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Increased production of tumor necrosis factor α and interleukin-1 β in the murine lung by Sho-saiko-to and interferon α/β

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Abstract

Examination was made of the effects of the herbal medicine Sho-saiko-to (SST) and interferon α/β (IFN α/β) on the production of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in lung tissue. SST (0.92 g/kg of body weight) was orally administered to mice for 3 weeks with or without intraperitoneal injection of IFN α/β (10^5 unit/kg of body weight, 2 times per week). SST and IFN α/β each increased TNF-α and IL-1β in the lungs and additively increased cytokines in that tissue. The present results provide indication of the effectiveness of SST for treating infectious lung diseases, since TNF-α and IL-1β are major proinflammatory cytokines essential to host defence system. The additive effect may possibly be related to the occurrence of interstitial pneumonitis, reported to occasionally occur as an adverse side effect due to SST administered with exogenous INF α/β in large amount.

Key words Sho-saiko-to (Xiao-Chai-Hu-Tang), 小柴胡湯, interferon α/β, tumor necrosis factor α, interleukin-1β, lung.

Abbreviations SST, Sho-saiko-to ; IFN α/β, interferon α and β ; IL-1β, interleukin-1β ; TNF-α, tumor necrosis factor-α ; PBS, phosphate buffered saline.

Introduction

Sho-saiko-to (SST), Xiao-Chai-Hu-Tang in Chinese, is a herbal medicine consisting of crude ingredients from seven medicinal herbs, Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus, Ginseng Radix, Glycyrrhizae Radix and Zingiberis Rhizoma. The pharmacological action of SST has been extensively studied, mainly in regard to immunological and inflammatory response.[1-6] This herbal medicine has been shown to express diverse activities such as immunostimulatory, anti-allergic and anti-inflammatory effects. The drug is thus widely used for treating various infectious diseases such as chronic viral hepatitis, bronchitis and the common cold.[6-8]

Although the effectiveness of SST for inflammatory lung diseases has been demonstrated,[9] it occasionally induces interstitial pneumonitis as an adverse effect, particularly when administered along with exogenous interferons (IFNs).[10,11] The mechanism for this adverse effect is little understood. Allergic reactions to the drugs and/or their synergistic effects on immunological and inflammatory response may possibly be involved.[10,11] SST and IFN α/β were previously shown to act synergistically in nitric oxide generation *in vivo*.[12] Further elucidation of the mechanisms for synergistic or additive effects of SST and INFs should provide some clarification of the effects of SST as medicine and adverse effects on lung physiology.

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SST and IFN α/β were shown in this study to additively increase TNF-α and IL-1β production in the lungs. TNF-α and IL-1β are major proinflammatory cytokines characteristically produced at the site of inflammation by macrophages/monocytes and are importantly involved in the inflammation and fibrogenesis of various tissue.[13, 14] The biological significance of this additive effect of SST and IFN α/β on TNF-α and IL-1β production in the lung is discussed, particularly in relation to pharmacological and adverse effects on lung physiology.

Materials and Methods

Preparation of spray-dried aqueous extracts of Sho-saiko-to : A spray-dried extract powder of Sho-saiko-to (TJ-9, lot No. 240009020, Tsumura & Co., Tokyo, Japan) was prepared from seven species of medicinal herbs according to traditional prescription. TJ-9 consists of hot water extracts from the following medicinal herbs mixed in the ratios shown in parentheses ; Bupleuri Radix (7.0 g), Pinelliae Tuber (5.0 g), Scutellariae Radix (3.0 g), Zizyphi Fructus (3.0 g), Ginseng Radix (3.0 g), Glycyrrhizae Radix, (2.0 g) and Zingiberis Rhizoma (1.0 g).

Experimental protocols : Six-week-old male Balb/c mice, obtained from Charles River Japan Inc., were housed five per cage in standard facilities, with free access to water and standard mice chow. Mouse IFN α/β was obtained from Paesel GmbH & Co. (Frankfurt, Germany). The animals were divided into 4 groups to undergo the following treatments.

1) Control group (n=10) ; received *i.p.* PBS (0.12 ml per animal) 2 times per week for 3 weeks, with free access to tap water.

2) SST group (n=10) ; received *i.p.* PBS (0.12 ml per animal) 2 times per week for 3 weeks, with free access to tap water containing 0.5 % SST.

3) IFN α/β group (n=10) ; received *i.p.* IFN α/β (10^5 units/kg of body weight in 0.12 ml of PBS) 2 times per week for 3 weeks, with free access to tap water.

4) SST+IFN α/β group (n=10) ; received *i.p.* IFN α/β (10^5 units/kg of body weight in 0.12 ml of PBS) 2 times per week for 3 weeks, with free access to tap water containing 0.5 % SST.

During each experiment, average daily water intake was 4.4 ml/mouse (23-24 g body weight). The daily intake of SST from drinking water was thus 22 mg/mice, or 0.92 g/kg of body weight. Intraperitoneal injections of PBS or IFN α/β were given at 10:00 a.m. on days 1, 4, 8, 11, 15, 18 and 22. The animals were sacrified 24 hr following the last injection. The lung was resected, weighed and frozen until tissue homogenate preparation. Blood was obtained at the time of sacrifice and from which, serum was separated by centrifugation after clot formation.

