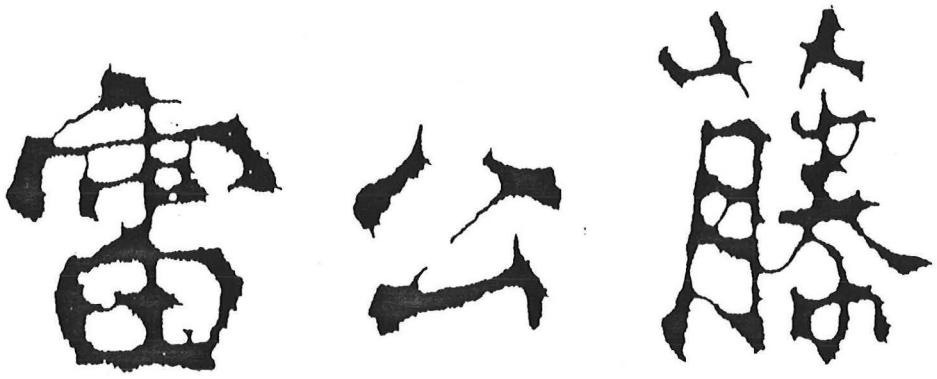

The Wisdom of the Orient Yields a New Treatment for Rheumatoid Arthritis: The Story of Thunder God Vine

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Crude preparations of Tripterygium have been used as medicinal herbs for many years in traditional Chinese medicine. The Compendium of Materia Medica compiled by Li Shi-Zheu in 1578 reports the use of Tripterygium for a variety of maladies. Tripterygium wilfordii Hook f and Tripterygium hypoglaucum (Levl.) Hutch have been most commonly used. The two plants are different species of the same family, the Celastraceae. Tripterygium wilfordii Hook f (Lei Gong Teng, Thundergod vine, seven step vine) grows in a wide area of the mountainous regions of southeast and southern China, whereas Tripterygium hypoglaucum (Levl.) Hutch grows primarily in Southwest China. Both plants are woody vine-like rambling shrubs that are believed to have similar properties (Figure 1). The active ingredients are thought to be primarily located in the roots of the plants. Moreover, the leaves, stock, flowers and skin of the roots are poisonous and capable of causing death when ingested (1,2). Even ingestion of honey containing the pollen of the plant has been reported to cause death. A variety of crude extractions and decoctions of the plants have been employed for medicinal purposes. According to the Supplement to the Compendium of Materia Medica and Great Dictionary of Chinese Medicine, Tripterygium has the following effects: stimulates blood circulation, relieves stasis, is anti-inflammatory and relieves edema, purges excess internal warmth and eliminates toxicity, among

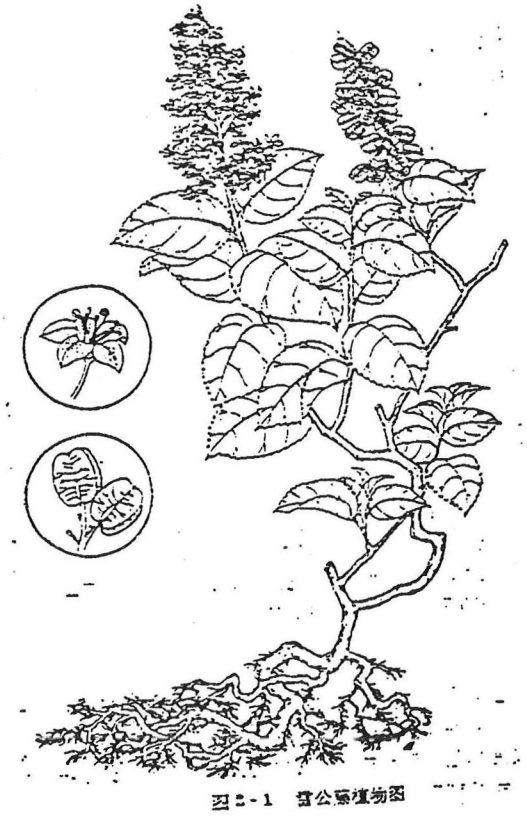


Figure 1

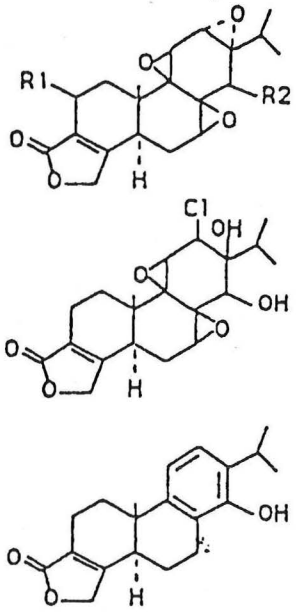
other activities. Despite this plethora of reported activities, because of its toxicity Tripterygium was not listed as an official drug of plant origin in the Chinese Pharmacopoeia, vol. 1 (1985). Moreover, it was not used in medical practice in China until recently, but rather was widely utilized as an agricultural insecticide.

Beginning at the end of the 1950's, the government of the Peoples Republic of China began to attempt to integrate western style medicine with traditional Chinese medicine. In 1966, Chinese life was disrupted by the beginning of the Cultural Revolution. As part of this social change, Chairman Mao ordered western-trained and oriented physicians to leave the cities, to learn about traditional Chinese medicine and to practice as "barefoot physicians" in the country-side. During this time, western-oriented physicians were forced to become familiar with many traditional medicinals. As a result of this re-education, many Chinese physicians and investigators became interested in the effect of Tripterygium and trials in a number of autoimmune and inflammatory diseases were organized and rapidly carried out. For example, by 1982 experience with treatment of more than 2000 patients with rheumatoid arthritis was reported (3). Since that time, a large number of clinical trials have strongly suggested the usefulness of Tripterygium and especially Tripterygium wilfordii Hook f (TwHf) in a variety of autoimmune and inflammatory conditions.

