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(5*R*)-5-hydroxytriptolide (LLDT-8), a novel triptolide analog mediates immunosuppressive effects in vitro and in vivo

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Abstract

A series of triptolide analogs have been successfully synthesized. In the present study we demonstrated one of them, (5*R*)-5-hydroxytriptolide (LLDT-8), showed low cytotoxicity and relative high immunosuppressive activities as compared with its parent compound triptolide in vitro. The CC_{50} values of triptolide and LLDT-8 were 2.1 ± 0.3 and 256.6 ± 73.8 nM, respectively. LLDT-8 significantly inhibited the proliferation of splenocytes induced by concanavalin A (ConA), lipopolysaccharide (LPS), or mixed lymphocyte reaction (MLR), and the IC_{50} values were 131.7 ± 32.4, 171.5 ± 17.3, and 38.8 ± 5.1 nM, respectively. LLDT-8 (25, 50, 100 nM) dose-dependently reduced the production of Th1 type cytokines (IFN-γ, IL-2) and inflammatory cytokines (TNF-α, IL-6) in vitro. Administration of LLDT-8 (at the low dose of 0.4 μg/kg, i.p.; 40 μg/kg, p.o.) intensively suppressed 2,4-dinitrofluorobenzene (DNFB)-induced delayed type hypersensitivity (DTH) reactions. Treatment with LLDT-8 (40 μg/kg, i.p. and p.o.) also markedly inhibited the sheep red blood cell (SRBC)-induced antibody production in BLAB/c mice. Most importantly, comparing with triptolide, LLDT-8 significantly reduced toxicity, with a 122-fold lower cytotoxicity in vitro and 10-fold lower acute toxicity in vivo. The results suggested that LLDT-8 had immunosuppressive activities in both cellular and humoral immune responses. LLDT-8 might be a potential therapeutic agent for immune-related diseases.

Keywords: (5*R*)-5-hydroxytriptolide; Immunosuppression

Abbreviations: LLDT-8, (5*R*)-5-hydroxytriptolide; ConA, concanavalin A; LPS, lipopolysaccharide; Sac, *Staphylococcus aureus* Cowan strain I; MLR, mixed lymphocyte reaction; DNFB, 2,4-dinitrofluorobenzene; DTH, delayed-type hypersensitivity; SRBC, sheep red blood cell; QHS, quantitative hemolysis of sheep red blood cells; CC_{50}, the cytotoxic concentration of the compound that reduces cell viability by 50%; IC_{50}, the inhibitory concentration of the compound that reduces cell proliferation by 50%.

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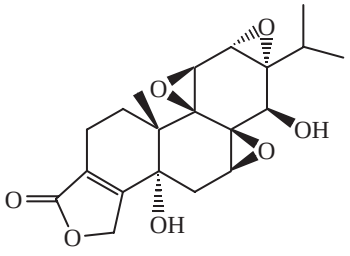
1. Introduction

Extracts of the Chinese traditional herb *Tripterygium wilfordii* Hook. f. (TWHF) have been widely used in the treatment of autoimmune and inflammatory diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus, dermatomyositis [1–5], and have beneficial effects on tissue and organ transplantation [6,7]. Triptolide, which has been defined as the active component in TWHF, has very strong immunosuppressive activities in vitro and in vivo [8–16]. However, its high toxicity limited for future clinical applications [17–20].

A series of the novel triptolide analogs have been successfully synthesized. We have made great efforts for searching the promising compounds from triptolide analogs, with low toxicity and relative high immunosuppressive activity. Through testing, analyzing and comparing with more than 20 of the new triptolide derivatives, we have found one of them, (5*R*)-5-hydroxytriptolide (LLDT-8) (Fig. 1), showed low cytotoxicity and relative high immunosuppressive activities when compared with its parent compound triptolide in vitro.

In this study, we report that the new triptolide analog, LLDT-8, effectively inhibits the murine immune responses in vitro and in vivo. We assessed LLDT-8′s cytotoxicity, in vitro immunosuppressive effects on mitogens- and alloantigen-induced splenocytes proliferations and cytokine productions, and in vivo immunosuppressive effects on 2,4-dinitrofluorobenzene (DNFB)-induced delayed type hypersensitivity (DTH) reaction and sheep red blood cells (SRBC)-induced antibody production. The acute toxicity of LLDT-8 was also tested in mice. We demonstrated that LLDT-8 had immunosuppressive activities similar to that of triptolide, but with greatly reduced toxicities. Moreover, we provide data that indicate that the immunosuppressive effects of LLDT-8 might be attributed to suppression of T cell and B cell activation and function, and Th1 type cytokine productions. Together these data strongly suggest that LLDT-8 may serve as an effective therapeutic agent for a number of human autoimmune diseases.



Formula: $C_{20}H_{24}O_7$

Molecular weight: 376.39

Fig. 1. Chemical structure of (5*R*)-5-hydroxytriptolide (LLDT-8).

2. Materials and methods

2.1. Reagents

(5*R*)-5-hydroxytriptolide (LLDT-8) was synthesized from triptolide that was separated from the Chinese traditional herb *T. wilfordii* Hook. f. (TWHF). LLDT-8 is the white amorphous powder with 99% pure by reversed-phase high performance liquid chromatography. Concanavalin A (ConA), lipopolysaccharide (LPS, *Escherichia coli* O55:B5), 3-[4,5-dimethylthylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), and 3,3′,5,5′-tetramethylbenzidine (TMB) were from Sigma (St. Louis, MO, USA) products. *Staphylococcus aureus* Cowan strain I (Sac) was obtained from Pansorbin cells (Biosciences, La Jolla, CA, USA). Mitomycin-C was purchased from Kyowa Hakko (Tokyo, Japan). RPMI (Roswell Park Memorial Institute) 1640 medium was purchased from GibcoBRL, Life Technologies (USA). Fetal bovine serum (FBS) was purchased from Hyclone Laboratories (Logan, Utah, USA). Mouse cytokine (IL-2, IFN-γ, IL-4, IL-6, IL-12p40, IL-10, and TNF-α) detecting ELISA kits were from BD Biosciences PharMingen (San Diego, CA, USA) products. [^{3}H]-thymidine (1 mCi/ml) was purchased from the Shanghai Institute of Atomic Energy. 2,4-Dinitrofluorobenzene (DNFB) and other reagents were PharMingen products (San Diego, CA).

2.2. Animal

Male and female BALB/c and C57BL/6 mice (6 to 8 weeks old) were purchased from Shanghai Experi-