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Review

Immunosuppressant discovery from *Tripterygium wilfordii* Hook f: the novel triptolide analog (5*R*)-5-hydroxytriptolide (LLDT-8)

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The Chinese traditional herb *Tripterygium wilfordii* Hook f (TwHF) has been widely used in the treatment of autoimmune and inflammatory diseases. Over the past few decades, great efforts have been made to explore modern preparations of TwHF with higher efficacy, solubility, and lower toxicity. In this study, we reviewed several examples both of naturally occurring compounds and their derivatives in TwHF, and summarized the preclinical evaluations with regard to autoimmune and inflammatory diseases. All of the candidate compounds described herein have been or are currently in clinical trials. Although some studies encountered problems, the data still provided valuable references for future studies. (5*R*)-5-hydroxytriptolide (LLDT-8, Leitengshu) is a novel triptolide derivative with potent immunosuppressive and anti-inflammatory activities developed at Shanghai Institute of Materia Medica. Indeed, a Phase I clinical trial for this compound has been completed in rheumatoid arthritis patients. The results will provide the basis for the further exploration of this ancient herb and encourage the research and development of valuable traditional Chinese medicine.

Keywords: *Tripterygium wilfordii* Hook f; triptolide; (5*R*)-5-hydroxytriptolide (LLDT-8); autoimmune diseases; immunosuppressants; clinical trials

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Introduction

For thousands of years, natural products have played an important role throughout the world in treating and preventing human diseases[1–5]. The discovery and development of immunosuppressants from natural sources have an impressive record: mycophenolic acid (MPA) and cyclosporin A (CsA), the fungal metabolites isolated in 1932[6] and 1970[7], respectively; rapamycin, found in 1975[8]; FK506, extracted from a culture filtrate of *Streptomyces tsukubaensis* in 1987[9, 10]; and fingolimod (FTY720), described in 1995[11]. Although the current industry model for drug discovery does not favor natural products, the resources are so vast as to seem unlimited, and these emerging tools will provide important discoveries, leading to new medicines[12]. Furthermore, the drugs already in use as immunosuppressants (*eg*, cyclophosphamide, methotrexate, azathioprine, cyclosporin) are associated with some significant problems, including toxicity, a lack of reversibility, and increased susceptibility to viral and other infections[13]. Indeed, exploring new and innovative immunosuppressants from natural sources has now become a focus of intense research.

Tripterygium wilfordii Hook f (TwHF) and its extracts have been widely used in the treatment of autoimmune and inflammatory diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and dermatomyositis (DM)[14–18], and have beneficial effects on tissue and organ transplantation[19, 20]. In 1993, the ethyl acetate (EA) extract of TwHF was entered into a Phase I study for the treatment of RA patients[21–25], and many clinical trials have tested triptolide for the treatment of RA and psoriasis[26, 27]. In addition, PG490-88/F60008 (Figure 1), a water-soluble prodrug of triptolide, has been approved for entry into a Phase I clinical trial for the treatment of solid tumors[28, 29].

The Shanghai Institute of Materia Medica (SIMM) has made great efforts to discover drugs from natural products that are of clinical value and have contributed to the treatment of autoimmune diseases, such as RA, SLE, and multiple sclerosis (MS). By combining basic and applied research efforts and through the collaboration between chemistry and biology, the SIMM has developed several series of immunosuppressive drug candidates against autoimmune diseases: LLDT-8 (Figure 1), a novel triptolide analog from the Chinese traditional herb

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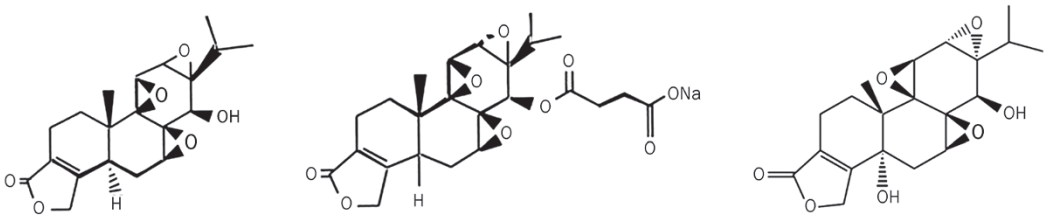

Figure 1. The structure of triptolide and its derivatives, PG490-88 (F60008) and LLDT-8.