TwHf has been documented to contain more than 70 components, including diterpenes, triterpenes, glycosides and alkaloids (4). A variety of crude preparations of TwHf have been studied. These include an aqueous decoction, an alcohol extract known as T1, an alcohol/ethyl acetate extract and a chloroform/methanol extract known as T2. Each has been

claimed to exert efficacy in a variety of clinical conditions. Although there are strong regional preferences, no trials have directly compared these various preparations.

Each of the various preparations of TwHf has been used in clinical trials in China, but most recent trials have employed one of two major preparations of TwHf. The major reason these two preparations have been favored relates largely to their apparent diminished toxicity, although this has been documented in only a few trials (5). The first preparation is an ethanol/ethyl acetate extract of the roots of TwHf manufactured by the Huanshi Pharmaceutical Company of Hubei Province. This material is known to contain multiple components including diterpenes, triterpenes, alkaloids and glycosides. Although, the active ingredient(s) of TwHf is still unclear, many investigators believe that the diterpene lactone, triptolide and its related compounds (Figure 2) account of the therapeutic effect of TwHf (6-9). Since triptolide is thought to be one of the most potent compounds and to account for much of the efficacy and toxicity of TwHf, the content of this component has been used to standardize the ethanol/ethyl acetate extract in China (10,11).



	R1	R2
Triptolide	H	OH
Tripdiolide	β-OH	OH
Triptonide	H	O

Tripchlorolide

Triptophenolide

Structures of the diterpenoids.

Figure 2

The second preparation was originally produced by the Tripterygium wilfordii study group of the Institute of Dermatology of the Chinese Academy of Medical Sciences in 1978 and

designated T2. It is a chloroform methanol extract of the woody portion of the roots that is currently manufactured by the Taizhou Pharmaceutical Company of Jiangsu Province. T2 is though to be enriched in glycosides, but also contains a variety of other components, including the diterpenes, triptolide and tripdiolide (12).

Clinical Experience

A wide variety of inflammatory and autoimmune conditions have been reported to be successfully treated with one or another preparation of TwHf (13). Most of this information derives from uncontrolled trials or retrospective analyses, but involves observations of many thousands of patients. Conditions reported to be successfully treated with TwHf include rheumatoid arthritis, psoriatic arthritis, psoriasis vulgaris, pustular psoriasis, Behcets' disease, discord lupus, systemic lupus erythematosus, allergic angiitis, Henoch-Schoenlein purpura, Sweets syndrome, reactional states of leprosy, eczema, contact dermatitis, polymorphous light eruption, palmoplantar pustulosis, erythema multiform, erythema nodosum, recurrent aphthous stomatitis, pemphigus vulgaris, dermatitis herpetiformis and dermatomyositis.

1. T2

More than 21 different inflammatory and autoimmune diseases have been treated in China with T2, including dermatologic disorders, a variety of nephropathies, rheumatoid arthritis, systemic lupus erythematosus, chronic lymphocytic thyroiditis and reactional states of leprosy. In general, impressive clinical benefit was noted (Table 1), although none of the

Table 1

Summary of the Chinese Experience with T2 used to Treat Various Inflammatory and Autoimmune Diseases

Disease	No.of patients treated	Efficacy (%) Significant improvement		Improvement	Ineffective	Reference
1. Rheumatoid arthritis	144	55.1		38.2	6.7	16,17
2. Systemic lupus erythematosus	36	33.0		33.0	34.0	16,5
3. Sweet syndrome	14	85.7		14.3	0	18
4. Erythema multiforme	10	90.0		10.0	0	19
5. Prurigo agria	8	37.5		62.5	0	18
6. Diffuse eczema	15	80.0		20.0	0	18
7. Pustular psoriasis	8	87.5		12.5	0	18
8. Allergic vasculitis	7	85.7		14.3	0	18
9. Psoriatic arthritis	5	80.0		20.0	0	18
10. Discoid lupus	9	22.2		77.8	0	18
11. Behcet's disease	41	76.0		20.0	4	16,18,19
12. Henoch-Schöelein purpura	20	<----	100	---->	0	20
13. Reactional states of leprosy	111	63-82		3-18	0-7	16,21,22
14. Childhood nephrotic syndrome	136	62-79		13-19	6-7	16,23,24
15. Adult nephritis	44	<----	56.8	---->	43.2	16
16. Idiopathic IgA nephropathy	17	82		18	0	25
17. Childhood nephritis	106	80		16	4	26,27
18. Chronic lymphocytic thyroiditis	12	<----	100	---->	0	28

trials was controlled and many were retrospective reports of experiences. A single prospective, randomized, double-blind, cross-over study of T2 in the treatment of rheumatoid arthritis has been reported (14,15). In this study, patients were randomly divided into two groups (A and B). Patients in group A receive T2 (20 mg tid) for the first 12 weeks (first course) and then were changed to placebo for the following 4 weeks (second course). Patients in group B received placebo during the first 12 week course and then were changed to T2 for 4 weeks (20 mg tid).

