baseline status. Assessments were then performed every visit (every 4–8 weeks) after beginning treatment with the EA extract. Patients who experienced > 20% improvement in tender joint count, swollen joint count, morning stiffness, and physician's global assessment as well as either ESR or CRP were categorized as responders. Patients who did not achieve these criteria were considered to be nonresponders.

Assessment of adverse reactions. Patients were actively queried for adverse events at each visit. A list of adverse events reported in the Chinese literature was read to the patients during each visit. When an adverse event was claimed, the timing relative to the administration of the EA extract was investigated. Blood pressure, blood cell count, and serum creatinine were examined before entry and at every visit (every 4–8 weeks) after the beginning of treatment with EA extract.

Statistical analysis. Outcome measurements before therapy and at the end of each dosage period were compared by the 2 tailed t test for comparison of means using Student's t test.

Table 1. Treatment plan.

Time After Starting Treatment, weeks	mg/day	Dosage Number and Frequency of Capsules per Day*
0	30	1
8	60	1, 1
16	120	2, 1, 1
24	180	2, 2, 2
28	240	3, 2, 3
32	270	3, 3, 3
36	300	3, 2, 2, 3
40	390	4, 3, 3, 3
44	480	4, 4, 4, 4
48–76	570	5, 5, 5, 4

* Each capsule contained 30 mg of EA extract.

RESULTS

Baseline characteristics of the patients. From 1993 to 1994, 13 patients with seropositive RA, 11 women and 2 men, were enrolled into the trial. The initial characteristics of the patients are summarized in Table 2. The mean age of the subjects was 55.2 years. The mean disease duration was 10.6 years. Most subjects had failed treatment with many DMARD, all of which had been discontinued for at least 4 weeks. All patients were taking NSAID. Eight patients were treated with prednisone as well, with an average dose of 5.8 mg/day. All patients had active disease as assessed by signs and symptoms as well as laboratory correlates of inflammation.

Table 2. Initial characteristics of the 13 patients with RA entered into the trial.

Variable	Value*
Age	55.2 ± 9.8
Female:male	11:2
Duration of RA (yrs)	10.6 ± 2.6
No. of DMARD failed	3.2 (2–6)
No. of patients taking NSAID	13
No. of patients taking prednisone	8
Prednisone dose (mg/day)	5.8 ± 1.4
AM stiffness (min)	265 (0–960)
Tender joint counts	24 (12–66)
Swollen joint counts	9.3 (5–22)
Patient global assessment (0–10 scale)	7 (2.9–9)
Physician global assessment (0–10 scale)	4.3 (3.1–7.5)
ESR (mm/h)	53 (12–155)
CRP (mg/dl)	2.6 (0.4–11.3)
Rheumatoid factor (IU/ml)	650 (108–3390)

* Data are median (range) or mean ± SD.

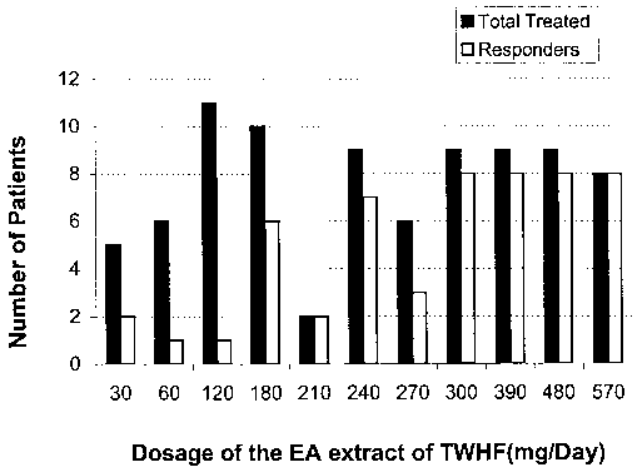

Figure 1. Clinical responses of patients to therapy with the EA extract of TWHF. Black bars indicate the number of patients treated with a given dose of EA extract; white bars indicate the total number who became clinical responders by ACR criteria.

