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Review

Immunosuppressive and anti-inflammatory activities of sinomenine

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ABSTRACT

Sinomenine (SN), a pure compound extracted from the *Sinomenium acutum* plant, has been found to inhibit T- and B-lymphocyte activation, proliferation and function and to interfere with the differentiation, recruitment and function of several other cell types, such as dendritic cells (DC). SN has demonstrated its potential anti-inflammatory role for treating immune-related disorders in experimental animal models and in some clinical applications. This review will summarize its potential effects, mechanisms and applications.

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1. Introduction

The root and stem decoctions of *Sinomenium acutum Rehd. et Wils.*, a medicinal plant identified as "Fang-chi" in ancient China, have been used as a folk remedy for centuries to treat neuralgia and rheumatoid diseases in many areas of the Far East. Since Sinomenine (SN) (7, 8-didehydro-4-hydroxy3, 7-dimethoxy-17-methyl-α, 13α, 14α-morphinan-6-one) was isolated by Ishiwari from this medicinal plant in the 1920s [1], a vast number of pharmacological and clinical studies performed in China and Japan have demonstrated that the pure alkaloid extract possesses anti-inflammatory and immunoregulatory properties, as well as mild sedative and analgesic actions due to its chemical structure, which is similar to morphine [1,2]. Within the past 30 years, the therapeutic efficacy and lower side effects of purified SN in patients with rheumatoid arthritis (RA) and mesangial proliferative nephritis have been confirmed in open clinical trials [3–5]. Furthermore, in vitro studies have shown that SN is able to inhibit lymphocyte proliferation and antibody production by B cells, as well as potently reduce production of inflammatory factors by macrophages [6–8]. Experimental studies using animal models have demonstrated that SN protects mice from endotoxin-induced fulminant hepatitis and exerts its synergistic effects when combined with suboptimal doses of cyclosporine (CsA), prolonging cardiac allograft survival and blocking tissue remodeling of chronic cardiac allograft rejection [9–13]. In terms of its immunosuppressive properties, SN appears to be a promising immunosuppressive drug for the treatment of autoimmune diseases or prevention of allograft rejection. This review focuses mainly on

Abbreviations: SN, sinomenine; CsA, cyclosporine; APC, antigen-presenting cells; DC, dendritic cells; MLR, mixed lymphocyte culture; IL, interleukin; AA, adjuvant-induced arthritis; CIA, collagen-induced arthritis.

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experimental studies of SN as a primary immunosuppressant in the treatment of inflammatory or immune-related diseases, such as autoimmune diseases and transplantation rejections, and in particular, focuses on related mechanisms that regulate the immune response.

2. Overview of SN

2.1. Effect of SN on T and B lymphocytes

Lymphocytes are the main cell type in the adaptive immune system, and they play key roles in autoimmune diseases. Studies have shown that SN suppresses the activities of lymphocytes by mainly inhibiting their proliferation, inducing the apoptosis of T lymphocytes, and attenuating the Th1/Th2 imbalance. Liu et al. [6] found that SN markedly inhibited [^{3}H] thymidine incorporation by mouse spleen cells activated with concanavalin A (ConA) or by two-way mixed lymphocyte culture (MLR) and also in human peripheral blood mononuclear cells activated with phytohemagglutinin (PHA) plus ionomycin. Furthermore, SN inhibition of lymphocyte proliferation was effective only when it was added at the onset of mixed lymphocyte culture and could be reversible by the addition of IL-2. The in vitro experiment described by Shu et al. showed that the immunosuppressive activity elicited by SN in CD4+ primary lymphocytes was largely attributed to caspase 3-dependent apoptosis, but not bcl-2-dependent apoptosis. Meanwhile, SN blocked the cell cycle progression from the G0/G1 phase to the S plus G2/M phases [14]. A clinical trial of Cheng et al. involved 25 patients with mesangial proliferative nephritis (MsPGN) who were treated with SN and followed for three months. This trial indicated that SN causes a decrease in T-bet mRNA expression, resulting in a drop in the T-bet/GATA-3 ratio, and elicits a decrease in the serum levels of IFN-γ. However, no changes in the expressions of GATA-3, IL-4, or IL-10 were observed following treatment [5]. The results suggested that SN might suppress T-bet expression and inhibit Th1 cell differentiation.

