Clinical and immunomodulatory effects of Fun-boi, an herbal medicine, on collagen-induced arthritis in vivo

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Abstract

Objective

Crude preparations of Fun-boi (Stephania tetrandra), a traditional antirheumatic herb, have been reported to have immunomodulatory effects on both cell-mediated and humoral immunity in vitro, but little is known about the mode of action in vivo. The objective of this study was therefore to evaluate the efficacy of Fun-boi against arthritis and its effect on the immune system.

Methods

Mice were divided into the following 3 groups of 7 mice each: 1) a normal group, not treated to cause collagen-induced arthritis (CIA), received water orally; 2) a control group with CIA received water orally; and 3) the Fun-boi group with CIA, received Fun-boi (3 mg/g body weight/day) orally. We analyzed the arthritis score, the serum anti-type II collagen (CII) antibody level, and the percentage of the following lymphocyte subsets from lymphoid organs: B220, CD3/CD4, CD3/CD8 and CD40L/CD4 lymphocytes from blood or lymph nodes; and CD4-CD8-, CD4+CD8+, CD4+CD8- and CD4-CD8+ from the thymus.

Results

Fun-boi therapy markedly reduced the severity of arthritis (p < 0.001) and tended to reduce the serum anti-CII antibody level (p = 0.06). Whereas CII immunization of DBA/1J mice caused a significant redistribution of CD3/CD8 lymphocytes from blood or lymph nodes, Fun-boi therapy caused significant normalization of the same types of lymphocyte subsets from lymph nodes, but did not affect the CD4 or CD4/CD40L lymphocyte subsets.

Conclusion

These results demonstrate that Fun-boi therapy exerts therapeutic effects in CIA mice, possibly by causing immunomodulatory effects at specific sites.

Key words

Fun-boi (Stephania tetrandra), traditional herbal (Kampo) medicine, immunomodulation.
Introduction

Crude preparations of Fun-boi (Stephania tetrandra S Moore), the tuberous root of the creeper Stephania tetrandra, have been used to treat rheumatic diseases in rural areas of China. Fun-boi and its principle active ingredient, tetrandrine, have been reported to have immunomodulatory effects on both cell-mediated and humoral immunity in vitro (1-3). In addition, we demonstrated previously that Fun-boi ameliorated the disease activity of patients with rheumatoid arthritis (RA), decreased IgM-rheumatoid factor and increased the CD3/CD8 lymphocyte subset in the peripheral blood of some RA patients (4).

Alterations in circulating T-cell levels might result from dysregulation of the T-cell maturation in thymus or from modifications of the T-cell subset redistribution within secondary lymphoid organs. However, to our knowledge, there has been no study of the effects of immunosuppressive agents such as Fun-boi on primary and secondary lymphoid organs. Therefore, in this study we investigated the development of arthritis and the modifications induced in lymphocytes from primary (thymus) and secondary (lymph node) lymphoid organs and peripheral blood in mice with collagen-induced arthritis (CIA), a widely used RA model (5), to evaluate the efficacy of Fun-boi against arthritis and its effect on the immune system.

Materials and methods

Animals

Eight-week-old male DBA/1(J) mice (Sankyo Laboratory Japan) were housed in an air-conditioned room at 22°C, fed a standard laboratory diet and given water ad libitum.

Agent

Fun-boi, prepared by Uchida Wakanyakaku Co. Ltd. (Tokyo, Japan), was extracted by boiling the roots of Stephania tetrandra in water for 50 minutes, and was then converted into a freeze-dried powder. For these experiments, we dissolved this Fun-boi extract in distilled water at a concentration of 300 mg/ml.

Induction and assessment of arthritis

Bovine type II collagen (CII; K-42, Cosmo Bio, Tokyo, Japan) was dissolved in 0.1 M acetic acid (2 mg/ml) and then emulsified with an equal volume of complete Freund’s adjuvant (Difco, Detroit, USA). The cold emulsion was injected intradermally at the base of the tail (0.2 ml: 200 µg of C II), followed by injection in the same manner 21 days later.