Global responses and adverse effects. The composite clinical responses of patients to therapy with the EA extract are shown in Figure 1. In the beginning, 5 patients were treated with 30 mg/day of EA extract. There were some beneficial responses with no side effects reported. Since the first 6 patients tolerated 60 mg/day of the EA extract with no adverse events, the next 5 patients were directly escalated to 120 mg/day. Afterwards, the daily dose was increased at 4 week intervals, with each increase being 60–90 mg per day. Three patients, who preferred to increase the dose more slowly after being treated with 180 mg/day of EA extract, increased it by 30 mg per day every 4 weeks thereafter. Six patients increased the dose by 30 mg/day for each escalation, after receiving a daily dose of 240 mg/day.

Three patients withdrew from the trial because they experienced no improvement during the first 16 weeks of dose escalation. They received the EA extract at doses of no more than 180 mg/day during this period. One patient withdrew from the trial because of increased diastolic blood pressure while receiving 180 mg/day. The remaining 9 patients went through the entire treatment program, eventually receiving a maximal dosage of 570 mg/day of EA extract, with the exception of one patient who discontinued therapy because of lack of efficacy after receiving 480 mg/day of EA extract. The mean duration of therapy in the 9 patients was 48 weeks, with the longest duration of therapy being 76 weeks. The highest dose that was administered was 570 mg/day.

An estimate of the percentage of patients who were clinical responders was obtained using an approach that antedated the adoption of the ACR criteria[21]. Patients who achieved a 20% improvement in the number of tender joints, the number of swollen joints, morning stiffness, and physician global assessment as well as ESR and CRP were considered responders. Figure 1 shows a clear dose-dependent response to the EA extract. Eight of 9 patients became responders when the dose reached 300 mg/day or more.

Adverse events are shown in Table 3. The most common side effect was decreased appetite, which developed in 8 patients (61.5%). Nausea or loose stools are noted by 2 patients (15.3%). Each of the following side effects was reported by one patient: stomatitis, oral ulcers, gastritis, weight changes, vomiting, and flatulence. Most of the adverse effects developed when the dose of the EA extract was increased above 390 mg/day. Most of the side effects were mild and transient without necessitating dose reduction or cessation of therapy. One patient developed increased

Table 3. Adverse events in patients taking EA extract.

One each: angular stomatitits, oral ulcers, gastritis, vomiting, weight gain, weight loss, diastolic hypertension, abdominal cramping, flatulence
2: diarrhea
2: nausea
8: dyspepsia

diastolic hypertension while receiving 180 mg/day of EA extract. The blood pressure returned to normal after discontinuing the EA extract.

Changes in clinical manifestations and laboratory findings. Figure 2 shows the changes in clinical manifestation in treated patients. Patients treated with EA extract improved in all clinical indices assessed. Significant improvement in morning stiffness was the first indication of efficacy, observed 8 weeks after the trial began while patients were receiving 240 mg/day of EA extract. Improvement was maintained during the entire course of treatment. The mean baseline morning stiffness was 265 min, which decreased to 40 min at a dose of 240 mg/day and still further decreased to 10 min at 390 mg/day. The number of tender joints was significantly reduced with a dose of 270 mg/day. Maximum improvement in number of tender joints was observed after starting a daily dose of 300 mg of EA extract. The mean baseline number of swollen joints was 9.3, which decreased to 6.0 at a dose of 240 mg/day and further decreased to 4.8 at a dose of 480 mg/day. The physician's global assessment on a 0–10 cm scale was decreased from the baseline of 5.0 to 3.0 at a dose of 480 mg/day of EA extract.

In parallel with the improvement in symptoms and physical signs of joint inflammation, the laboratory findings were also improved. As shown in Figure 3, ESR was the first and most significant response to the treatment, decreasing from the baseline of 53 mm/h to 30 mm/h at a dose of 240 mg/day. Maximum improvement was obtained after the dose of the EA extract was increased to 480 mg/day, when the mean ESR was 22 mm/h, close to the normal range. Significant decreases in RF were found after the dose of EA extract reached 390 mg/day. A similar pattern was noted for the changes in CRP. More than a 2-fold decrease in CRP was found at a dose of 300 mg/day, which dropped to < 1 mg/dl (within the normal range) as the dose increased to 480 mg/day or more.

