Immunomodulatory effects and mechanisms of plant alkaloid tetrandrine in autoimmune diseases

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ABSTRACT

Autoimmune diseases characterized by activation of immune effector cells and damage of target organs are currently treated with a combination of several disease-modifying antirheumatic drugs (DMARDs) that preserve different immunomodulatory mechanisms. Such a combination treatment strategy not only provides synergistic effects but also reduces side effects from individual drug. Tetrandrine (Tet), purified from a creeper Stephania tetrandra S Moore, is a bis-benzylisoquinoline alkaloid and has been used to treat patients with silicosis, autoimmune disorders, and hypertension in Mainland China for decades. The accumulated studies both in vitro and in vivo reveal that Tet preserves a wide variety of immunosuppressive effects. Importantly, the Tet-mediated immunosuppressive mechanisms are evidently different from some known DMARDs. The synergistic effects have also been demonstrated between Tet and other DMARDs like FK506 and cyclosporin. These results highlight Tet a very potential candidate to be considered as one of DMARDs in the treatment of autoimmune diseases, especially rheumatoid arthritis. This review summarizes evidence-based in vivo and in vitro studies on this potential Chinese immunosuppressive herb.

INTRODUCTION

The immune system is a well-organized and well-regulated system and its dysregulation may lead to the development of autoimmune diseases. The prototype of such a group of illnesses is rheumatoid arthritis (RA), a disease with progressive and massive destruction of joints accompanied with or without other organ involvement. Although many contributing factors were considered to play roles in causing rheumatoid arthritis, the etiology remains unclear. While no single agent was proven to be enough to control disease progression, current acceptable therapy for rheumatoid arthritis is aimed to attenuate disease activity with a combination of disease-modifying antirheumatic drugs (DMARDs) such as methotrexate, sulfasalazine, leflunomide, hydroxychloroquine, cyclosporin, gold, azathioprine… etc[1-3]. The purpose of a combination therapy is to obtain synergistic therapeutic effects of drugs with different immunomodulatory mechanisms and, in the meantime, to reduce side effects from each drug by decreasing their dosages. Along the way of investigating the pathogenesis...
of autoimmune diseases, the activated immune effector cells such as T cells, B cells, monocytes/macrophages, and dendritic cells have been consistently found in the peripheral blood and the involved organs. Among these immune effector cells, T cells have been considered to be the most important because the regulation of T cell activation requires antigen specificity and a great amount of cytokines released in autoimmune responses are from T cells\[^4\-7\].

It is clear that full activation of T cells requires the integration of two signals: one is from a T cell receptor signal and the other is from a costimulatory signal\[^8\]. Among the molecules expressing costimulatory activities on T cells, only CD28 could in combination with the activation of T cell receptor induce detectable levels of interleukin-2 and prevent anergy, a status of unresponsiveness\[^9, 10\]. The importance of CD28 costimulation in T cell activation is substantiated by the accumulated data showing that 1) blockade of CD28 costimulatory signal leads to the reduction of disease severity and proteinuria as well as the prolongation of survival in lupus-prone mice\[^11\]; 2) blockade of CD28 costimulation results in the increased survival of the transplanted organs such as heart, kidney, bone marrow and pancreas\[^12\-16\]; 3) enhanced activation of CD28 by immobilized anti-CD28 monoclonal antibodies (mAbs) leads to the reduction of virus load as well as the expansion of CD4\(^+\) T cells in HIV-infected patients\[^17\]; 4) blockade of CD28 costimulation causes improvement of symptoms in psoriasis patients\[^18\]; 5) introduction of CD28 costimulation elicits tumor rejection in animal models\[^19\-21\].