TwHF, that will enter into a Phase II clinical trial involving RA patients in China[30–40]; SM934, a water-soluble derivative of artemisinin; and periplocoside E, a pregnane glycoside identified from *Periploca sepium* Bge.

This review focuses on the drug candidates isolated from TwHF. Regardless of the success or failure in treatment, the experience gained through the exploration and practice is invaluable.

TwHF, a representative Chinese medicinal herb showing immunosuppressive and anti-inflammatory activities

TwHF is a deciduous climbing vine that grows up to 12 meters and has brown, angular, downy twigs. The leaves are light green, smooth adaxially, and pale gray with light-colored hairs abaxially. As a well-known Chinese medicinal herb, TwHF is distributed widely in southern China, including Fujian, Zhejiang, and Anhui Provinces. The Chinese herb Lei Gong Teng is derived from the roots of TwHF and has been used in traditional Chinese medicine for more than two thousand years. The description of TwHF has been traced to the period of the Three Kingdoms (220–280 AD) when these plants were recorded as "Mangcao" in *Shennong's Chinese Materia Medica*. In the ensuing thousand years, much Chinese literature, including *Materia Medica of South Yunnan (dian nan ben cao)* and *Guidelines and details on roots and herbs (Bencao gangmu)* of the Ming dynasty, *Chinese herbal Iconographia Plantarum* of the Qing dynasty, and *Icons of Chinese medicinal plants* in the 20th century, has recorded the resources, efficacy and medicinal application of TwHF. Modern research on the pharmacology of TwHF focuses on active component identification, structure modification and novel derivative discovery.

For centuries, Chinese people have used TwHF and its extracts for the treatment of autoimmune and inflammatory diseases, including RA, SLE, and DM. Contemporary researchers have attempted to standardize the TwHF extract for further development and investigate its efficacy in autoimmune diseases, and some progress had been achieved in recent years. The ethyl acetate (EA) extract of TwHF was entered into a Phase I study that included 13 patients with established RA in 1993[21–25]. The EA extract of TwHF at dosages up to 570 mg/d appeared to be safe, and doses >360 mg/d were associated with clinical benefits in the patients with RA[21]. The dosage was normalized to previous extracts by assessing the content of triptolide and tripdiolide. A randomized, controlled, 24-week study was then conducted in 2004 in patients with active RA and 6 or more painful and swollen joints[22]. The results demonstrated that the treatment with a standardized extract from the peeled roots of TwHF administered (60 mg 3 times daily) over 24 weeks may be both effective and safe in treating patients with active rheumatoid arthritis.

With the recent technological developments in the isolation and structural identification of compounds, more than 100 components have been isolated from TwHF, with most of them having a potent therapeutic efficacy for a variety of autoimmune and inflammatory diseases. Among the reported active components from this herb, including triterpene, diterpene and macrocyclic alkaloids, the most noteworthy component is triptolide. Triptolide, an oxygenated diterpene, was identified as the most active component, accounting for the immunosuppressive effects of TwHF[41, 42]. Over the past few decades, much research has been conducted on the clinical use of triptolide for the treatment of autoimmune and inflammatory diseases, such as RA, SLE, nephritis[26, 43–52], psoriasis[27], and Crohn's disease[53], and kidney transplantation[54]. However the strong toxicity, particularly with regard to male reproductive toxicity, limits the application of triptolide to a great extent[55–58].

To identify more effective compounds with less toxicity and higher solubility, the structural modification of triptolide was studied, and derivatives were synthesized and evaluated for their biological activities. In the past few years, new water-soluble triptolide derivatives have been designed and synthesized, including PG490-88 or F60008. PG490-88 or F60008, a prodrug of triptolide, is converted to triptolide *in vivo* by plasma esterases following intravenous administration[59-67]. Table 1 summarizes the preclinical pharmacological study of triptolide and PG490-88/F60008 against autoimmune diseases and transplantation rejection. Although PG490-88 or F60008 has been approved for entry into a Phase I clinical trial for the treatment of solid tumors, two lethal events were observed in twenty patients, and the high inter-individual variability rendered PG490-88 or F60008 a far from optimal derivate of triptolide[28, 29].