The clinical severity of arthritis was evaluated daily using the arthritis score of Holmadahl et al. (6): 0 point, no changes; 1 point, detectable swelling in one joint; 2 points, swelling in more than one but not all joints; 3 points, severe swelling of the entire paw and/or ankylosis. Each limb was graded, and thus the maximum possible score was 12 for each animal. A mouse with a score of 1 or more was regarded as arthritic. All clinical evaluations were made by an investigator blinded to the mouse treatment.

Protocol

Fun-boi extract at 3 mg/g/d, a dose 15 times higher than the human daily dose, or water at the same volume-body-weight, was orally injected to normal or CIA mice from the day of the first immunization until the end of this experiment. The mice were divided into three groups during these experiments: 1) non-immunized mice, treated with water (NOR); 2) CIA mice, treated with water (CONT), and 3) CIA mice, treated with Fun-boi (FUN).

Preparation of cell suspension

Four weeks after the second immunization, heparinized blood was collected by heart puncture under pentobarbital sodium anesthesia, and lymph nodes from the inguinal and axillary sites and from the thymus were separately harvested. The tissues were mechanically dissociated into phosphate-buffered saline (PBS). Lymphocytes were isolated by density centrifugation on Ficoll Hypaque (Lymphoprep, Nycomed, Oslo, Norway). The lymphocytes separated from the blood, lymph nodes or thymus were collected by...
Flow cytometric analysis of lymphocyte subsets in the blood, peripheral lymph nodes and thymus

The suspended cells were incubated with saturating concentrations of directly labeled monoclonal antibodies to various cell surface determinants. Phenotypic markers on the surface of these cells were characterized by automated two-dimensional flow cytometry in our laboratory (EPICS XL, Coulter, USA). The following lymphocyte subpopulations were determined (antibodies except that for CD40L were obtained from Immunotech, USA): anti-CD3 (FITC) with anti-CD4 and anti-CD8 (PE), anti-B220 (FITC) and anti-CD4 (FITC) with anti-CD40L (PE) for cells from blood or lymph nodes, and anti-CD4 (FITC) with anti-CD8 (PE) for cells from the thymus. At least 10,000 cells per monoclonal pair were analyzed repeatedly.

Measurement of anti-CII antibodies

An enzyme-linked immunosorbent assay (ELISA) was used to quantitate antibody to CII. Sera were collected at 4 weeks after the second immunization and kept at -20°C until use. Ninety-six-well micro-ELISA plates were coated with 100 µl/well of soluble bovine type II collagen at a concentration of 10 µg/ml in PBS by incubation overnight at 4°C. The plates were washed three times with PBS (pH 7.4) containing 0.05% Tween 20 (PBS-Tween) and blocked by incubation with 100 µl/well of 3% bovine serum albumin-containing PBS overnight at 4°C. The plates were then thoroughly washed three times with PBS-Tween, and incubated with serum diluted 1:1000 in PBS for 2 hours at room temperature. After the plates were washed 3 times with PBS-Tween, alkaline phosphatase-conjugated goat anti-mouse IgG antibody (BioRad Laboratories U.S.) diluted 1:3000 in PBS was added to each well. After 2 hours of incubation at room temperature, the plates were washed thoroughly in PBS-Tween and then the bound enzyme was quantified by incubation with a paranitrophenol-containing substrate buffer. The absorbance was determined using a micro-plate reader (Model 450, BioRad, CA, USA) at a wavelength of 405 nm and a reference wavelength of 490 nm. Each sample was tested in triplicate. The titer of specific anti-CII IgG in each group was expressed as the mean± standard deviation (SD) of the OD value.

Statistical analysis

All data were expressed as mean ± SD. Statistical evaluation was made using repeated measures ANOVA or one-way factorial ANOVA (Fisher’s protected LSD test as the post hoc test). P < 0.05 was considered significant.

Results

The therapeutic effect of Fun-boi extract

All CIA mice developed arthritis, but Fun-boi therapy significantly reduced the severity of the disease (Fig. 1) and slightly delayed the onset of arthritis (p = 0.06) compared with that in mice treated with water.