Table 2

Clinical Features of Patients Entering the Controlled Trial of T2 in Rheumatoid Arthritis

	First Treatment Course (12 weeks)		Second Treatment Course (4 weeks)	
	Group A T2	*Group B* Placebo	*Group A* Placebo	*Group B* T2
Number of Patients	35	35	27	31
Male/Female	3/32	4/31	1/26	4/27
Mean age, years	46.3	48.0	46.2	47.7
Mean disease duration (years)	5.9	6.1	5.8	6.0
Stage of Disease				
(1)	6	6	4	5
(2)	14	16	11	13
(3)	12	10	10	9
(4)	3	3	2	4

In comparison with patients in group B, patients in group A showed significant improvement in all parameters. The most significant improvement was observed in tenderness

scores, ESR, CRP, and IgG, IgA and IgM levels ($p<0.001$). A significant difference between the two groups was also seen in the physician's and patients' overall assessments ($p<0.005$) after the completion of the first course. By the end of the second course of treatment, patients in group B significantly improved in most clinical and laboratory parameters (Table 2-6). These results confirm the short-term utility of T2 in the treatment of patients with rheumatoid arthritis. More extended controlled trials have not been carried out to date.

Table 3

Changes in Clinical Parameters in Patients
Completing the First Course of Treatment

		Group A T2 (n=27)	*Group B* Placebo (n = 31)	*p
Morning stiffness (hours)	Before	2.4 ± 0.4	1.1 ± 0.2	
	After	0.9 ± 0.2	2.3 ± 1.4	<0.01
Joint tenderness score	Before	25.1 ± 1.9	25.5 ± 1.7	
	After	7.9 ± 1.3	21.9 ± 2.1	<0.001
Number of swollen joints	Before	9.2 ± 0.9	7.8 ± 0.7	
	After	4.3 ± 0.6	7.4 ± 1.1	<0.01
Grip strength (mean of both sides, mm Hg)	Before	49.0 ± 0.4	73.6 ± 7.7	
	After	84.4 ± 7.5	81.2 ± 8.9	<0.05
15 meter walking time (seconds)	Before	36.6 ± 6.6	37.0 ± 2.4	
	After	21.6 ± 1.5	31.9 ± 3.6	<0.05

*Group A vs Group B

Table 4

Changes in Laboratory Parameters in Patients
Completing the First Course of Treatment

		Group A T2 (n=27)	Group B Placebo (n=31)	*p
ESR (mm/hour)	Before	69.2 ± 6.4	63.9 ± 5.2	
	After	41.0 ± 5.9	67.2 ± 6.6	<0.001
CRP u/ml)	Before	29.4 ± 5.7	31.6 ± 4.1	
	After	10.4 ± 3.9	43.7 ± 7.0	<0.001
RF (titers)	Before	87.1 ± 23.2	86.1 ± 35.5	
	After	48.0 ± 13.4	63.4 ± 10.9	NS
IgG (u/ml)	Before	227.5 ± 4.6	231.9 ± 14.2	
	After	117.4 ± 9.5	180.4 ± 29.8	<0.001
IgM (u/ml)	Before	302.8 ± 40.3	284.5 ± 32.2	
	After	105.2 ± 11.1	261.3 ± 29.3	<0.001
IgA (u/ml)	Before	289.6 ± 29.4	257.6 ± 25.2	
	After	149.0 ± 15.5	280.4 ± 29.8	<0.001

*Group A vs Group B

Table 5

Changes in Clinical and Laboratory Parameters in Patients Completing the Second Course of Treatment

		Group A Placebo (n=24)	*p	*Group B* T2 (n=25)	*p
Morning stiffness (hours)	Before	1.8 ± 0.2		2.5 ± 1.7	
	After	0.8 ± 0.2	NS	1.3 ± 0.9	NS
Joint tenderness score	Before	7.9 ± 1.4		22.2 ± 2.4	
	After	11.0 ± 2.6	NS	13.5 ± 2.0	<0.001
Number of swollen joints	Before	4.2 ± 0.8		7.0 ± 1.2	
	After	4.4 ± 0.9	NS	3.5 ± 0.5	<0.05
Grip strength (mean) of both sides, mm Hg)	Before	87.5 ± 8.0		80.1 ± 9.2	
	After	70.2 ± 9.5	<0.05	97.1 ± 13.2	<0.05
15 meter walking time (seconds)	Before	20.3 ± 1.7		31.5 ± 5.9	
	After	17.1 ± 0.6	NS	18.9 ± 2.3	NS
ESR (mm/hour)	Before	42.3 ± 6.0		68.5 ± 6.9	
	After	31.7 ± 7.3	NS	22.0 ± 4.9	<0.001
RF (titer)	Before	49.3 ± 13.5		67.2 ± 12.1	
	After	32.0 ± 12.3	NS	32.0 ± 19.1	<0.05

*After vs before treatment

Table 6

Overall Effect of T2 in Patients with Rheumatoid Arthritis

Clinical Responses Compared to the Beginning of the Trial	First Course Treatment				Second Course Treatment			
	Group A T2 (n=27)		Group B Placebo (n=31)		Group A Placebo (n=24)		Group B T2 (n=25)	
	No.	%	No.	%	No.	%	No.	%
Remission	2	7.4	0	0	0	0	0	0
Improvement								
Patient's assessment	25	93	7	23	20	82	20	80
Physician's assessment	25	93	7	23	19	79	22	88
Clinical criteria	22	82	7	23	19	79	11	44
Laboratory evaluation	23	85	4	13	18	75	13	52

An estimate of the incidence of adverse events related to administration of T2 can be obtained from analysis of reports of treatment of large groups of patients with diverse diseases. One such group of 537 patients included 144 with rheumatoid arthritis, 114 with various forms of nephritis, 54 with hepatitis, 57 with reactional states of leprosy, 51 with recurrent mouth ulcers, 15 with SLE and 3 with unclassified disease. The length of treatment of these patients varied from 10 days to 3 months for different patients. The daily dose of T2 was 1.0-1.5 mg/kg body weight. No patient was taking steroids, cytotoxic drugs or other anti-inflammatory drugs during treatment with T2 (15).