Sixty percent of patients in this trial were considered to be responders to 180 mg/day, whereas 8 of 9 patients (88.8%) responded well to 300 mg/day of EA extract evaluated as described. One of the 8 patients achieved disease remission by the ACR preliminary criteria for clinical remission in RA[22].

Figure 4 shows the responses of the patient who was considered to have achieved disease remission after treatment with EA extract. This patient had severe, refractory RA for more than 15 years and incomplete responses to various therapies. His disease was completely controlled by 390 mg/day of EA extract. Four weeks after he was treated with 390 mg/day of EA extract, he experienced no morning stiffness (300 min pretreatment). Physical examination noted a swollen joint count of 0 (2 pretreatment), tender joint count 0 (21 pretreatment), and MD-VAS of 0.5 (3 pretreatment).

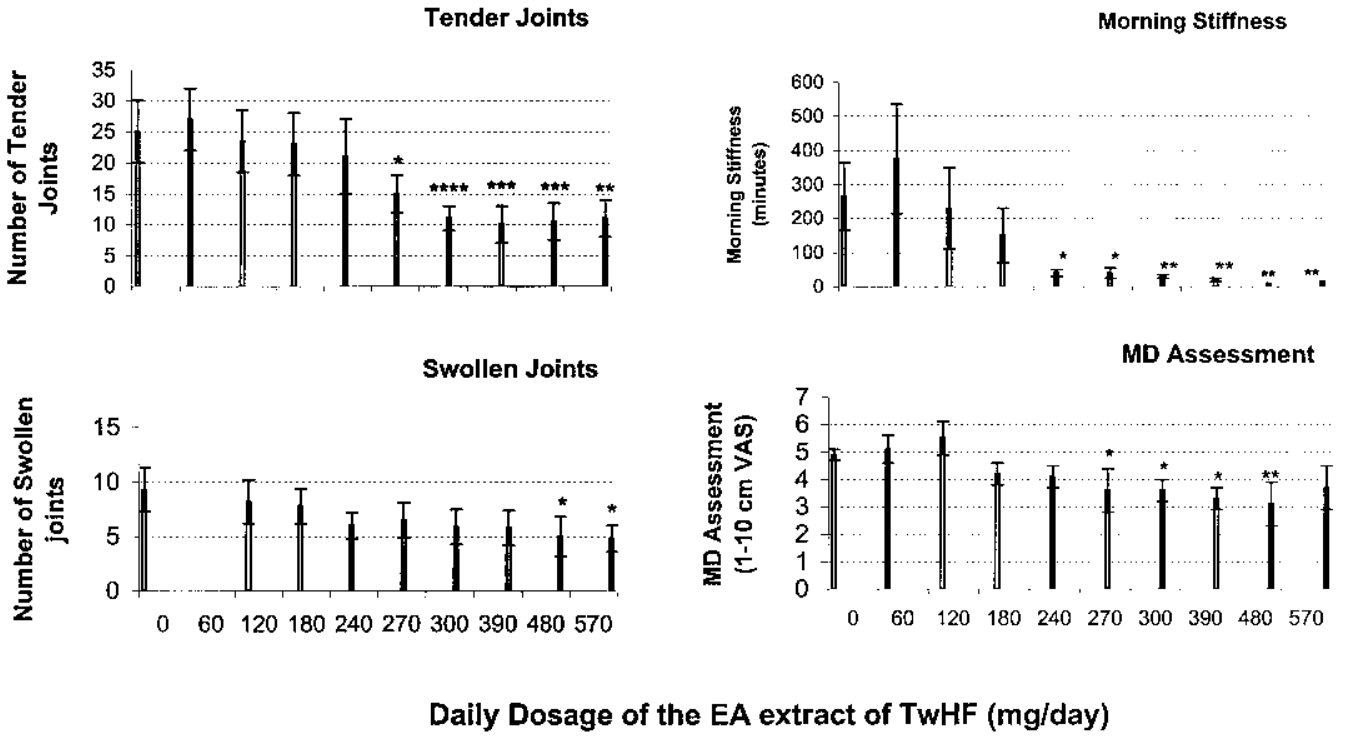