Aside from the activation of immune effector cells, the defective apoptosis (programmed cell death) mechanism also plays a crucial role in disease progression of RA and other autoimmune diseases\[^22\-28\]. Apoptosis is a natural protective mechanism for embryogenesis, for thymic organ to eliminate inappropriate T cells and for immune-privileged sites to protect from inflammatory cell invasion\[^26, 27\]. Different from the necrotic process, the apoptotic process does not induce any inflammatory response because dead cells or their degraded products are rapidly phagocytosed before any leakage of cellular contents. Since the etiology of autoimmune diseases is largely unknown, the immune reaction towards the apoptotic bodies released or apoptotic antigens expressed from dead cells has been implied as one of the mechanisms leading to autoimmune diseases\[^26\-31\]. After development of autoimmune diseases, fail to execute the appropriate apoptotic program may result in sustention of inflammatory process\[^32\-38\]. In light of the significance of apoptotic process, the apoptosis-based therapy has been suggested as one of the approaches to control the progression of autoimmune diseases\[^39\].

Altogether, both the inappropriate activation of immune effector cells and the ineffective deletion (through apoptosis) of these cells may lead to the development and progression of autoimmune disorders (Fig 1). The therapeutic approaches for autoimmune diseases may rely on both the inhibition of cell activation and the maintenance or enhancement of the apoptotic program of immune effector cells.

Fig 1. Regulation of immune effector cells leads to autoimmune diseases. Two major pathways contribute to the development of autoimmune diseases; one is the over-expanded proliferation or activation of immune effector cells and the other is the defective execution of the apoptotic program of immune effector cells, especially those already being activated cells.

Although the significance of T cells in autoimmune pathogenesis is very clear, the T-cell directed biological therapies for RA has been unsuccessful due to both lack of efficacy and serious side effects. The use of pharmacological agents such as cyclosporin A and leflunomide that block T cell activation and proliferation give more promising therapeutic outcomes (reviewed in \[^40\]). Han-Fang-Chi is the dried tuberous root of the creeper Stephania tetrandra S Moore. The purification of Han-Fang-Chi yields an active ingredient tetrandrine (Tet) that accounts for its major biological activities\[^41\]. Tet has been used in China for several decades to treat patients with silicosis and rheumatic diseases that are associated with the activation and infiltration of immune effector cells at lesion sites. In a clinical study of Tet effects in silicosis patients, the administration of 200 mg or 300 mg per day with aver-

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agely total 120 gm(s) of Tet to silicosis patients results in significant improvement of pulmonary function and reduces the progression of silicosis\(^4\). Additionally, such a therapeutic strategy only causes limited side effects during the three-year follow-up. These side effects include abdominal distension, diarrhea, dry eye, itching, hyperpigmentation and mildly elevated liver enzymes. All these symptoms and signs resolve spontaneously after discontinuance of the medication\(^4\). This clinical observation highlights not only the effectiveness but also the acceptable tolerance of Tet for clinical use.

**EFFECTS ON T CELLS**

The early observation that Tet inhibits delayed-type hypersensitivity (DTH) responses in mice model suggests that Tet may have some direct effects on T cells\(^4\). Subsequently, Kondo et al\(^4\) showed that Tet inhibited plaque-forming cell response to a T-cell-dependent antigen, sheep red blood cell, but it has no effect on such a response to a T-cell-independent antigen, lipopolysaccharide. Furthermore, the severity of arthritis is greatly reduced by late or prophylactic administration of Tet in an adjuvant-induced chronic arthritis model of rats\(^6\).

Based upon these observations, a series of studies examining directly the effects of Tet on T cells were performed. In purified human peripheral blood T cells, Tet inhibits the CD28-costimulated T cell proliferation and cytokine production. Both T helper 1 (Th1) and Th2 cytokines are susceptible to Tet suppression\(^4\). However, Tet may or may not directly down-regulate CD28 signaling pathway because T cell receptor-mediated signaling events can also be inhibited by Tet. The investigation of Tet-regulated T cell receptor downstream signaling pathways clearly shows that Tet specifically inhibits protein kinase C-dependent but not calcium-dependent signaling events\(^7\). In addition, these observations suggest that the immunosuppressive effects of Tet may not relate to its calcium-channel blocking properties\(^7\). Since there are more than 11 protein kinase C isoenzymes identified so far\(^8\), whether Tet targets one or several protein kinase C isoenzyme-mediated signaling pathways is currently not known. Because the activation of protein kinase C isoenzymes can readily be detected in many signaling pathways in a variety of immune and non-immune effector cells, these results also partly explain the broad-spectrum anti-inflammatory properties of Tet\(^8,9\). Molecular dissec-