As can be seen in Table 7, adverse effects were common. The most common side effects of T2 were gastrointestinal tract disturbances. However, only a few patients had to discontinue T2 because of these adverse reactions. Both leukopenia and thrombocytopenia were transient and disappeared soon after T2 was discontinued. Different side effects occurred after different lengths of exposure to T2. Early in the course of treatment with T2, a number of adverse reactions were noted, including gastrointestinal tract disturbances, loss of appetite, anorexia, vomiting, abdominal pain, diarrhea, and esophageal burning. These reactions usually occurred within days of starting T2 treatment. Leukopenia and thrombocytopenia were also observed during the early period of treatment. Development of oral ulcers, skin pigmentation and disturbances of the menstrual cycle occurred several weeks or months after the beginning of T2 treatment. The development of amenorrhea appeared to be related to the cumulative dose of T2. There were no deaths among the 537 patients treated. All adverse events appeared to be dose related and reversible with cessatation of medication.

Table 7

Adverse Events Related to T2 Treatment of 537 Patients

Clinical manifestation	No. of cases	%
Loss of appetite	31	5.8
Nausea or vomiting	19	3.5
Dryness of mouth	60	11.2
Diarrhea	4	0.76
Constipation	7	1.3
Abdominal pain	11	2.0
Esophageal burning	2	0.4
Other GI disturbances	17	3.2
Leukopenia	34	6.3
Thrombocytopenia	3	0.6
Irregular or delayed menstrual period	19	3.5
Amenorrhea	10	1.9
Skin pigmentation	57	10.6
Skin rash	29	5.4
Herpetic rash	11	2.0
Skin ulceration	2	0.4
Fatigue	8	1.5
Dizziness	11	2.0
Gynecomastia	3	0.6

A similar array of adverse responses was noted in the double-blind trial of T2 in rheumatoid arthritis patients (13,14), although amenorrhea was more frequent (Table 8). This might relate to the somewhat older age of the treated females in this study (Table 2). of female patients having received T2 for 12 weeks, 31% developed amenorrhea, whereas only 5.5% of patients noted it after 4 weeks of treatment in group B. The amenorrhea disappeared several months after withdrawal from T2 in all the patients except 2 who were aged 46 and 49, respectively. A total of 4 patients withdrew from this trial because of the severity of side-effects.

Table 8

Adverse Events During the Double-Blind Trial
of T2 in Rheumatoid Arthritis

	First course				Second Course			
	Group A (n=35)		Group B (n=35)		Group A (n=27)		Group B (n=31)	
	Case	%	Case	%	Case	%	Case	%
Skin rash	15	42.8	1	2.8	0	0	7	22.5
Diarrhea	6	17.1	0	0	0	0	2	6.4
Anorexia	2	5.7	0	0	1	3.7	0	0
Abdominal pain	2	5.7	1	2.8	0	0	0	0
Amenorrhea	5	31.3*	0	0	5	31.3	1	5.5**
Postmenopausal vaginal bleeding	1	10.0***	0	0	0	0	0	0

* Number of female patients aged 49 or less in group A=16

** Number of female patients aged 49 or less in group B=18

***Number of female patients aged 49 or more in group A=10.

2. Ethanol/Ethyl Acetate Extract

There has been a broad uncontrolled experience with the ethanol/ethyl acetate extract of TwHf in a number of inflammatory and autoimmune diseases. The reported results, in general, are comparable to those noted with patients receiving T2. A multicenter open-label trial in 155 patients with rheumatoid arthritis was carried out by the Cooperative Study Group of Hubei Province in 1979 (29). Results of this study showed that 92.4% of patients experienced a positive response, with significant improvement in both clinical parameters and laboratory findings in 29.7% and remission of disease in 23.8% of patients treated with the ethanol/ethyl acetate extract for 3 months or more. Similar results were observed in a second open-label trial involving treatment of 270 patients with rheumatoid arthritis (30). Longer term trials have not been carried out, although many patients have been treated for long periods of time in practice in China.

A phase I dose escalation trial of the ethanol/ethyl acetate extract of TwHf prepared at UT Southwestern Medical Center in patients with rheumatoid arthritis has been initiated (31). To date 13 patients have been treated. Although uncontrolled, the majority of patients receiving 180 mg per day or more improved. Side effects were noted in 5 patients (38%), but were mild and did not necessitate discontinuation of therapy.

Side-effects caused by the ethanol/ethyl acetate extract were comparable in severity and frequency to those noted with T2 administration. Fifty-six of the 155 rheumatoid arthritis patients (36.1%) developed adverse reactions (29). Most common side effects were

gastrointestinal tract disturbances and amenorrhea. It was not necessary to stop the ethanol/ethyl acetate extract because of toxicity in any patient. Functional abnormalities of heart,liver or kidney were not observed. In the second trial of the ethanol/ethyl acetate extract involving 270 patients with rheumatoid arthritis a similar frequency of side effects was noted. Besides the common side effects, such as gastrointestinal tract discomfort and amenorrhea, skin rash, mucous membrane erosions and leukopenia were also occasionally observed (30).

