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Review Article

Immunosuppressive and antiinflammatory effects of triptolide and its prodrug PG-490-88

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Introduction

Triptolide is a diterpenoid triepoxide purified from a Chinese herb called *Tripterygium wilfordii* Hook F (TWHF) (1). Triptolide was first isolated in 1972 and was initially shown to possess antileukemic effects (1, 2). During the same period of time, the crude preparation of TWHF was demonstrated to be effective in treating inflammatory lesions associated with leprosy (3) and rheumatoid arthritis (4). The efficacy of TWHF in treating autoimmune diseases accelerated the process of testing individual components from this herb (5, 6). Although numerous compounds were purified (5), the diterpenoid components, especially triptolide, are recognized as the major active compounds eliciting immunosuppressive and antiinflammatory effects (7). The identification of triptolide as a potent immunosuppressive and antiinflammatory agent promoted the use of this compound in other autoimmune and inflammatory diseases (8, 9) as well as in organ (10-13) and bone marrow transplantation (14, 15). These studies also led to research on the mechanism(s) of action of the compound. Significant data have been accumulated in recent years. More importantly, the current data confirm in multiple assays that triptolide is a very potent, unique immunosuppressive and antiinflammatory agent. This review will summarize the updated data from studies using purified triptolide and its prodrug PG-490-88.

Origin, isolation and purification

Triptolide was originally purified from TWHF (1, 16). TWHF is a woody shrub of the Celastraceae family (1, 17, 18). An example of the isolation and purification procedure was published by Gu *et al.* (16), where 1 mg of triptolide was obtained from 47 g of the dried xylem of crude TWHF root.

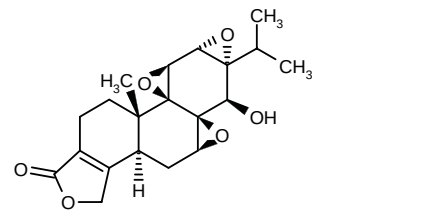
Chemistry

Triptolide ($C_{20}H_{24}O_6$) is the first recognized diterpenoid triepoxide containing the 18(43) *abeo*-abietane skeleton (1). Stable modification of the 12,13-epoxide group resulted in the loss of immunosuppressive and antiinflammatory activity (19), implying that the 12,13-epoxide group is critical for its immunosuppressive and antiinflammatory effects. The hydroxyl group at 14-C of triptolide (14-OH) is also very important for its immunosuppressive effect because replacing the 14-OH of triptolide significantly reduced immunosuppressive activity (19). Triptolide has been totally synthesized successfully (20, 21).

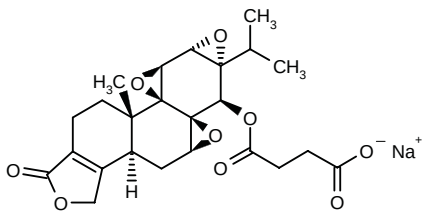
The chemical structures of triptolide and its prodrug PG-490-88 are shown in Figure 1.

Physical properties

Triptolide is composed of white to off-white crystals with a melting point of 226-240 °C (11). The water solubility of triptolide is poor, but it can be dissolved in a variety of organic solvents such as dimethyl sulfoxide (22) and ethol/cremophor (23). Mao *et al.* studied the stability of triptolide in a variety of conditions (24). Triptolide was very stable in chloroform solution (no degradation after 3 years). The order of stability of triptolide in other solvents was as follows: ethanol > methanol > dimethyl sulfoxide. The stability of triptolide is affected by pH (pH4 < pH5 < pH6 > pH7 > pH8 > pH9). The shelf-life of triptolide in 5% ethanol and at 25 °C is 204 days. Degradation pathways of triptolide in a light-protected environment include hydrolysis, isomerization and oxidation.



Triptolide



PG-490-88

Fig. 1. Chemical structures of triptolide and its prodrug PG-490-88.

Toxicity

In 7-day toxicity studies in dogs, a dose of 20 μg/kg/day was safe, doses of 40 or 80 μg/kg/day induced reversible leukopenia and myocardial damage, and a dose of 160 μg/kg/day was lethal (25). The LD_{50} of triptolide in mice was 1.93 mg/kg (10), while the effective doses for prolonging allogeneic organ grafts (10, 11) and preventing graft-*versus*-host disease (GVHD) (14, 15) in mice and rats were within the range of 0.1-0.5 mg/kg/day. These data suggest that the therapeutic window of triptolide may be narrow.

Mechanisms of action

Molecular level

1) T lymphocytes

The mechanisms by which triptolide inhibits T cell activation have been studied in detail by Qiu *et al.* (26). Their initial observations indicated more inhibition of interleukin (IL)-2 expression by triptolide than by ciclosporin, inhibition of IκBα mRNA expression and enhancement of NF90 mRNA expression by triptolide but not ciclosporin. These data suggest that triptolide modulates gene expression through distinctly different mechanisms than ciclosporin. Moreover, triptolide inhibited ciclosporin-resistant T cell activation pathway triggered through the costimulatory receptor CD28. Overexpression of calcineurin on Jurkat T cells did not shift the dose-dependent inhibition curve of IL-2 transcription, suggesting that calcineurin may not be the target of triptolide. Further analyses revealed that triptolide inhibited transcriptional activation but not DNA binding of NF-κB through NF-κB transactivation domains TA1 and TA2. The target was at the purine-box/ARRE/NF-AT and NF-κB target sequences after specific binding to DNA.