Fig 2. Tetrandrine targets protein kinase C-NF-κB signaling pathway. The T cell receptor signal is conducted via the CD3 complex that contains several subunits. This signal then causes both increase of intracellular calcium and activation of protein kinase C isoenzymes. The increase of intracellular calcium leads to the activation of nuclear factor of activated T cells (NFAT) and the activation of protein kinase C results in the stimulation of NF-κB transcription factors. CD28 costimulation has unique effects, distinct from those through T cell receptor, on the activation of NF-κB. Tet is supposed to inhibit the T cell receptor-mediated protein kinase C-NF-κB signaling pathway. Whether Tet has direct effect on CD28-mediated NF-κB activation is currently unclear. In contrast, cyclosporin is a suppressor of calcium-dependent NFAT activation.

Both in vivo and in vitro studies also suggest that the T cell receptor signaling pathway targeted by Tet is different from that targeted by cyclosporin that inhibits C\(^{a2+}\)-dependent calcineurin activity\(^4,5\). Importantly, the combination of both Tet and cyclosporin provides a synergism at much lower therapeutic concentrations of each drug\(^7\). Indeed, in diabetes animal models, Lieberman et al\(^5\) also showed that Tet in combination with FK506, an immunosuppressive agent functionally similar to but more potent than cyclosporin, synergistically prevent the development of diabetes.
EFFECTS ON OTHER IMMUNE EFFECTOR CELLS

The immunosuppressive effects of Tet could also be demonstrated in other immune effector cells, including macrophages, monocytes, B cells, neutrophils, and mast cells. At therapeutic concentrations, Tet inhibits the production of tumor necrosis factor alpha (TNF-α) from monocytes stimulated with killed *Staphylococcus aureus* [52]. Both fangchinoline and isotetrandrine, two Tet analogues, also inhibit interleukin-1 (IL-1) and TNF-α production from *Staphylococcus aureus* Cowan 1-stimulated human peripheral blood mononuclear cells [53]. Such an effect is mediated at least through transcriptional regulation of mRNA expression of these cytokine genes. Further expansion of these observations demonstrates that Tet inhibits not only IL-1 and TNF-α but also other cytokines like IL-6 and IL-8 production from activated monocytes as well as inhibits the antibody production from activated B cells [54]. Moreover, Tet significantly blocks the extent of inflammation of uveitis induced by endotoxin and IL-1α administration in rats [55]. Therefore, aside from the inhibition of cytokine production, Tet also suppresses cytokine-mediated tissue damage, a suggestion that Tet may directly block cytokine-mediated signaling pathways. The results of these studies may somehow be explained in part by an early observation showing that Tet significantly inhibits the production of nitric oxide, a critical mediator of inflammation, in lipopolysaccharide-stimulated macrophages [56]. In cultured human umbilical vein endothelial cells, Tet dose-dependently inhibits the secretion of a chemotactic factor and then blocks the migration of monocytes [57]. In addition, Tet also suppresses the IL-1α and platelet-derived growth factor (PDGF)-induced tube formation of rat vascular endothelial cells, an important step in angiogenesis [58]. The inhibitory potency is measured to be about 100-fold stronger than hydrocortisone [59].

