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Suppressive effects of fisetin on mice T lymphocytes *in vitro* and *in vivo*

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ABSTRACT

Background: Most of the immunosuppressive drugs have satisfactory therapeutic effects on organ transplantation and autoimmune disease. However, their clinical application is limited by side effects. Therefore, new and safe immunosuppressive drugs against acute and chronic rejections are eagerly awaited. Fisetin, a flavonoid present in various types of vegetables and fruits, has few side effects and low level of toxicity, which would be a desirable clinical feature. In the present study, we investigated the immunosuppressive effects and underlying mechanisms of fisetin against T-cell activation *in vitro* and *in vivo*.

Methods: We measured the effect of fisetin on T-lymphocyte proliferation, T-cell subsets, cell cycle progression, cytokine production, and nuclear factor activation *in vitro*, as well as its influence on T cell–mediated delayed-type hypersensitivity reaction *in vivo*.

Results: In vitro, the results showed that fisetin significantly suppressed mouse splenocytes proliferation, Th1 and Th2 cytokine production, cell cycle and the ratio of $CD4^+/CD8^+$ T cells. Furthermore, fisetin exerts an immunosuppressive effect in mouse T lymphocytes through the suppression of nuclear factor kappa B activation and nuclear factor of activated T cells signaling in a dose-dependent manner. *In vivo*, fisetin treatment also significantly inhibited the dinitrofluorobenzene-induced delayed-type hypersensitivity reactions in mice.

Conclusions: Fisetin had strong immunosuppressive activity *in vitro* and *in vivo*, suggesting a potential role for fisetin as an immunosuppressive agent.

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1. Introduction

T cells play a pivotal role in immune reaction and have been implicated in mediating many aspects of immune diseases. During T-cell development, a common precursor population of $CD4^+CD8^+$ double-positive thymocytes gives rise to two main cell lineages of αβ T cells: CD4 and CD8 T cells [1,2]. CD4 T cells recognize peptides bound to class II major histocompatibility complex and have helper activity. CD8 T cells recognize peptides bound to class I major histocompatibility complex and have cytotoxic activity [3]. $CD4^+/CD8^+$ coreceptor expression and effector function are central for our understanding of T-cell development.

Cytokines are also potential targets for immunomodulation. Immunologic cytokines are classified into two types: Th1 and Th2 cytokines. The two types of cytokines work together to modulate the immune function. The Th1–Th2 cytokine balance is an important indicator of the disease state, and its imbalance can lead to immunologic disorders [4,5]. Thus, modulation of Th1–Th2 cytokine balance has become a new paradigm for immunomodulatory therapy.

Nuclear factor of activated T cells (NFAT) and nuclear factor kappa B (NF-κB) signaling pathways play important regulatory roles in T-cell activation. NFAT has been reported to be involved in the activation of T lymphocytes and production of cytokines. NF-κB has a central role in the maturation and survival of T lymphocytes and the expression of a wide variety of genes that control the immune system [6]. NFAT and NF-κB have been shown to be useful targets for the inhibition of T-cell activation and proliferation by the immunosuppressants cyclosporin A and tacrolimus (FK506) [7].

Fisetin (3, 7, 3, 4-tetrahydroxy flavone) is a member of flavonoids, and it is commonly found in plants such as the smoke tree and various types of fruits and vegetables such as strawberries, grapes, onions, and cucumber at concentrations ranging from 2 to 160 μg/g [8–10]. Lately, studies have demonstrated that fisetin exhibits a wide variety of activities, including anticancer, antiangiogenic, neuroprotective, neurotrophic, antioxidant, anti-inflammatory, antiproliferative, and apoptosis effects [11–18]. Previous research indicated that fisetin has immunosuppressive effects in human mast cells [19]. Furthermore, fisetin was found to inhibit NF-kB activation via suppressing its upstream signaling molecules, including transforming growth factor beta activated kinase 1 and inhibitors of κB in cancer cells [20]. Fisetin may protect against the progression of inflammatory diseases by limiting interactions between mast cells and activated T cells [21]. These findings indicate that fisetin has potential immunosuppressive function through its effect on T cells. However, there is a little information about the mechanism concerning T lymphocytes immunosuppressive effects of fisetin. In this experiment, we investigated the *in vitro* and *in vivo* immunosuppressive effect of fisetin on BALB/c mice T lymphocytes and explored the potential mechanism underlying this effect.

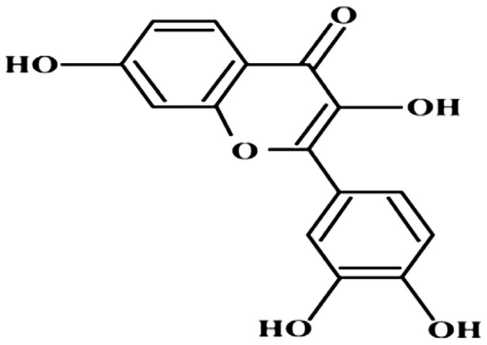

Fig. 1 – Chemical structure of fisetin.

2. Materials and methods

2.1. Chemicals

Fisetin (purity >98%, Fig. 1) was ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Changchun, Jilin, China). Concanavalin A (ConA) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO). Fisetin was diluted in saline with 0.1% DMSO and then sterile filtered before used.

2.2. Reagents

Interleukin (IL) 2, interferon gamma (IFN-γ), IL-4, and IL-6 enzyme-linked immunosorbent assay kits were purchased from BioLegend (San Diego, CA). Roswell Park Memorial Institute-1640 (RPMI-1640) medium and fetal bovine serum were obtained from Invitrogen-Gibco (Grand Island, NY). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Thermo (Thermo Fisher). Primary antibodies used for Western blot analysis Inhibitors of κB (IκB) and P-IκB were purchased from Cell Signaling (Beverly, MA); NFAT2 antibodies were purchased from Abcam (Cambridge, MA); β-actin was obtained from Tianjin Sungene Biotech Co, Ltd (Tianjin, China); goat anti-mouse IgG and goat anti-mouse fluorescein isothiocyanate (FITC) were purchased from Protein Tech Group, Inc (Chicago, IL); PerCP/Cyanine 5.5–anti-CD3, FITC–anti-CD4, and phycoerythrin–anti-CD8 antibodies were purchased from BD Pharmingen. 2, 4-Dinitrofluorobenzene (DNFB) and cyclophosphamide (CTX) were obtained from Sigma-Aldrich.

2.3. Experimental animals

Male BALB/c mice (grade II, 6- to 8-wk-old), weighing 18–22 g, were purchased from Jilin University Experimental Animal Center (Changchun, Jilin, China). The mice were housed for 1 wk in microisolator cages and received food and water *ad libitum* with a temperature of 24°C ± 1°C and humidity of 40%–80% to adapt them to the environment before experimentation. All the procedures were in strict accordance with the