Lymphocyte subsets

Whereas CII immunization of DBA/1J mice significantly increased the proportion of B220 lymphocytes from lymph node and significantly decreased CD3/CD8 lymphocytes from blood or lymph nodes, Fun-boi therapy significantly normalized the proportion of CD3/CD8 lymphocytes from lymph nodes alone. There were no significant changes of the proportion of CD3/CD4 lymphocytes or CD4/CD40L lymphocytes from lymph nodes or blood in the three groups. In the thymus, we found no significant difference of the lymphocyte subsets that we analyzed among the three groups (Fig. 2).

Serum level of type II collagen-specific immunoglobulins

Fun-boi therapy tended to reduce the serum level of antibodies against type II collagen, although there were no significant differences between control and Fun-boi group (Fig. 3).

Discussion

This study demonstrated that Fun-boi has therapeutic effects in CIA mice, possibly exerting these effects through immunomodulation of secondary lymphocyte organs through redistribution of CD3/CD8 T lymphocytes from the blood or lymph nodes in response to local immunization of DBA/1J mice against CII. The treatment of CIA mice with Fun-boi extract prevented the
Fig. 2. The effect of Fun-boi on the partitioning of lymphocyte subsets in peripheral blood, lymph nodes, and thymus in CIA and non-immunized mice.
NOR: non-immunized mice, treated with water; CONT: CIA mice, treated with water; FUN: CIA mice, treated with Fun-boi. Differences among the three groups were analyzed using one-way factorial ANOVA (with Fisher's protected LSD test as the post hoc test). *p < 0.05 compared with control group.
development of arthritis, and caused normalization of the levels of these molecules in lymph nodes only. The production of anti-CII antibody tended to decrease in Fun-boi-treated mice. However, Fun-boi therapy did not modify the proportions of CD4 or CD4/CD40L T lymphocytes.

To study the basis of the development of RA and the efficacy of therapeutic agents for RA, the CIA model has been widely used (5). The development of CIA is known to be related to both cellular and humoral immune responses to CII (5, 7). Recently, the importance of CD40-CD40L complex formation in the development of RA has been demonstrated in several murine models (5, 8-10). CD40L (ligand) (CD154), a T cell surface glycoprotein that is transiently expressed on activated CD4+ T cells, plays a crucial role in the CIA-related cell-cell signaling process by binding to CD40, which is expressed on various cell types such as B cells, monocytes/macrophages, dendrite cells and fibroblasts. The interaction of CD40L on CD40 helper T cells with CD40 on B cells causes B cell proliferation and immunoglobulin production. Activation of monocytes/macrophages and dendrite cells by CD40L-CD40 signaling leads to the production of several chemokines and inflammatory cytokines. Thus, CD40L-CD40 interactions are involved in humoral and numerous cell-mediated immune responses (8). The present study showed that lymphocytes from lymph nodes and peripheral blood were altered in DBA/1J mice with C II, but not thymus. Similar observations were reported about the influence of CII on the lymphoid organs (11, 12). An increase of anti-CII antibodies in CIA mice may reflect a significant expansion of the B cell population (B220+ cells), resulting in the overproduction of antibodies. In contrast, the expression of CD4/CD40L cells did not differ between the control and normal groups. These findings permit us to speculate that the increase of B cells seen in this study might not have been directly associated with the interaction between CD40 on B cells and CD40L on CD4+ cells.

In this study, there was a tendency (although it was not significant) for the concentration of anti-CII antibodies to differ between the control and Fun-boi groups. This effect may have been induced by the normalization of the CD3/CD8 cell population in CIA mice. This normalization was observed only in lymph nodes, suggesting that Fun-boi therapy induces immunomodulatory effects through the lymph nodes. Importantly, Fun-boi therapy resulted in the normalization of the proportion of CD3/CD8 cell population in the lymph node, as well as the decreased development of arthritis in CIA mice. We guess that immunomodulatory effects similar to those in lymph nodes may be expressed in the joint synovium, which is a major pathological change in CIA mice. To test this possibility, immunopathological investigations of the synovium will be required.

In conclusion, Fun-boi therapy has therapeutic effects in CIA mice, possibly by exerting immunomodulatory effects on secondary lymphocyte organs or specific local sites. This herbal medicine might be a useful agent especially for seropositive RA patients.

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References