With regard to innate immune responses, Tet has been shown to down-regulate tumor promoter and phorbol myristate acetate-induced adherence of neutrophils [60]. The phagocytic effects of neutrophils are also greatly inhibited by Tet [54]. In an animal model of myocardial ischaemia-reperfusion injury, Tet effectively suppresses the up-regulation of Mac-1 on neutrophils and therefore blocks their migration and reduces the injury-induced myocardial infarct size and ventricular tachyarrhythmia [61].

INDUCTION OF PROGRAMMED CELL DEATH (APOPTOSIS)

Since the apoptotic process is defective in autoimmune diseases [33-35], any drug that could potentially induce the apoptosis of activated immune effector cells may have its additional advantage to control the inappropriate expansion of immune responses. Several Western anti-rheumatic drugs such as corticosteroid, nonsteroidal anti-inflammatory drugs and hydroxychloroquine, and a Chinese anti-rheumatic drug, *Tripterygium wilfordii* Hook f., have been shown to preserve the capacity of inducing cellular apoptosis [61-64]. Reasonably, the apoptotic effects of Tet may also play a role in the control of autoimmune disorders.

Tet at 0.1 µmol/L to 100 µmol/L concentration-dependently reduces the viability of mouse peritoneal macrophages, guinea-pig alveolar macrophages and mouse macrophage-like J774 cells [65]. In the example of other permanent cell lines, U937 and HL-60, Tet also dose-dependently inhibits cellular proliferation and induces cellular apoptosis [66,67]. In contrast to the cellular apoptosis induced by glucocorticoids in CEM-C7 cells, the induction of apoptosis by Tet is much more rapid (40 h compared to 4 h, respectively) [68]. In addition, the Tet-induced cellular apoptosis appears not to require de novo protein synthesis [68]. A finding suggests that glucocorticoid-induced and Tet-induced cellular apoptosis may be mediated through different mechanisms. Aside from the results shown in immortal cell lines and murine cells, Tet could also induce the apoptotic program in human peripheral blood lymphocytes [67].

Consistent with the results in other immune effector cells, Tet effectively causes apoptosis of human peripheral blood T cells [47,69]. Importantly, compared to resting T cells, Tet preserves much stronger killing capacity towards activated T cells [69]. When drug-induced apoptosis was readily compared, the results suggest that the Tet-induced T cell apoptotic mechanism is different from the one induced by hydroxychloroquine, a commonly used Western anti-rheumatic drug [69]. While Tet-induced T cell apoptotic DNA damage requires caspase-3 activity, hydroxychloroquine-induced T cell apoptotic DNA damage is mediated through a caspase-3- and caspase-8-independent but Z-Asp-Glu-Val-Asp fluomethyl ketone-sensitive signaling pathway [69]. The differential usages of caspase-mediated apoptotic
mechanisms by Tet and hydroxychloroquine may also provide a possible synergism of prescribing both drugs for the treatment of autoimmune diseases.

ANTI-OXIDATIVE EFFECTS

One of the important effects of Tet that may explain many of its biological activities is the anti-oxidative property. In measuring both hypoxanthine/xanthine oxidase and erythrocytes auto-oxidation systems, Tet effectively scavenges the generated superoxide anions at concentrations from 7.5 to 15 μmol/L[70]. Other evidence also shows that Tet inhibits freshly fractured quartz-induced lipid peroxidation[71]. In evaluation of active oxygen production from neutrophils, the results indicate that the generation of superoxide and luminol-dependent chemiluminescence induced by opsonized zymosan, arachidonic acid, formylmethionyl-leucyl-phenylalanine, or by phorbol myristate acetate decreases significantly when Tet or its analogues are added into the reaction[72]. In addition, Tet inhibits hexose-phosphat mono-phosphate shunt activity and hydrogen peroxide production in neutrophils[73]. The anti-oxidative reaction rate constant of Tet with *OH is determined to be 1.4x10^10 mol^-1 s^-1, a value that is comparable with other anti-oxidants such as ascorbate, glutathione, and cysteine[74]. When human mononuclear cells were examined, Tet greatly inhibits irradiation-induced superoxide production from these cells[75]. In animal models, via inhibiting Ca^{2+} influx and reactive oxygen species formation, Tet suppresses neutrophil adhesion to fibrinogen[76]. Part of the effects is mediated through G protein modulation that prevents Mac-1 up-regulation in neutrophil activation[77].