Figure 2. Changes in clinical manifestations during the treatment course with EA extract of TWHF. The number of patients at each dose is the same as in Figure 1. The bars and brackets represent mean ± SD. Student's t test was used to analyze each variable at each dose compared to baseline of the same patients. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001.

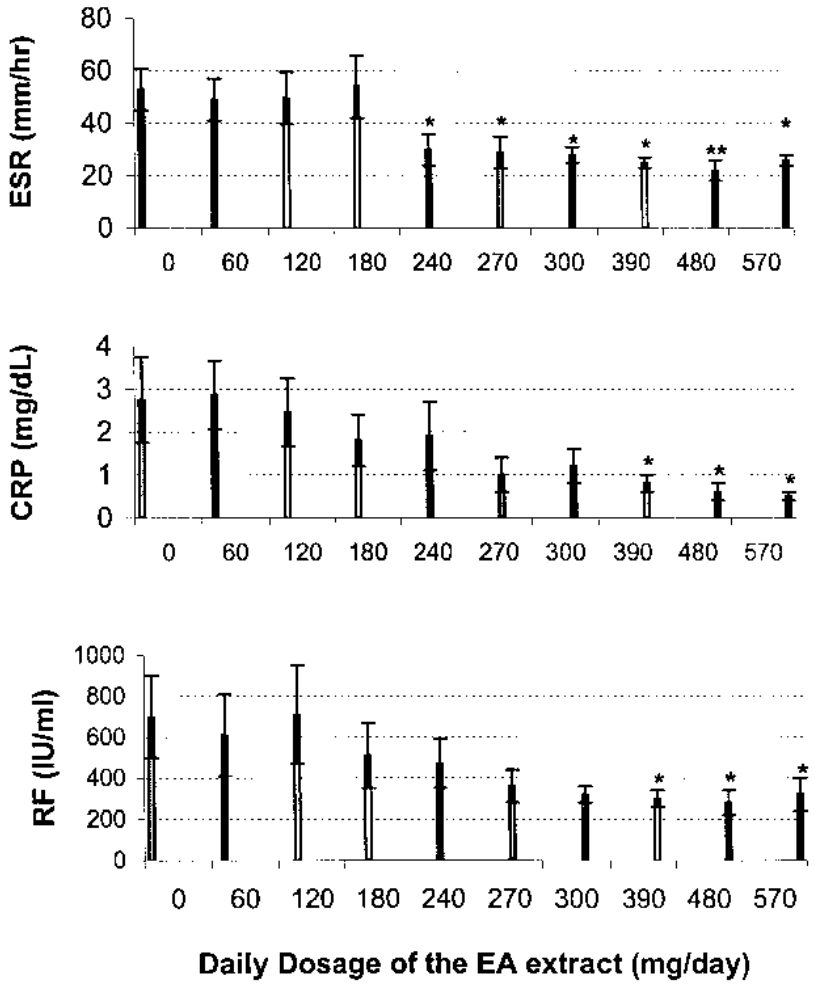

Figure 3. Changes in laboratory findings during the treatment course with EA extract of TWHF. The number of patients at each dose is the same as in Figure 1. The bars and brackets represent mean ± SD. Student's t test was used to analyze each variable at each dose compared to baseline of the same patients. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001.

His ESR and CRP returned to normal from a baseline of 175 mm/h and 11.5 mg/dl, respectively. His RF titers, as high as 3500 IU/ml before entry, decreased to 250–300 IU/ml after the dose reached 300 mg/day. He experienced slight dyspepsia and loose stools at this dose. None of the side effects necessitated cessation of therapy. His disease improvement lasted for 10 weeks after cessation of the EA treatment.