3. Purified Components

TwHf has been documented to contain more than 70 compounds (4). One of these, a diterpenoid lactone, triptolide (Figure 2), has attracted attention because of its potent anti-proliferative effects and alkylating properties (32,33). Because of the possibility that the content of triptolide might account for the therapeutic effects of TwHf, a clinical trial to compare the effects of triptolide with that of the ethanol/ethyl acetate extract in patients with rheumatoid arthritis has been carried out (34). Thirty patients were entered in this study (15 patients in each group). The two groups were treated with a standard dose of the ethanol/ethyl acetate extract (120 mg/day) or the comparable amount of triptolide (0.5-0.75 mg/day). Results from this uncontrolled study indicated that there was significant improvement in both clinical and laboratory parameters in most patients treated with either triptolide or the ethanol/ethyl acetate extract. The improvements in clinical and laboratory parameters of the two groups were comparable except that significant decreases in rheumatoid factor titers were seen in the patients treated with the ethanol/ethyl acetate extract, but not in those receiving triptolide. Side effects caused withdrawal of 47% and 20% of the triptolide and ethanol/ethyl acetate extract

groups, respectively. Triptolide induced considerable serious toxicity including urinary abnormalities, changes in the ECG indicative of myocardial damage, leukopenia, as well as increased levels of SGPT. The comparable therapeutic effect and milder adverse reactions of the ethanol/ethyl acetate extract compared with triptolide have suggested that triptolide might be therapeutically active, but also can account for considerable toxicity, whereas other components of the ethanol/ethyl acetate extract may also have therapeutic efficacy but with less toxicity. One additional preliminary study has suggested that an alkaloid, wilforine, might also be effective in the treatment of rheumatoid arthritis (35). However, subsequent analysis of the wilforine preparation indicated that it was contaminated with triptolide that might have accounted for the therapeutic benefit. No other studies of isolated components of TwHf have been carried out to date.

Animal Studies

A number of *in vivo* studies have suggested that components of TwHf function to suppress various immune and inflammatory responses. A large number of studies have been carried out in China examining the anti-inflammatory and immunosuppressive activities of different extracts of TwHf in various models in mouse, rat and guinea pig (Table 9). Striking immunosuppressive and anti-inflammatory effects were noted, although these were usually observed with much larger concentrations of material than are used to treat human disease. More recently, an extract of TwHf has been shown to decrease proteinuria and prolong survival in the MRL-lpr/lpr model of systemic lupus erythematosus (45,46). Similarly, extracts of TwHf have been reported to suppress allergic encephalomyelitis (47) as well as collagen-induced arthritis in mice (48).

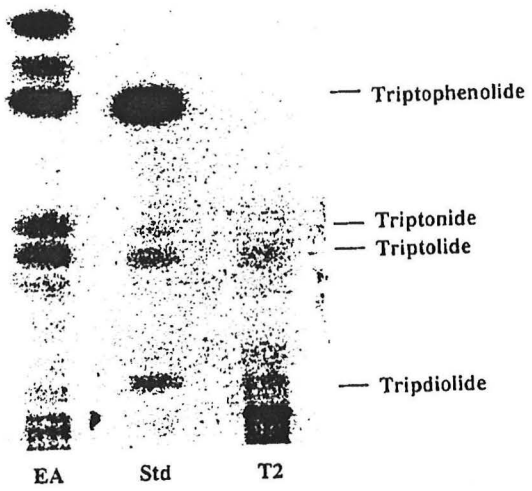
Table 9

In Vivo Anti-Inflammatory and Immunosuppressive Effects of TwHf

Model	Animal	Extract	Daily TwHf Dosage	Ref.
Egg white induced paw edema	rat	Ethanol/ethyl acetate	40 mg/kg	36
Egg white induced paw edema	rat	Decoction	5 gm/kg	37
Egg white induced paw edema	rat	Ethanol	150 mg/kg	38
Agar induced paw edema	rat	T2	30 mg/kg	39
Cotton wad induced granuloma	rat	Ethanol/ethyl acetate	40-80 mg	36
Croton oil induced ear swelling	mouse	triptolide	10 μg/kg	40
Delayed type hypersensitivity	mouse	Decoction	10 g/kg	41
Delayed type hypersensitivity	guinea pig	Decoction	2.5 g/kg	42
Delayed type hypersensitivity	mouse	T2	12 mg/kg	39
Antibody production to SRBC	mouse	Ethanol/ethyl acetate	40 mg/kg	36
Antibody response to SRBC	mouse	triptolide	100-200 μg/kg	40
Adjuvant arthritis	rat	Ethanol/ethyl acetate	40 mg/kg	36
Adjuvant arthritis	rat	Decoction	10 gm/kg	41
Heart transplantation	mouse	Decoction	0.1 gm/day	43
Skin graft	mouse	T2	50 mg/kg	44

In Vitro Studies

In vitro studies have defined the action of components of TwHf more precisely. Initial studies carried out in China noted that various extracts of TwHf suppressed a number of activities of mouse and rat lymphocytes in vitro (49-53). More recent studies have defined the major immunosuppressive activities of TwHf more precisely (12,54-56). Both T2 and the ethanol/ethyl acetate extract of TwHf were found to suppress transcription of the interleukin 2 gene and related cytokine genes,such as the gamma interferon gene that were regulated by related promoter elements. By contrast, cytokines regulated by different promoter elements, such as interleukin 1 and interleukin 6, were not suppressed. The data, furthermore, indicated that transcription of target cytokines was directly suppressed, whereas no impact on membrane proximal signaling events was apparent. Although the action of T2 and the ethanol/ethyl acetate extract were superficially similar to that of cyclosporine and FK-506, the extracts of TwHf had no inhibitory effect on the phosphatase activity of calcineurin, the known target of cyclosporine and FK-506. Thus, the extracts of TwHf inhibit transcription of a variety of genes involved in promoting immune and inflammatory responses by a novel mechanism. This effect could well explain the clinical effects of the extracts of TwHf. Of note, two of the diterpene esters found in both T2 and the ethanol/ethyl acetate extracts of TwHf, triptolide and tripdiolide, appeared to be sufficient to explain the immunosuppressive activity of each (Figure 3, Table 10).



