Effect of *Tripterygium wilfordii* Hook *f*, a Chinese Traditional Herb, on Macrophage Function in Mice. II. Inhibition of Inflammatory Mediator Production in vivo

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Abstract: The effect of the chloroform extract of *Tripterygium wilfordii* Hook *f* (TWH) on inflammatory mediator production was examined in vivo in BALB/c mice. Autoclaved Teflon chambers (30 × 10 mm) were implanted into the backs of BALB/c mice. After 10 days, 1% zymosan A in a volume of 300 μl was injected into the chamber to induce local inflammatory responses. Exudate fluid in the chamber was withdrawn at various intervals after zymosan injection to measure the levels of inflammatory mediators, interleukin-1β, tumor necrosis factor-α and prostaglandin E2. After zymosan injection, levels of inflammatory mediators increased rapidly, reached maximums 1 to 2 days later, then slowly decreased to baseline levels. Oral treatment with TWH extract 2 hours before zymosan injection reduced in a dose-dependent manner concentrations of both interleukin-1β and tumor necrosis factor-α in exudate fluid obtained from mice 24 hours after injection. However, TWH extract reduced prostaglandin E2 concentrations at only the highest dose used.

Key words: *Tripterygium wilfordii*, BALB/c mouse, inflammation, in vivo, mediator

Introduction

*Tripterigium wilfordii* Hook *f* (TWH), a traditional Chinese herb, is a vine-like plant indigenous to a large area of southern China. This herb has been used as an agricultural insecticide for many years but had not been used as a medicine. The therapeutic effectiveness of TWH in rheumatoid arthritis was first reported in 1981 by Chang and Tao[1,2]; since then, many controlled, double-blind trials performed in China have found TWH to be useful in the treatment of other immune-related diseases, such as systemic lupus erythematosus, psoriasis, and Behçet’s diseases[3,4].

Several immunologic and pharmacologic studies of TWH have been performed in vitro to clarify the mechanisms that might account for its therapeutic effects. These studies show that TWH inhibits immunoglobulin production by B cells[5], antigen- and mitogen-induced T-cell proliferation[6], and the generation of cytotoxic T cells in mixed-lymphocyte...
culture\textsuperscript{7}. TWH also suppresses production T cell cytokines, including interleukin (IL)-2, IL-4, and interferon-\(\gamma\) \textsuperscript{8,10}. These observations suggest that TWH might exert immunosuppressive or immunomodulatory actions on both T and B cells and be effective in the treatment of immune-related diseases. Rheumatoid arthritis and systemic lupus erythematosus are chronic inflammatory diseases characterized by high levels of inflammatory cytokines, such as IL-1 and TNF-\(\alpha\), in local areas of disease\textsuperscript{11}. However, there are few reports concerned with the effect of TWH on inflammatory cytokine production in vivo.

A new type of tissue chamber has been developed by Dawson \textit{et al.} which involves the subcutaneous implantation of sterile Teflon chambers into the backs of mice\textsuperscript{12,13}. This tissue chamber allows numerous samples to be obtained from the same animal to measure inflammatory mediator production at a site of inflammation. Therefore, we used this tissue chamber model to examine the effect of TWH on local inflammatory mediator production.

**Materials and Methods**

**Mice**

Specific-pathogen-free, male BALB/c mice, 5 weeks of age, were purchased from Charles River Japan Inc. (Atsugi, Japan). The mice were allowed to adapt to our animal room conditions (50\(\pm\)5 % humidity; 12-hour light/dark cycle) for at least 1 week before use.

**Drug and chemical materials**

The chloroform extract of TWH (TWH extract) of human oral administration grade was purchased from Zhuzhou Pharmaceutical Co., Ltd., (Zhuzhou, China). Each tablet contained 33.0\(\mu\)g of TWH extract. For oral administration into mice, randomly selected tablets were ground, suspended in distilled water, and homogenized with a sonic dismembrator at 60 W for 30 minutes in an ice-cold water bath just before use. The following chemical materials were purchased from the indicated companies: zymosan A (Sigma Pure Chemical Co., St. Louis, MO, USA); pentobarbital sodium (Abbott Laboratories, North Chicago, IL, USA); dexamethasone (Sigma Pure Chemical Co.); and pyrogen-free saline (Otsuka Pharmaceutical Co., Ltd., Osaka, Japan).

**Implantation of tissue chambers**

Mice were anaesthetized by intraperitoneal injection with 1.0 mg/kg of pentobarbital sodium. Then, 5.0 ml of sterile air was injected through a 0.22\(\mu\)m filter (Millipore Co., Ltd., Yonezawa, Japan) into the backs of mice to disrupt the subcutaneous connective tissue. Autoclaved Teflon tissue chambers donated by Dr. Dawson (Sandoz Pharm. AG., Basel, Switzerland) were then implanted into the backs through a small incision under aseptic conditions. The incision was closed with a surgical wound clip (Diener Co., Ltd., Frankfurt, Germany). The chambers were checked for sterility 10 days after implantation by culturing 10\(\mu\)l of chamber fluid on Brain-Heart infusion agar for 24 hours at 37\(^\circ\)C.