To examine the durability of response after cessation of therapy, patients were monitored after the trial was completed and the EA extract discontinued. In most patients, active disease recurred 8 weeks after cessation of the EA extract, but remained at a modest level for an additional 8 weeks thereafter despite no DMARD therapy.

DISCUSSION

Our results suggest that most patients tolerate the doses of the EA extract that induced maximum therapeutic effect, suggesting safety and tolerability of this treatment.

Although various preparations of TWHF have been used for more than 2 decades in the treatment of RA in China[1], the current study is the first to use a preparation of TWHF made and used outside China in the treatment of RA. Our data suggest that the minimum effective dose of the EA extract of TWHF was 180 mg/day. For maximum therapeutic effect, however, 300 to 480 mg/day containing 132 to 158 μg of triptolide and tripdiolide was required. This amount of the EA extract was well tolerated by most patients enrolled in the trial and is comparable to the amount used in China, normalized for the content of triptolide. Chen has reported that 131 to 262 μg of triptolide was contained in a daily therapeutic dose of EA extract made in China[18]. Therefore, the effective dose of the EA extract used in the current trial appeared to contain a comparable amount of triptolide as therapeutic amounts of the EA extract used in China.

Eight of 9 patients who received 300 mg/day or more of the EA extract were considered to be responders to the treat-

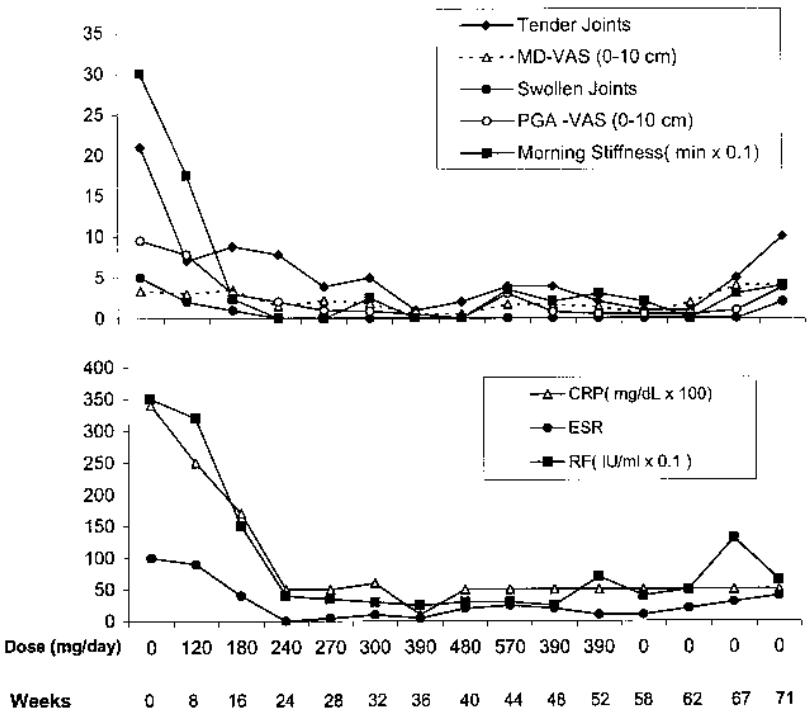

Figure 4. Responses of one patient who achieved remission after therapy with EA extract of TWHF. MD-VAS: physician's global assessment. PGA-VAS: patient's global assessment.

ment (88.8%) evaluated as described. This is comparable to the effect noted in clinical trials of either the EA extract or the polyglycoside preparation in the treatment of RA reported by Chinese investigators[12-14,23].