STRUCTURAL MODIFICATION

A great potential for Tet being one of the DMARDs is the advantage that there are several structurally similar Tet analogues. Importantly, the subtle structural difference among Tet analogues appears to have great impact on their differential immunosuppressive activities. Based upon their structural similarity and the difference in biological activity, structural modification of this group of drugs can lead to the discovery or development of more potent yet less toxic immunosuppressive drugs.

Tet and berbamine are two purified plant alkaloid analogues. Differ by only one substitution in the side chain of one of the benzene rings, these two drugs preserve quite different immunosuppressive activities. For example, Tet but not berbamine inhibits the chronic inflammation of adjuvant-induced arthritis in rats[45]. The incidence of relapsing experimental allergic encephalitis (EAE) is reduced by 41 % and 65 % for Tet and berbamine treatment, respectively[78]. Tet is shown to preserve 6-18 times more potent than berbamine in suppressing the production of IL-1 and TNF-α from the stimulated monocytes and macrophages as well as the production of TNF-β by activated lymphocytes[79]. Investigation of the direct cytokine effects in rat subcutaneous air pouch model of inflammation reveals that both compounds are equipotent in suppressing leukocyte infiltration into air pouches induced by IL-1 and TNF[80]. Nevertheless, Tet is more potent than berbamine as a suppressant of platelet activating factor-induced mononuclear cell infiltration and is less effective than berbamine in carageenin-induced polymorphonuclear cell infiltration[80]. While Tet has stronger suppressive effects on adherence and locomotion of neutrophils, berbamine preserves greater capacity for inhibition of natural killer cell cytotoxicity[81]. Along the arachidonic acid metabolizing pathway, Tet but not berbamine potentially inhibits the lipooxygenase-dependent leukotriene production from activated human monocytes and neutrophils. However, both compounds were equally potent in suppressing cyclooxygenase-dependent prostaglandin generation[82]. In the context of T cell response, both Tet and berbamine are equipotent in suppressing the induction and expression phases of DTH responses to sheep red blood cell antigens in mice[83]. When human peripheral blood T cells were examined, the results also indicate that both Tet and berbamine equipotently block CD28-costimulated T cell activities, including cytokine production, cell surface activation marker expression as well as cell proliferation[46]. This study also identifies dauricine as the most potent one among Tet analogues that inhibit CD28-costimulated T cell activities[46]. Surprisingly, the accumulated evidence also suggests that Tet but not berbamine preserves strong anti-oxidative activity[72, 81]. The results in a series of these studies are summarized in Tab 1.

CONCLUSION

Tet preserves very extensive immunosuppressive effects through inhibiting the activation of various immune effector cells and inducing the apoptosis of these cells. The molecular target of Tet is currently unknown, but likely to be involved in T cell receptor – protein
kinase C-NF-κB signaling pathway in the example of T cells. Although only minor structural difference is present among Tet and its analogues, great biological differences exist among these analogues. These observations also give us important insights into structure-activity relationships of Tet and its analogues and the opportunity to design novel analogues that are potentially more potent and less toxic than currently available drugs for the treatment of autoimmune disorders.

Aside from its calcium-channel blocking effects, there are at least two major fields about Tet that are not covered in this review. One is its anti-cancer effect like the increase of chemotherapeutic agent drug sensitivity in multi-drug resistance cells and the other is its anti-silicosis effect like the inhibition of pulmonary macrophage and pulmonary fibroblast activation. In this context, the clinical application of this drug may not be limited to the currently known knowledge on this drug.

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REFERENCES

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* The symbol †(strong) and ↓(weak) indicate the suppressive intensities.