**Induction of inflammation by zymosan**

Before induction of inflammation, 300 \(\mu\)l of tissue chamber fluid was removed percutaneously with a 23-gauge needle from each mouse. These samples were stored at \(-40^\circ\)C and designated day 0 samples. An inflammatory response in the implanted chamber was induced by injection of 300 \(\mu\)l of 1 % sterile zymosan A suspended in pyrogen-free saline\textsuperscript{12,13}. Control mice received 300 \(\mu\)l of sterile pyrogen-free saline.

**Drug treatment**

TWH extract suspended in distilled water was administered orally into mice as a single
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Fig. 1. Interleukin-1β (A, IL-1β) and tumor necrosis factor-α (B, TNF-α) levels in tissue chamber fluids during zymosan inflammation. Exudate fluid was collected at various intervals after saline (□) or zymosan (■) injection from tissue chambers implanted into the backs of BALB/c mice and assayed for cytokine concentrations with ELISA. Each point represents the mean ± SD of five mice.

Dose of either 60, 120, or 200 μg/kg9,10) once a day for 2 days starting 2 hours before the injection of zymosan. Dexamethasone was first dissolved in 99.5% ethanol at a concentration of 50.0 mg/ml, then diluted with sterile phosphate-buffered saline to obtain a concentration of 5.0 mg/kg. The diluted dexamethasone was injected subcutaneously into mice as a single dose of 30.0 mg/kg13) in a similar manner.

**Assay for inflammatory mediators**

Tissue chamber fluid was obtained on days 1, 3, 6, and 9 after zymosan injection from five mice not treated with TWH extract and 24 hours after zymosan injection from five mice treated with TWH extract. Fluid samples were examined for concentrations of IL-1β, TNF-α, and prostaglandin E2 (PGE2). The levels of both IL-1β and TNF-α in chamber fluids were assayed with commercially available mouse cytokine enzyme-linked immunosorbent assay test kits (Genzyme Inc., Cambridge, MA, USA). Levels of PGE2 in chamber fluids were also analyzed with a commercially available mouse PGE2 enzyme immunosorbent assay kit (Cayman Chemical Co., Ltd., Ann Arbor, MI, USA). Both assays were performed in duplicate with each sample according to the manufacturers' instructions, and the results are expressed as means ± SD.

**Results**

**Concentration of inflammatory mediators in tissue chamber fluid**

The IL-1β concentration in tissue chamber fluid on day 0, before induction of inflammation, was less than 100 pg/ml (Fig. 1A). After injection of zymosan, IL-1β levels increased rapidly, reached a peak on day 1, and decreased gradually thereafter. The kinetics of the TNF-α concentration in tissue chamber fluid was similar to that of IL-1β concentration: the concentration of TNF-α was extremely low on day 0 (typically less than 100 pg/ml) but increased immediately after zymosan injection, was maximal on day 1, and...
Fig. 2. Prostaglandin E₂ (PGE₂) levels in tissue chamber fluids during zymosan inflammation. Exudate fluid was collected at various intervals after saline (□) or zymosan (■) injection from tissue chambers implanted into the backs of BALB/c mice and assayed for PGE₂ levels with EIA. Each point represents the mean ± SD of five mice.

Fig. 3. Effects of dexamethasone and the chloroform extract of Tripterygium wilfordii Hook f (TWH extract) on the concentrations of both interleukin-1β (A, IL-1β) and tumor necrosis factor-α (B, TNF-α) induced by zymosan injection into tissue chambers. BALB/c mice were treated with either dexamethasone (30 mg/kg, subcutaneously) or TWH extract (orally) 2 hours before zymosan injection into the tissue chamber. Exudate fluid was collected 24 hours later and assayed for cytokine concentrations with ELISA. Each point represents the mean ± SD of five mice.

then decreased gradually (Fig. 1B). The concentration of PGE₂ in tissue chamber fluid samples on day 0, before induction of inflammation, was typically less than 100 pg/ml (Fig. 2). The PGE₂ concentration increased rapidly after zymosan injection, reached maximal levels on days 1 and 2, then returned to baseline levels by day 6 (Fig. 2).