CD4+CD25+ T regulatory cells (Treg) and Th17 were newly identified as different lineages distinct from Th1 and Th2 cells; Th17 are required for the triggering of several autoimmune diseases, including collagen-induced arthritis, experimental autoimmune encephalitis (EAE) and inflammatory bowel disease, and in contrast, Treg cells play an important role in the induction and maintenance of immune tolerance. Although no reports suggested whether SN could exert direct effects on CD4+CD25+ T cells and Th17 differentiation, our studies demonstrated that SN inhibits dendritic cell (DC) maturation and induces bone marrow progenitors derived from IL-10-producing Ia^{low} regulatory DCs, which consequently induce the CD4+CD25+ regulatory T cell generation that contributes to the induction of tolerance. Moreover, our studies showed that SN may not inhibit the differentiation of Th17 cells from naive CD4+ T cells in culture medium in the presence of CD3/CD28 mAb and cytokines including IL-6 and TGFβ; however, SN can selectively inhibit the differentiation of Th17 cells from naive CD4+ T cells and DCs co-cultured in the presence of cytokines including IL-6 and TGFβ and cytokine mAbs (IL-2mAb), indicating that SN may be a novel anti-inflammatory agent that targets DCs to block IL-6 production, which in turn would terminate Th17 cell development (unpublished data). Interestingly, a novel derivative of SN, termed 1032, can directly inhibit Th17 cell differentiation and in turn reduce inflammatory symptoms in experimental autoimmune encephalomyelitis [15].

Compared to the research regarding the suppressive activities of SN on T lymphocytes, there is notably less research on B lymphocytes and/or antibody production. Hojo et al. tested the immunosuppressive effect of SN in mice and found a decrease of plaque-forming cells (PFC) to T cell-dependent antigen, sheep red blood cells in vivo. In contrast, SN failed to suppress the PFC response to lipopolysaccharide, a T cell-independent antigen [7]. Further investigation on the effect of SN on B cells is warranted.

2.2. Effect of SN on DC/macrophages and monocytes

DCs are the most important antigen-presenting cells (APC) in the immune system, holding an essential role in regulating innate and adaptive immune responses, and are considered important target cells for immunosuppressants. We investigated the effect of SN on the function of DCs generated from mouse bone marrow progenitors and found that SN inhibits the bone marrow-derived DC expression of Ia, CD86, and CD40; the production of IL-12, TNF-α, and IL-1β; and the antigen-presenting capacity in a dose-dependent manner. Interestingly, SN induces DCs to secrete more IL-10, indicating that regulatory DCs with low Ia expression and high IL-10 secretion may be generated from bone marrow (BM) progenitors in the presence of SN. SN-treated DCs induce donor-specific T cell hyporesponsiveness and trigger the generation of IL-10-producing T regulatory-like cells in vitro, and pretreatment of recipients with SN-treated DCs can significantly prolong allograft survival. Importantly, pretreatment of recipients with SN-treated DCs in combination with SN administration profoundly prolonged allograft survival, resulting in the long-term survival of 30% of allografts along with increased generation of CD4+CD25+ Treg cells and enhanced microchimerism in the recipients, thus outlining a new mechanistic explanation for SN as an immunosuppressant.

SN may also inhibit human monocytes-derived DC differentiation and maturation. Chen et al. found that SN significantly inhibits LPS-triggered DC expression of CD83, CD86, B7-H1, and CD40 in a dose-dependent manner. In contrast, CsA has little effect on B7-H1 and B7-DC expression even at the concentration of 4000 ng/ml, which is far beyond the therapeutic blood concentration (200–250 ng/ml) [16]. It is interesting that SN and CsA might have, at least in part, different mechanisms of action on inhibiting DC maturation and function. However, several signaling pathways are involved in LPS-induced DC activation and maturation, including MAP kinase, PI3 kinase, P38 SAP kinase, and NF-κB pathways [17]. The research on the effects of SN on cytokine expression of macrophages by Wang et al. suggested that NF-κB activity was significantly suppressed by SN [18]. Because NF-κB activity seems to be a critical signaling pathway leading to DC maturation, inhibition of NF-κB activation might be an important mechanism to inhibit DC maturation by SN. Zhao et al. [19] also concluded that SN inhibited DCs through the NF-κB binding activity and RelB (a subunit of NF-κB and is more important in regulating differentiation and maturation of DCs) migration from the cytoplasm to the nucleus; this inhibition was dependent on a decrease of I-κBα phosphorylation but not on the expression of RelB and p38 stress-activated protein kinase (p38SAPK).

Given the significant role of monocytes/macrophages in inflammation, they are ideal therapeutic targets for pharmacological modulation. Many studies have investigated the potential immunosuppressive mechanisms of SN on monocytes/macrophages and/or monocyte-derived DC. Singhal et al. [20] reported that NO, an important inflammatory factor, could induce the apoptosis of RAW264.7 macrophages. However, these results were contrary to the conclusion that SN could strongly inhibit the production of NO by macrophages when RAW 264.7 cells were treated with LPS and IFN-γ [21]. Our experiment also found that NO production by RAW264.7 cells was inhibited by SN and that SN could inhibit the proliferation of RAW264.7 macrophages by inducing apoptosis in a dose- and time-dependent manner [22]. Therefore, the authors began to search for another mechanism for SN-induced macrophage apoptosis, and they found that apoptosis required the activation of extracellular signal-regulated protein kinase (ERK), which was involved in stress-induced apoptosis. Subsequently, ERK activation increased the expression of p27 and Bax to induce the apoptosis of macrophages.

SN reduces the invasion and migration ability of monocytes. Ou et al. [23] reported that SN significantly inhibited the invasion and migration ability of human monocytes and synoviocytes in a