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RESEARCH ARTICLE

Protective effects of Jiayan Kangtai granules on autoimmune thyroiditis in a rat model by modulating Th17/Treg cell balance

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Abstract

OBJECTIVE: To investigate the protective effects of Jiayan Kangtai (JYKT) granules, consisting of 9 Chinese herbs, in a rat model of autoimmune thyroiditis (AIT), and the possible underlying mechanism.

METHODS: Female Lewis rats (6-8 weeks) were randomly apportioned to 5 groups of 10, including a normal control. AIT was induced in the untreated AIT-model group, and rats treated subsequently with daily low, medium, or high dose JYKT granules. After 12 weeks, plasma levels of thyroid autoantibodies and morphological changes in the thyroid were detected by enzyme-linked immunosorbent assay and histological examination, respectively. The presence of interleukin (IL)-6, IL23p19, and IL-2 in thyroid tissue was assessed by immunohistochemical staining. The percentages of T helper (Th)17 cells and regulatory T cells (Tregs) in the peripheral blood were analyzed by flow cytometry. Relevant levels of cytokines and proteins were examined *via* bead-based multiplex flow cytometry and ELISA, respectively. Expressions of genes and proteins regulated by Th17 cells and Tregs were shown by real-time PCR and Western blot.

RESULTS: Compared to the control, AIT-model rats had higher plasma concentrations of thyroid autoantibodies. The high-dose JYKT rats showed significantly lower levels of thyroid autoantibodies compared with the AIT model group. Rats in the AIT-JYKT groups also had fewer thyroid lesions and less lymphocytic infiltration, a lower percentage of Th17 cells, and a higher percentage of Tregs, compared with the AIT-model. Rats given high-dose JYKT had a significantly lower Th17/Treg ratio compared with the AIT model. Differences in plasma cytokine concentrations and relevant gene and protein expressions in the spleens of JYKT-treated rats and the AIT group suggested an association between JYKT treatment and lower Th17 cell percentage and higher Treg activity.

CONCLUSION: JYKT treatment appeared to be protective against AIT in rats, possibly *via* the regulation of the Th17 cell/Treg imbalance in AIT.

Keywords: thyroiditis, autoimmune; Th17 cells; T-Lymphocytes, regulatory; Herbal medicine; Jiayan Kangtai granules

INTRODUCTION

Autoimmune thyroiditis (AIT), also known as Hashimoto's disease, is an organ-specific autoimmune disease involving lymphocyte reactivity to the host thyroid.[1] AIT is characterized by lymphocytic infiltration into the thyroid tissue,[2] an enlarged thyroid gland, and the presence of serum autoantibodies against thyroglobulin and thyroid peroxidase. Additional clinical symptoms include pernicious anemia, diabetes, and other autoimmune diseases that compromise patients' quality of life.[3] The rate of AIT incidence is much higher in women than in men.[4]

The exact mechanism of AIT pathogenesis is not completely known. It is believed that genetic background, environmental factors, and endogenous factors contribute to the breakdown of immune tolerance, production of thyroid self-antigens, and subsequent activation of T lymphocytes. These interactions appear to trigger an inflow of lymphocytes into the thyroid gland and the production of anti-thyroid autoantibodies.[5]

Helper T cells (Th cells), also known as CD4+ T cells, are a crucial component of immunological responses. Th1 and Th2 cells, differentiated from naive T cells, participate in the progression of AIT.[6,7] In addition, multiple studies have indicated that Th17 and regulatory T cells (Tregs) also contribute to the pathogenesis and development of AIT.[7-9] An imbalance between Th17 cells and Tregs has been observed, not only in a mouse model of AIT but also in clinical human samples. Specifically, an AIT mouse model indicated the induction of Tregs and reduced expression of a specific transcriptional factor, forkhead box P3 (FOXP3). This subsequently enhanced the Th17-specific transcription factor isoform: RAR-related orphan receptor gamma t (RORγt, encoded by the gene RAR-related orphan receptor C, or Rorc).[10]

Another independent study confirmed that peripheral blood mononuclear cells (PBMCs) from patients with AIT had a higher percentage of Th17 cells and higher mRNA expression of Rorc, accompanied by fewer Tregs and reduced FOXP3 levels compared with the healthy control group.[7] A separate study showed that the AIT patients not only had significantly elevated intra-thyroid infiltrating Th17 cells, but also higher serum concentrations of interleukin (IL)17 and IL22 compared with control subjects. Moreover, the IL17 levels in the thyroid were positively associated with local fibrosis, whereas the serum IL17 concentration inversely correlated with patients' residual thyroid function.[8] Thus, all these studies indicate that regulating the balance between Th17 cells and Tregs may be an important therapeutic strategy in AIT.

Currently, no effective therapeutic strategies are available for AIT. The main methods, all of which are limited and risk severe side effects, include surgical treatment, thyroid hormone therapy, or immune-modulatory therapy. Thus, identification of a new effective agent for AIT treatment is urgently required. In this regard, we have focused our research attention on Traditional Chinese Medicine (TCM), which has a long history in the treatment of autoimmune diseases.[11] Clinical evidence supports that TCM exerts beneficial effects in AIT treatment.[6,12] For example, using an experimental rat model of AIT, it was observed that ginsenoside treatment led to lower levels of interferon (IFN)-γ in the peripheral blood. Ginsenoside also produced a biphasic effect on IL-4 secretion, where low and moderate doses promoted, but a high dose inhibited, its secretion.[12] Another study evaluated the clinical efficacy of ruanjian xiaoying decoction on chronic lymphocytic thyroiditis.[13] Similarly, another TCM recipe, xiaoyin, putatively functions by modulating the balance of Th1/Th2 cells.[6]

The Jiayan Kangtai (JYKT) is a kind of traditional Chinese formula. We have observed that JYKT has demonstrable effects on AIT using a classic rat model of the disease.[14] The work is the subject of a pending patent (Patent Application No. CN106421633A) filed by Beijing University of Chinese Medicine. However, the mechanisms that underlie the therapeutic effects of treatment with JYKT remain unclear.

This study was aimed to investigate the protective effects of JYKT granules in an AIT rat model, and the possible underlying mechanism was explored.

METHODS

Herbs and animals

The JYKT granules were obtained from Eastern Hospital of Beijing University of Chinese Medicine, China. It consists of 9 Chinese herbs: Chaihu (*Radix Bupleuri Chinensis*) 10 g, Yujin (*Radix Curcumae Wenyujin*) 20 g, Xiakucao (*Spica Prunellae Vulgaris*) 30 g, Wumei (*Fructus Mume*) 15g, Zhebeimu (*Bulbus Fritillariae Thunbergii*) 15 g, Xuanshen (*Radix Scrophulariae*) 10 g, Chuanshanlong (*dioscorea nipponica*) 10 g, Shancigu (*Pseudobulbus Cremastrae*) 6 g, and Huangqi (*Radix Astragali Mongolici*) 30 g.

Female specific pathogen-free Lewis rats (aged 6-8 week, 120-150 g) were purchased from Beijing Vital River Laboratory Animal Technology, China (Certificate of quality No. SCXK [jing] 2016-0006). The rats were housed in the Animal Facility of Beijing Institute for Drug Control, China at (24 ± 2) ℃ and − 40% hu-