(5*R*)-5-hydroxytriptolide (LLDT-8, Leitengshu), a novel triptolide analog in clinical trials

Great efforts have been made at the SIMM in the search for promising triptolide analogs with a low toxicity and relative high immunosuppressive activity, and a series of novel triptolide analogs have been successfully synthesized. We have identified one derivative, (5*R*)-5-hydroxytriptolide (LLDT-8, Leitengshu), which demonstrates potent immunosuppressive and anti-inflammatory activities[31]. Over the course of ten years, the biological activity of LLDT-8 has been evaluated, and the underlying mechanisms have been investigated with regard to many autoimmune and inflammatory diseases. The administration of LLDT-8 reduced the incidence and severity of collagen-induced arthritis in DBA/1 mice[33]. To assess the long-term effectiveness of LLDT-8, 3-month-treatment experiments were performed. The oral administration of LLDT-8 (0.125, 0.25, and 0.5 mg/kg, starting at 1 d before the booster immunization) consistently attenuated the severity of CIA compared with untreated mice. LDP, Leigongteng Duodai Pian, is the prescription drug of the extracts of TwHF used for treating RA in China. The preventive effect of LLDT-8 at a dose of 0.25 mg/kg was similar to LDP at 20 mg/kg in CIA mice. We also tested the therapeutic effect of LLDT-8 after the establishment of RA, and the inhibitory profile was persistent during the 3-month observation. LLDT-8 also exerted therapeutic effects on experimental autoimmune encephalomyelitis (EAE)[36], concanavalin A-induced acute hepatitis[37], graft-versus-host disease (GVHD)[32], allograft rejection[38], and bleomycin-induced lung fibrosis[39]. LLDT-8 effectively inhibited human T cell immune responses without affecting the NK cytotoxic activity, and this immunosuppressive activity was parallel to that observed for murine immunity[40]. The mechanism of LLDT-8 involves a variety of immune cells and molecules and includes limiting T cell function and proliferation, inhibiting macrophage activation, inducing regulatory T cell expansion, and interfering with IFN-γ-related signaling[31–40]. More importantly, compared to triptolide, LLDT-8 displayed a much lower toxicity both *in vitro* and *in vivo*. The CC_{50} value of LLDT-8 was 256.6±73.8 nmol/L, and the CC_{50} value of triptolide was 2.1±0.3 nmol/L in murine splenocytes. The immunosuppressive effects of LLDT-8 and triptolide were also tested in mitogen- and alloantigen-induced lymphocyte proliferation assays. The IC_{50} values of triptolide for inhibiting ConA-induced T lymphocyte proliferation, LPS-induced B lymphocyte proliferation, and mixed lymphocyte reaction (MLR) were 6.7±0.2, 8.6±2.8, and 2.7±0.6 nmol/L, respectively, with the IC_{50} values of triptolide being close to or even higher than the CC_{50} values. This result indicated that the activities of triptolide were largely dependent on its cytotoxicity. However, the IC_{50} values of LLDT-8 for inhibiting the lymphocyte proliferation caused by ConA, LPS, or MLR were lower than its CC_{50} values (IC_{50}=131.7±32.4, 171.5±17.3, and 38.8±5.1 nmol/L, respectively), thus excluding the possibility that the inhibitory activities of LLDT-8 were attributable to its cytotoxicity[31]. When administered in mice, the lethal dose for 50% of the animal test population of LLDT-8 is 9.3 mg/kg (intraperitoneal), whereas that of triptolide is 0.86 mg/kg, with a 10-fold lower acute toxicity *in vivo*[30]. Table 2 summarizes the preclinical pharmacological study of LLDT-8 as an immunosuppressant drug candidate. Female RA patients who were over the 35 years of age and menopausal or did not have birth demands were enrolled in a tolerability and pharmacokinetic study. The tolerability and pharmacokinetic properties of LLDT-8 and its initial therapeutic efficacy were assessed and determined. According to the pharmacokinetic and pharmacodynamic results reported using experimental animals, the initial dose should be set at 0.25 mg/d. In accord with the dose escalation scheme, the highest dose could be set at 4 mg/d in the phase I clinical trial for LLDT-8[30]. The results of the clinical trial will provide the basis for the further exploration of this novel derivate of triplide and encourage the research and development of valuable traditional Chinese medicine.