Effects of TWH extract on inflammatory mediator production in the tissue chamber.
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Fig. 4. Effect of dexamethasone and the chloroform extract of *Tripterygium wilfordii* Hook f (TWH extract) on the concentrations of prostaglandin E$_2$ (PGE$_2$) induced by zymosan injection into tissue chambers. BALB/c mice were treated with either dexamethasone (30 mg/kg, subcutaneously) or TWH extract (orally) 2 hours before zymosan injection into the tissue chamber. Exudate fluid was collected 24 hours later and assayed for PGE$_2$ concentrations with ELA. Each point represents the mean ± SD of five mice.

Treatment of zymosan-injected mice with TWH extract significantly reduced the concentration of IL-1β in chamber fluid in a dose-dependent manner; the highest dose (200 μg/kg) of TWH extract reduced IL-1β concentrations to levels similar to those observed in dexamethasone-treated mice (Fig. 3A). TWH extract also dose-dependently reduced the concentration of TNF-α in a chamber fluid (Fig. 3B). Furthermore, TWH extract suppressed IL-1β levels to a much greater degree than did dexamethasone (Fig. 3A and B). However, dexamethasone reduced the PGE$_2$ concentration to baseline levels, whereas TWH extract was effective at only the highest dose (200 μg/kg) used (Fig. 4).

Discussion

In China, TWH extract has been used extensively and has been found effective for the treatment of autoimmune diseases, especially rheumatoid arthritis. Several in vitro studies have clearly shown that TWH extract suppresses the function of both T and B cells. These suppressive effects were confirmed by our recent in vivo studies in mice: TWH extract given subcutaneously for 3 weeks after cell transplantation attenuated chronic-graft-versus-host reactions by inhibiting production of IL-4 by T cells and of IgE by B cells. TWH extract has also been shown to suppress production of several cytokines, such as IL-2 and IFN-γ, by T cells prepared from mice treated with TWH extract and cultured in vitro with specific antigens. These findings suggest that the therapeutic effects of TWH extract on autoimmune diseases might be explained, at least in part, by the inhibition of the function of both T and B cells. In the autoimmune diseases, all cellular and humoral components of the immune system are activated and coordinately contribute to disease pathology. On the other hand, autoimmune diseases are primarily associated with inflammation in local areas of disease. Furthermore, the levels of inflammatory mediators, such as IL-1β and TNF-α, are also correlated with the severity and strongly implicated in the pathogenesis of
autoimmune diseases\textsuperscript{16,17}). However, few studies have examined the effects of TWH extract on inflammatory mediator production \textit{in vivo}.

The present results show that injection of zymosan into the lumen of a tissue chamber transiently increases concentrations of both IL-1$\beta$ and TNF-$\alpha$ in chamber fluid and that these concentrations are reduced significantly by treatment with dexamethasone. TWH extract also reduces the concentrations of both IL-1$\beta$ and TNF-$\alpha$ in a dose-dependent manner. The suppression by dexamethasone of IL-1$\beta$ and TNF-$\alpha$ production was expected because anti-inflammatory steroids, such as predonisolone and dexamethasone, are active in other animal models of arthritis and inflammation\textsuperscript{18,19}). However, the mechanisms of action of TWH extract on IL-1$\beta$ and TNF-$\alpha$ production are not clear. Previous studies with the tissue chamber model have clearly shown that IL-1$\beta$ is produced by phagocytic macrophages in granuloma tissue which have engulfed zymosan particles\textsuperscript{20)}. Furthermore, T cells are a source of TNF-$\alpha$ and are able to mediate TNF-$\alpha$ production by macrophages; however, zymosan-induced production of TNF-$\alpha$ in the tissue chamber model is unrelated to the presence of T cells\textsuperscript{20}). Our present results suggest that TWH extract directly suppresses production of IL-1$\beta$ and TNF-$\alpha$ by macrophages and inhibits the functions of T and B cells.

TWH extract significantly reduced zymosan-induced PGE$_2$ production at only the highest dose used. Anti-inflammatory steroids, such as predonisolone and dexamethasone, block PGE$_2$ production by inhibiting the cyclo-oxygenase enzyme system\textsuperscript{21}). Therefore, TWH extract administered orally into mice might inhibit cyclo-oxygenase and suppress zymosan-induced PGE$_2$ production.

The recommended therapeutic dose of TWH extract in inflammatory diseases, 1.0 mg/kg/day, does not produce adverse effects in humans\textsuperscript{3,4}). The present results indicate that the dose of TWH extract that most effectively inhibits production of the zymosan-induced inflammatory mediators is 200 $\mu$g/kg, which is one fifth the human therapeutic dose. The reason for this discrepancy is not clear; however, the metabolic pathway of the active molecules of TWH extract may differ between humans and mice. Further experiments are needed to clarify this point.

Chronic inflammatory diseases are characterized by a marked increase in cell migration and influx of inflammatory mediators to local areas of disease. Therefore, the present results may suggest that TWH extract inhibits inflammatory mediator production and decreases the severity of chronic inflammatory diseases.

References


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