Diterpenoid compounds detected in the EA extract and T2. T2 or the EA extract or the purified diterpenoid standards was dissolved in chloroform and filtered. The samples were applied to an aluminum oxide column and then eluted with ethanol. After concentration, the samples were spotted on a silica gel plate. The samples were resolved with a mixture of chloroform and ether (1:4). Diterpenoid molecules were visualized with the Kedde reagent, which detects the α,β-unsaturated lactone ring of the diterpeniod compounds.

Figure 3

However, additional diterpenes and alkaloids in the extracts also exhibited immunosuppressive potential (12).

Toxicity in Animals

In China, acute toxicity testing in mice (Hubei white) indicated that the LD50 of the ethanol/ethyl acetate extract was 608-858 mg/kg. This varied with the source of plant material and the season of harvest. Examination of mice dying during the acute toxicity test of the extract (714-1400 mg/kg every 3 days, p.o.) showed that the most significant changes developed in lymphatic organs, including induction of atrophy of the thymus. Microscopic examination demonstrated changes that were especially noteworthy in the lymphatic system. These included decreased numbers of nodules and lymphocytes in lymph nodes, spleen and intestine. These findings suggested that the major target organ was the lymphatic system, which is consistent with the immunosuppressive action of the ethanol/ethyl acetate extract observed

Table 10
Comparison of the Concentrations of Diterpenoids in the Ethanol/Ethyl Acetate Extract and T2 to Their Immunosuppressive Activity

T Cell Function		EC_{50} (ng/ml)	Amount of Component in Extract at EC_{50} (ng/ml)			
			Triptolide	Tripdiolide	Triptonide	Triptophenolide
Proliferation	EA extract	806.67 ± 72.34	0.87 ± 0.07	0.25 ± 0.02	0.64 ± 0.02	3.30 ± 0.13
	T2	983.33 ± 61.10	0.35 ± 0.02	0.67 ± 0.09	0.03 ± 0.01	0.04 ± 0.13
	Triptolide	0.61 ± 0.11				
	Tripdiolide	0.86 ± 0.13				
	Triptonide	2.46 ± 0.21				
	Triptophenolide	830.11 ± 102.23				
IL-2 Production	EA extract	497.67 ± 51.93	0.54 ± 0.06	0.15 ± 0.02	0.39 ± 0.02	2.04 ± 0.01
	T2	946.67 ± 141.89	0.34 ± 0.05	0.64 ± 0.10	0.03 ± 0.01	0.04 ± 0.01
	Triptolide	0.61 ± 0.11				
	Tripdiolide	0.86 ± 0.13				
	Triptonide	2.46 ± 0.21				
	Triptophenolide	830.11 ± 102.23				

[a]The inhibitory effect of ethanol/ethyl acetate (EA) extract, T2 or the individual diterpenoids on IL-2 production and DNA synthesis by PHA-stimulated T cells was examined. The concentrations causing 50% inhibition of responsiveness (EC_{50}) were then calculated.

both *in vitro* and *in vivo*. Mild weight loss and atrophy of the testes along with a decrease in the number and degeneration of spermatocytes were also found in these mice (57).

Subacute toxicity testing was conducted by treating rats with 40 mg/kg/day of the ethanol/ethyl acetate extract for 5 weeks,or dogs with 10 mg/kg/day for 90 days. Sub-chronic toxicity testing was also carried out by feeding rats different doses of the extract for 6 months. Histological examination of the tissue of the animals receiving these various doses of the ethanol/ethyl acetate extract revealed changes in the lymphatic system, and reproductive organs, but of a milder nature than that observed in the acute toxicity test. The minimum dose required to induce these changes was 50 mg/kg/day. Pathological changes in both the lymph system and testes were dose-dependent. There were no microscopic abnormalities observed in liver, kidney, lung and adrenal gland of the animals (58).

T2 induced toxicity has also been examined in animal models (59-61). The LD_{50} for acute toxicity in mice was 159.7 ± 14.3 mg/kg. Administration of T2 at 60 mg/kg for 60-80 days, which was higher than that used for the studies of efficacy, did not affect body weight or the histology of most visceral organs except that of the thymus in mice. Long term treatment of dogs with 5 mg/kg of T2 for 14.5 months did not affect body weight or hepatic or renal function. No pathological changes of the testes of the treated animals were observed. However, treatment of female mice with T2 at a dosage equal to 4.2% of the LD_{50} (~21 mg/kg) for 5 months reduced the frequency of pregnancies and the number of fetuses in pregnant mice. T2 treatment also decreased the activity of sperm of rats in a time dependent manner. Treatment of dogs for 14.5 months with 10 mg/kg of T2 also caused significant atrophy of the testes.

These results suggest that administration of the various extracts of TwHf at dosages which were effective in the treatment of autoimmune diseases in animal models is relatively safe. Long term, high dose administration of the various extracts can, however, induce damage to the reproductive organs.

Summary

Tripterygium wilfordii Hook f has been widely used to treat patients in China with a variety of autoimmune and inflammatory disorders. Despite the absence of properly controlled trials, the magnitude of the experience and the wide acceptance of the material by patients suggest efficacy. Extensive biochemical and pharmacologic analyses in China have identified a number of components of the extracts that are candidates to explain their apparent efficacy. More recent work in the west has suggested a unique immunosuppressive and anti-inflammatory mode of action of these specific components. More clinical and biochemical analyses seem warranted to document the full therapeutic potential of components of Tripterygium wilfordii Hook f.

References

1. Yunsheng J and Zhao S: Tripterygium wilfordii Hook induced acute toxicity with kidney involvement in 17 cases. Chin J Kid Dis 3:167, 1987.