Concluding remarks

Research on TwHF has long been an intense issue. During the past few decades, several drug candidates from this herb have been or are currently in clinical trials. However, it has been demonstrated that some compounds cannot be considered the optimal derivative of triptolide. For other compounds, for example, LLDT-8, we are still awaiting the results of the clinical studies. Regardless of the results of the trials, research on this important ancient medicinal herb will continue.

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Abbreviations

TwHF, *Tripterygium wilfordii* Hook f; TCM, traditional Chinese medicine; LLDT-8, (5*R*)-5-hydroxytriptolide; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; DM, dermatomyositis; EA, ethyl acetate; MPA, mycophenolic acid; CsA, cyclosporin A; FTY720, fingolimod; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; GVHD, graft-versus-host disease.

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Table 1. The preclinical pharmacological study of triptolide and PG490-88/F60008 against autoimmune diseases and transplantation rejection.

Compounds	Disease	Models	Regimens	Reported responses	Possible mechanism	Refs
Triptolide	Rheumatoid arthritis	Type II collagen-induced arthritis (CIA) in Lewis rats	Oral administration of 0.1 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ for 28 d	Significant delay in time to onset of arthritis, as well as significantly decreased arthritis incidence, clinical arthritis severity score, histopathological arthritis severity score, and *in vivo* cell-mediated immunity to collagen	Suppressed cell-mediated immune responses	16
	Rheumatoid arthritis	Collagen-induced arthritis in DBA/1 mice	Oral administration (8, 16, and 32 $\mu g{\cdot}kg^{-1}{\cdot}d^{-1}$, started when the first clinical signs of disease were beginning, and continued for 21 d	Arthritis incidence was reduced in all groups after receiving triptolide (8–32 μg/kg), arthritis scores was suppressed at 16 and 32 μg/kg doses	Suppression of MMPs, up-regulation of TIMPs, interference with the gene expression of proinflammatory cytokines and PGE_2 production, inhibition of NF-κB	44
	Arthritis	Adjuvant-induced arthritis in rat	ip administrations from day 14–20 after immunization at a dose of 0.1, 0.2, and 0.4 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$	Thickness index decreased in all administration groups and maximal inhibition occurred at a dose of 0.4 mg/kg	Over-expression of MCP-1, MIP-1α, and RANTES at both mRNA and protein levels were inhibited	45
	GVHD	C57BL/6 to BDF1 murine BMT model	Oral or intraperitoneal treatment for only 14 d	Prevented GVHD induction and development, produced long-term survival	Induction of anergy and a deviation away from a proinflammatory phenotype	52
	Transplantation	F344 donor to Lewis recipient rat cardiac transplantation	Intraperitoneal treatment at doses of 0.04, 0.08, 0.16, and 0.32 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ starting on the day of transplantation	The median survival time (MST) was 8 d for placebo; 9.5, 11, 14, and 19 d for triptolide monotherapy at doses of 0.04, 0.08, 0.16, and 0.32 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, respectively	/	46
	Transplantation	Mouse model of cardiac transplantation	ip administration of 3 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ of triptolide at d 0–7, 9, 11, 13, and 15 post transplantation	MST in vehicle group: 7.66±0.8 d, in triptolide group: 23.5±5.3 d	/	47
	Transplantation	Skin allograft rejection	0.1 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$	Prolonged the graft survival when triptolide was given for 9 d after transplantation, but not before transplantation.	Inhibits lymphocyte activation at a relatively late stage	49
	Transplantation	Rat cardiac and renal allograft (from Brown Norway into the abdomen of Lewis rats)	ip dosing of 2.5, 5, 10, 20, or 30 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ for 16 d; oral administration for 16 d at doses of 5, 10, or 30 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ starting 1 d before transplantation	MST of heart allografts was significantly longer in ip dosing of 10, 20, or 30 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, also in oral administration at doses of 5, 10, or 30 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ MSTs of renal allograft in ip dosing of 20, 30 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ significantly longer	/	55
PG490-88 (F60008)	GVHD	Bone marrow and spleen cells transplantation (B10.D2 to BALB/c mice)	ip at 0.535 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ for the first 3 weeks after transplantation	Protected from developing GVHD up to 100 d	Inhibition of alloreactive T cell expansion through interleukin-2 production	59
	Transplantation	MHC-mismatched renal transplanted into cynomolgus monkeys	0.03 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ by gavage	PG490-88 monotherapy failed to prolong allograft survival	Inhibition of T-cell activation and a decrease in IFN-γ production and NF-AT/NF-κB activity	61
	Transplantation	Dog renal transplantation model	Oral administration at 0.06 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$	Prolonged graft survival from a MST of 6 d to 11 d	Inhibition of complement activation and T-cell infiltration	62