Severe intoxication has been reported[2,3,24,25] in China. Most of the cases were associated with administration of herbal decoction of TWHF or overdose with polyglycoside tablets. It was suggested that 4 times conventional dosage could cause severe intoxication[2]. Most patients in the current trial experienced one or more side effects during the treatment course. However, no patient had to stop the treatment with EA extract of TWHF because of side effects, except one who developed diastolic hypertension. This adverse effect has never been reported in patients receiving similar preparations of TWHF[1]. Therefore, the relationship between the onset of hypertension and administration of the EA extract of TWHF in this case is uncertain. Further clinical studies with larger numbers of patients may answer this question.

The pattern of the adverse reactions developed in this group of patients was similar to that noted in patients treated with other TWHF preparations. However, the incidence and the severity of the side effects in this group of patients appeared to be less than that reported in the literature[12-14,25,26]. We did not observe skin rash and skin pigmentation, 2 of the most common side effects of TWHF preparations in China. The lower toxicity of the EA extract used in our trial could be related to the method of EA extract preparation, including the use of skinned roots rather than whole roots. The skin of the root is felt to contain additional toxic components that might be avoided when extracts are prepared from skinned roots[27].

There were no adverse effects on the reproductive system in this group of patients despite previous evidence that reproductive dysfunction was a frequent adverse event in trials of all preparations of TWHF[14,23]. It is possible that this related to the age of the patients in this trial (mean 55.2 years). Notably, however, there were no postmenopausal symptoms associated with TWHF treatment in this study. Whether patients in the current trial have better tolerance to the EA extract of TWHF than Chinese patients needs to be evaluated more completely.

The preliminary data were obtained from an open trial with a small number of patients and without a placebo treated control group and therefore, to delineate the therapeutic value of the EA extract in RA, a prospective, blinded, controlled trial of TWHF in a larger population of patients with RA is currently being conducted. However, results of this Phase I study are encouraging for the high ratio of responders and patients experiencing disease remission. Notably, our results were consistent with the reported efficacy noted in China. Even the occurrence of remission was comparable to that noted in China (5.7%–17.6%)[14,28]. The relatively rapid (3–4 weeks) but profound clinical response

correlated with remarkable decreases in CRP, ESR, and RF, suggesting that besides the direct antiinflammatory effect, a modifying effect on the immune responses of the patients explained the mechanism by which the EA extract exerted its therapeutic effect. This idea can be supported by the results of *in vitro* and *in vivo* preclinical studies showing that the EA extract inhibited upregulation of COX-2 expression and prostaglandin production as well as antigen and mitogen induced upregulation of IL-2 gene expression[29,30].

Chen and colleagues compared the therapeutic effect of different fractions of TWHF including diterpenoids, total alkyloids, and ducitol in patients with RA, and found that the fraction containing diterpenoids gave a much better therapeutic benefit than the other fractions, suggesting that the diterpenoid components account for most of the antirheumatic activities of TWHF[31]. Seven diterpenoid components with an epoxide lectone structure have been isolated from the polyglycoside preparation of TWHF[32]. Comparing these components to each other and to the entire polyglycoside preparation in terms of the antiinflammatory and immunosuppressive effect revealed that many of the diterpenoid components were potent, and triptolide and tripdiolide were the 2 most active[20]. Previously, we reported that the activity of the total amount of triptolide and tripdiolide explained most of the immunosuppressive properties of either the EA extract or the polyglycoside preparation of TWHF[17]. By screening high performance liquid chromatography fractions of the EA extract for *in vitro* immunosuppressive activity, we found that fractions containing triptolide and tripdiolide were the most effective despite accounting for about 0.1% of the weight of the extract (unpublished data).

Even though the EA extract contained a complex of many components from the roots of TWHF, and therefore its quality relied on the identity of the source of the plant materials and the reproducibility of the manufacture protocol, its activity could be controlled by identification and quantification of the spectrum of diterpenoids. Our results confirm this idea because clinical benefit was noted when the daily dose of the diterpenoids approached that previously reported in Chinese literature to be effective.

Treatment with therapeutic doses of the EA extract was safe, with tolerable side effects for most patients with RA who achieved clinical benefit in this trial. The safety and efficacy of the EA extract of TWHF is currently being evaluated in a double blind controlled Phase II study.

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