2. Chen L, et al: Clinical analysis of 10 cases of Tripterygium wilfordii Hook induced toxicity. Proc Sym. Clinical Application of Tripterygium wilfordii Hook. Hubei, China, 1987.

3. Zheng J, et al: Clinical and experimental studies on Tripterygium. J Tradit Chin Med 23:714-718, 1982.

4. Zhand L, et al: The achievements in the studies of chemical components of TWH family. J Chin Pharm Univ 21:251, 1990.

5. Xue Z-Y: Treatment of 21 cases of systemic lupus erythematosus with Tripterygium wilfordii Hook f. Chin J Derm 7:201, 1981.

6. Chen Z, et al: Proceedings in the study of the active compounds of tripterygium wilfordii Hook F. Zong Guo Yi Yaun Yao Xue Za Zhi 6:26, 1986.

7. Chen Z, et al: Study of the active compounds and the preparation of Tripterygium wilfordii Hook F. Zi Liao Hui Bian 6-9, 1988.

8. Lu X, et al: The isolation of the structure of triptochlorolide (T4) from Tripterygium wilfordii. ACTA Academiae Medicinae Sinica 12:157, 1990.

9. Ma P, et al: 16-hydroxytriptolide, a new active diterpene isolated from tripterygium wilfordii. ACTA Pharmaceutica Sinica 26:759, 1991.

10. Chen Z, et al: Quantitative measurement of triptolide content in the tablets of Tripterygium wilfordii Hook F. Zi Liao Hui Bian, 1985.

11. Fang G, et al: Quantitative measurement of triptolide contained in the ethyl acetate extract of tripterygium wilfordii Hook F and its tablets. Zhong Yao Tong Bao 11:38, 1986.

12. Tao XL, Cai JJ and Lipsky PE. The identity of immunosuppressive components of the ethyl acetate extract and chloroform methanol extract (T2) of Tripterygium wilfordii Hook f. J Pharm Exp Ther 272:1305, 1995.

13. Xu W, Zheng J and Lu X: Tripterygium in dermatologic therapy. Int J Derm 24:152, 1985.

14. Tao X, et al: A prospective, controlled, double-blind, cross-over study of Tripterygium wilfordii Hook F in treatment of rheumatoid arthritis. Chin Med J 102:327, 1989.

15. Tao XL, Dong Y and Zhang N-Z: A double-blind controlled trial of Tripterygium wilfordii glycosides in rheumatoid arthritis. Arthritis Rheum 30:S59, 1987.

16. T2 study group of Jiang Su, China: Summary on the clinical trials of T2 in the treatment of 554 patients with various diseases. Ann Chin Acad Med Sci 3:3, 1982.

17. Yu D: Clinical observation of 144 cases of rheumatoid arthritis treated with glycoside of radix Tripterygium wilfordii. J Tradit Chin Med 3:125,1 983.

18. T2 study group of Chinese Institute of Dermatology: Clinical observation on the treatment of skin disorders with polyglycoside of Tripterygium wilfordii Hook F. ACTA Acad Med Sinicae 1:6, 1979.

19. Xie DF: Experience in the treatment of Behcet's disease with polyglycosides of Tripterygium wilfordii Hook f. Zohg Xi Yi Jie He Za Zhi 3:349, 1983.

20. Peng S-Y: Report on 20 patients with Henoch-Schölein purpura treated with polyglycosides of Tripterygium wilfordii Hook (T2). Jiang Su Yi Yao 11:38, 1983.

21. Feng B: Observation on the effect of polyglycosides of Tripterygium wilfordii Hook (T2) in 50 cases of leprosy reactive status. Clin Derm J 4:211, 1985.

22. Wu YZ: Treatment of neuralgia of leprosy reactive status with polyglycosides of Tripterygium wilfordii Hook f (T2). Chin J Derm 19:217, 1986.

23. Jiang X-Y: Exploration of the treatment of nephrotic syndrome with polyglycosides of Tripterygium wilfordii Hook f (T2). Chin J Ped 20:201, 1982.

24. Li X-W: Clinical observation on the treatment of 53 cases of nephrotic syndrome with polyglycoside of Tripterygium wilfordii Hook f (T2). Chin J Ped 20:203, 1982.

25. Wang QW, et al: Clinical investigation of treatment with Tripterygium wilfordii Hook f of idiopathic IgA nephropathy. Jian Su Yi Yao 1:7, 1991.

26. Li X-W: Treatment of 50 cases of childhood purpura nephritis with polyglycosides of Tripterygium wilfordii Hook f. (Clinical observation). Jiang Su Yi Yao 12:644, 1987.

27. Pan Y-R: Treatment of purpura nephritis with Tripterygium wilfordii Hook f. ACTA Acad Med Sinicae 9:2, 1987.

28. Cheng R-L: Observation of the therapeutic effect of polyglycosides of Tripterygium wilfordii Hook (T2) combined with thyroid gland tablets on chronic lymphocytic thyroiditis. Zhong Xi Yi Jie He Za Zhi 8:676, 1988.

29. Hubei Cooperative Study Group of Tripterygium wilfordii Hook: Clinical study of the extract of tripterygium wilfordii hook in the treatment of rheumatoid arthritis: Wu Han Yi Xue Yuan Xue Bao 4:62,1981.

30. Shu D, et al: Report of 270 cases of rheumatoid arthritis treated with ethyl acetate extract of tripterygium wilfordii Hook. Zhong Yao Li Yu Lin Chuang 5:40, 1989.

31. Cush J, Garrett M, Nichols L, Cai J, Tao X and Lipsky P: Phase I dose escalation trial of an ethyl acetate extract of Tripterygium wilfordii Hook F in rheumatoid arthritis. Arthritis Rheum. (Submitted).