(To be continued)

Compounds	Disease	Models	Regimens	Reported responses	Possible mechanism	Refs
	Transplantation	Chronic rejection in rat kidney (F344 to LEW rats)	0.5 mg·kg^{-1}·d^{-1} for 10 d	Moderate histological changes on d 90	Suppression of intragraft gene expression	63
	Transplantation	Acute renal rejection across the ACI-to-LEW rat strain	Orally administered at 0.3, 0.5, or 1.0 mg·kg^{-1}·d^{-1}d for 10 d	Dose-dependent prolongation of kidney allograft survival at 0.5 and 1.0 mg·kg^{-1}·d^{-1}	/	64
	Transplantation	Mouse heterotopic tracheal allograft model of oblitera-tive airway disease	Intraperitoneal injection at 0.25 mg·kg^{-1}·d^{-1}	Attenuates airway obliteration and inhibits accumulation of inflammatory cells	Direct effect on DC or an indirect effect resulting from increased T-cell-mediated apoptosis to be elucidated	65
	GVHD	Murine allogeneic BMT model (B10.D2 to BALB/c)	ip administration at 0.0535 mg/mL daily for the first 3 weeks after BMT	Protected from lethal GVHD for more than 100 d	Induction of responding T cells anergy or TH_2 responses	59, 66
	Lung fibrosis	Bleomycin-induced lung fibrosis	ip administered at 0.25 mg/kg on the same day or 5 d after bleomycin installation	Blocks both inflammation and fibrosis in the bleomycin model of mouse lung fibrosis	Inhibition in TGF-β gene expression	67

Table 2. The preclinical pharmacological study of LLDT-8 as an immunosuppressant drug candidate.

Compounds	Disease	Models	Regimens	Reported responses	Possible mechanism	Refs
LLDT-8	Rheumatoid arthritis	Collagen-induced arthritis in DBA/1 mice	*po* administrations for 3 months (0.125, 0.25, and 0.5 mg/kg, starting from 1 d before booster immunization)	Attenuated the severity of CIA	Blockade of IFN-γ signaling, IFN-γ related cytokine and chemokine	33
	Multiple sclerosis	MOG 35-55 induced EAE in mice	1 mg·kg^{-1}·d^{-1} LLDT-8 *ip* from the day of EAE induction	Reduced the incidence and severity of EAE	Suppression of T cell proliferation and activation	36
	GVHD	Allo-BMT murine model (BLAB/c, H-2d to C57BL/6, H-2b) of aGVHD	1 mg·kg^{-1}·d^{-1} administered orally starting on day of allo-BMT until the end of experi-ment	Prevented weight loss and death, extended survival of allo-BMT mice	Increased the $CD4^+CD25^+$T cells and up-regulated Foxp3 expression	32
	Transplantation	Balb/c to C57BL/6 murine cardiac transplantation model	LLDT-8 (1, 0.25 mg·kg^{-1}·d^{-1}) was admini-stered orally starting on the transfer day until the end of experiment	Induced the survival prolongation of allogeneic cardiac graft	Reduced expression of chemokines and its receptor	38
	Acute Hepatitis	ConA-induced hepatitis in mice	Pretreatment with 0.5, 1, or 2 mg/kg LLDT-8 (by ip) four times (on d -3, -2, -1, and 1 h before conA injection)	Significantly increased the survival rates to 83%, 86% and 100%, respectively	Blockade of IFN-γ/STAT1/IRF-1 signaling- and inflammatory medi-ators	37
	Lung fibrosis	Bleomycin-induced lung fibrosis	LLDT-8 (0.5, 1, and 2 mg/kg, ip) admini-stered once daily for 7 or 14 consecutive days	Protective against bleomycin-induced lung fibro-sis, alleviated the body weight loss and lung index increase caused by bleomycin, reduced neutro-phils and lymphocytes in the BALF	Anti-inflammation, antioxidant, and cytokine inhibition	39

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