2) Inflammatory-related molecules

Using a cDNA array and clustering algorithm analysis (27), triptolide was shown to inhibit multiple gene expression in human bronchial epithelial cells stimulated by PMA. These genes included proinflammatory cytokine and mediator genes tumor necrosis factor-α, IL-8, macrophage inflammatory protein-2α, vascular endothelial growth factor, granulocyte-macrophage colony-stimulating factor, GATA-3, FRA-1, Sp3 and adhesion molecule genes integrin β6 and intercellular adhesion molecule-1. A possible explanation is that triptolide inhibited IL-8 expression at least in part through inhibition of NF-κB transcription activation (27). Transforming growth factor-β mRNA expression in normal human lung fibroblasts was also inhibited by triptolide (9). Triptolide suppressed the mRNA expression of platelet-derived growth factor-A, which is considered an important growth factor in the pathophysiology of arteriosclerosis such as in the myocardium from transplanted hearts (12, 13).

3) Apoptosis

Triptolide was shown to induce apoptosis of T cells by activating caspases (28). A cDNA array analysis (27) revealed that triptolide inhibited constitutively expressed cell cycle regulator and survival genes including the cdc-2-related protein kinase PISSLRE, cdc protein 25, G_1/S-specific cyclin D1, G_2/M-specific cyclins A and B1, GADD-45, bcl-x, and transcription factors c-jun and Jun D. Inhibition of cyclins may mediate cell cycle arrest and inhibit cell growth. Inhibition of bcl-x may induce apoptosis.

Cellular level

1) T lymphocytes

Triptolide significantly inhibited T cell proliferation mediated by mitogens (10, 29), alloantigens (16, 22, 29) and IL-2 (22) in mice (10, 16, 29), rats (22) and humans (29) with EC_{50} values of ≤ 6 ng/ml. Triptolide was also reported to inhibit constitutive proliferation of a T cell hybridoma (28). Most reports demonstrated that IL-2 production mediated by both Ca^{2+}-dependent (26, 28) and Ca^{2+}-independent pathways (26) could be inhibited by triptolide (EC_{50}: 10-40 ng/ml). Triptolide was able to block the generation of cytotoxic T lymphocytes upon alloantigen challenge (EC_{50}: < 2 ng/ml) (10). Triptolide was also reported to induce apoptotic death of T cell hybridomas and peripheral T cells but not thymocytes (28).

2) B lymphocytes

Very limited data on B cells have been reported. Up to 10 ng/ml of triptolide was unable to inhibit B cell proliferation upon lipopolysaccharide challenge (29). The same group also reported that triptolide was unable to inhibit IL-2 production.

3) Proinflammatory cytokines and mediators

Proinflammatory cytokines and mediators are very important components in the pathogenesis of inflammation (30). Triptolide inhibited the expression of IL-6 and IL-8 by normal and transformed human bronchial epithelial cells upon challenge with phorbol 12-myristate 13-acetate, tumor necrosis factor-α and IL-1β (27). Triptolide also inhibited prostaglandin E_2 production by a variety of cell types stimulated by lipopolysaccharide (31). In a bleomycin-induced lung fibrosis model (9), triptolide suppressed the level of transforming growth factor-β in the bronchoalveolar lavage fluid.

4) Induction of specific tolerance

In a bone marrow transplantation model, specific tolerance induced by the prodrug PG-490-88 was not due to deletion of alloreactive T cells and suppressor cells were not involved in the maintenance of tolerance (15). PG-490-88 appeared to cause allogeneic tolerance through the induction of a state of antigen-specific anergy or through induction of Th2 cell responses (15).

In vivo effects

Autoimmune and inflammatory diseases

Numerous studies have been performed in both humans and animals using crude extracts of TWHF (5, 6, 18). However, there are relatively few studies using purified triptolide. The prodrug PG-490-88 blocked bleomycin-induced lung fibrosis in mice, probably through inhibition of TGF-β activity (9). A small clinical trial using triptolide to treat rheumatoid arthritis in humans has been reported (32). Triptolide treatment was associated with significant improvement. However, about half of the patients withdrew from the trial due to serious treatment-related side effects, including urinary disorders, myocardial damage and leukopenia.

Transplantation

Triptolide (0.1-0.5 mg/kg/day) was first reported to prolong the survival of major histocompatibility complex (MHC)-mismatched allogeneic skin grafts in mice in 1992 (10). Inhibitory effects of triptolide (3.75 and 37.5 μg/kg/d) were observed on graft coronary arteriosclerosis (12, 13), which is related to chronic rejection of heart grafts. Triptolide (0.25 and 0.5 mg/kg/day) significantly prolonged renal grafts in rats (11). PG-490-88 produced a marked increase in the survival of allogeneic heart grafts in rats (11). Moreover, triptolide acted synergistically with ciclosporin in prolonging the survival of allogeneic heart grafts in rats (11). In a GVHD model, PG-490-88 completely prevented development of the disease (14). In this model, PG-490-88 induced a state of specific tolerance to donor cells (15).

Summary

Triptolide is a diterpenoid triepoxide purified from *Tripterygium wilfordii* Hook F, an herb found in China. Triptolide inhibits T cell activation mainly through inhibition of interleukin-2 production. In contrast to the target of ciclosporin and FK506, the target of triptolide is at the purine-box/ARRE/NF-AT and NF-κB target sequences after specific binding to DNA. Triptolide also induces apoptosis of T cells by activating the caspase cascade. Moreover, triptolide can suppress the expression of multiple proinflammatory cytokines and mediators, which play important roles in the pathogenesis of autoimmune diseases, transplantation rejection and GVHD. Although data in humans is very limited, triptolide has been successfully used to treat rheumatoid arthritis in humans and prevent bleomycin-induced lung fibrosis in mice. Triptolide significantly prolongs the survival of allogeneic grafts in rats and completely prevents lethal GVHD in mice. Despite its narrow therapeutic window, triptolide is a very potent immunosuppressant and antiinflammatory agent with unique mechanisms of action.

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