32. Kupchan SM, Court WA, Dailey RG, Gilmore CJ and Bryan RF: Triptolide and tripdiolide, novel antileukemic diterpenoid triepoxides from Tripterygium wilfordii. J Am Chem Soc 94:7194, 1972.

33. Kupchan SM and Schubert RM: Selective alkylation: a biomimetic reaction of the antileukemic triptolides? Science 185:791-793, 1974.

34. Shu DF, Li RL and Sun Y: Comparative study of triptolide and the ethyl acetate extract of Tripterygium wilfordii Hook F in the treatment of rheumatoid arthritis. Zhong Cao Yao 10:144, 1990.

35. Shu SY: Preliminary study of the treatment of rheumatoid arthritis with wilforine: Zhong Cao Yao 11:33, 1980.

36. Li L, et al: Pharmacological study of the ethyl acetate extract of Tripterygium wilfordii Hook f. Zhong Cao Yao 13:27, 1982.

37. Hubei Cooperative Study Group of Tripterygium wilfordii Hook: Preliminary study on the pharmacological effect of Huangteng. Hubei Wei Sheng 1:72, 1979.

38. Zhang B, et al: Study on the pharmacological action of total alkaloids in Tripterygium hypoglaucum Hutch. Zhong Cai Yao 16:24, 1985.

39. Zheng J, et al: Studies on pharmacological actions of total glycosides in Tripterygium wilfordii Hook. Acta Acad Med Sinicae 5:1, 1983.

40. Li L, et al: Effect of triptolide on inflammation and immune function. Zhong Guo Yao Li Xue Tong Bao 2:25, 1986.

41. Zheng Y, et al: Effect of Tripterygium wilfordii Hook decoction and its effective fraction on immune functions. Fujian Yi Yao Za Zhi 4:30, 1982.

42. Qian O and Y Gao: Experimental study of the effect of Tripterygium wilfordii Hook on allergic and toxic contact dermatitis. Chin J Derm 18:2, 1985.

43. Zhao P, et al: Prolonged servival of syngeneic heart graft in mice by treatment with Tripterygium wilfordii Hook. Zhong Xi Yi Jie He Za Zhi 8:31, 1988.

44. Chang J-L, et al: A preliminary study on the immunosuppressive activity of mixed glycoside of TWH. Chin J Micro Immunol 4:331, 1984.

45. Zhang XY, et al: Prolonged survival of MRL-lpr/lpr mice treated with Tripterygium wilfordii Hook F. Clin Immun Immunopathol 62:66, 1992.

46. Gu W-Z, Banerjee S, Rauch J and Brandwein SR: Suppression of renal disease and arthritis, and prolongation of survival in MRL-lpr mice treated with an extract of Tripterygium wilfordii Hook F. Arthritis Rheum 35:1381, 1992.

47. Cheng HW, et al: Cellular immunological study on experimental allergic encephalopathy and the exploration of the effect of Tripterygium wilfordii Hook on it. Immunol Bull 5: 7, 1985.

48. Gu W-Z, Brandwein SR, and Banerjee S: Inhibition of type II collagen-induced arthritis in mice by an immunosuppressive extract of Tripterygium wilfordii Hook f. J Rheumatol 19:682-688, 1992.

49. Zuo DM and Shang SL: Different effect of Tripterygium reglii on T and B cell function. Chin J Immunol 2:232, 1986.

50. Zhang LS: Inhibitory effect of celastrol on murine lymphocyte proliferation. Acta Phamacol Sinicae 7:85, 1986.

51. Nan J, et al: Effect of T II and Huang Jian (Po) on lymphocytes: Zi Liao Xuan Bian 10:5, 1979.

52. Kuang YD, et al: Effect of TWH on IL-2 production and IL-2 receptor expression. Shanghai J Immunol 8:250, 1988.

53. Zhu XY, et al: *In vitro* observation on the effect of Tripterygium hypoglaucum hutch on immune responses. Physiol Sci 8:417, 1988.

54. Tao XL, Davis LS and Lipsky PE: Effect of an extract of the Chinese herbal remedy Tripterygium wilfordii Hook F on human immune responsiveness. Arthritis Rheum 34:1274-1281, 1991.

55. Li XW, Weir MR: Radix Tripterygium wilfordii - a Chinese herbal medicine with potent immunosuppressive properties. Transplant 50:82-86, 1990.

56. Tao XL, Davis LS, Hashimoto K and Lipsky PE: The Chinese herbal remedy, T2, inhibits mitogen-induced cytokine gene transcription by T cells, but not initial signal transduction. J Pharm Exp Ther, 1995, in press.

57. Zhang Y, et al: An experimental pathological study of intoxication by the ethyl acetate extract of Lei Gong Teng (Tripterygium wilfordii Hook F). Yao Wu Yan Jiu 4:367, 1983.

58. Wang Z, et al: An experimental pathological study of subchronic toxicity by ethyl acetate extract of tripterygium wilfordii Hook. Tong Ji Ti Ke Da Xue Bao 20:247, 1991.

59. Zheng J, Liu J, Hsu LF, Gao J and Jiang BL: Studies on toxicity of total glycosides in Tripterygium wilfordii. Acta Acad Med Sinicae 5:73, 1983.

60. Zheng JR, et al: Effect of total glycosides of Tripterygium wilfordii on animal reproductive organs. 1. Experiments of male rats. Acta Acad Med Sinicae 5:73, 1985.

61. Zheng JR, et al: Effects of total glycosides of Tripterygium wilfordii on reproductive organs of experimental animals. II. Experiments in female rats. Acta Acad Med Sinicae 7:256, 1985.