Measurement of TNF-α and IL-1β : TNF-α and IL-1β in lung tissue homogenates were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits for mouse IL-1β and TNF-α (PerSeptive Diagnostics, USA). Lung tissue (100 mg) was homogenized in 1 ml homogenate solution [10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml pepstatin A and 20 μg/ml 4-(2-Aminoethyl) benzenesulfonyl Fluoride Hydrochloride] with a Polytoron homogenizer, followed by sonication for 10 seconds with an ultrasonic generator (model US-50, Nissei). Each homogenate was then centrifuged at 105,000×g at 4°C for 45 min. The supernatant fraction was subjected to cytokine measurement. Protein concentration in the soluble fraction of lung homogenate was determined using a Bio-Rad protein assay kit. Cytokine concentration was expressed as pg/mg protein of lung homogenate.

Statistical analysis : The results were determined as means±S.E.M.. Significance of difference was assessed by the paired Student's t test. P less than 0.05 was considered statistically significant.

Results

TNF-α in lung tissue of mice treated with SST and/or IFN α/β (Fig. 1)

TNF-α in lung tissue of the control group was 128.47±11.74 pg/mg protein (n=10). Treatment of mice with either SST or IFN α/β caused slight increase in lung TNF-α. Lung TNF-α in mice that had received SST or IFN α/β for 3 weeks was 215.08 ±46.87 pg/mg protein (n=10) and 237.19±49.0 pg/mg protein (n=10, $p<0.05$ vs. control), respectively. Mice treated with SST and IFN α/β showed a TNF-α value of 352.39±72.16 pg/mg protein (n=10, $p<0.05$

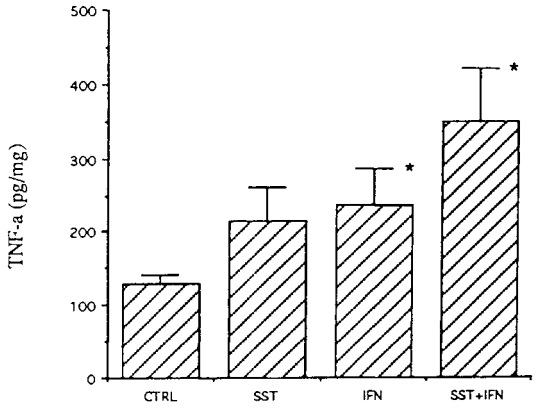

Fig. 1 TNF-α in lung tissue of mice treated with Sho saiko to (SST) and/or interferon α/β (IFN α/β). Mice were treated with SST and/or IFN α/β for three weeks following the procedure described in Materials and Methods and TNF α was measured in supernatants of lung tissue homogenates by ELISA. Valus represent means±S.E.M.(n=10). *$p<0.05$ compared with control.

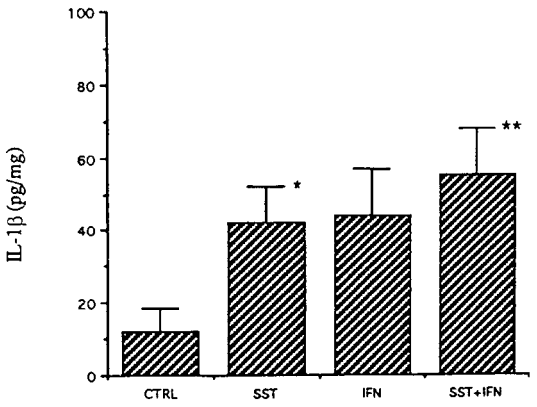

Fig. 2 IL-1β in lung tissue of mice treated with Sho saiko to (SST) and/or interferon α/β (IFN α/β). Mice were treated with SST and/or IFN α/β for three weeks following the procedure described in Materials and Methods and IL 1β was measured in supernatants of lung tissue homogenates by ELISA. Values represent means+S.E.M. (n=10). *$p<0.05$ compared with control. **$p<0.01$ compared with control.

vs. control), this being nearly three times that of the control group.

IL-1β in the lung tissue of mice treated with SST and/or IFN α/β (Fig. 2)

IL-1β in lung tissue of the control group was 12.08 ±6.23 pg/mg protein (n=10). Treatment of mice with SST or IFN α/β resulted in a three fold to four fold increase in IL-1β in the lung homogenates ; lung IL-1β in mice that had received SST or IFN α/β for 3 weeks was 42.30±10.08 pg/mg protein (n=10, $p<0.05$ vs. control) and 44.24±12.84 pg/mg protein (n=10), respectively. SST and IFN α/β showed additive increase in lung IL-1β. IL-1β was 55.54±12.68 pg/mg protein (n=10, $p<0.01$ vs. control), this being nearly five times that of the control group.

Discussion

Clinical studies have indicated SST to exert therapeutic effects in many infectious diseases.[6-8] One purpose of this study was to provide some clarification of the mechanism(s) for the effectiveness of SST toward infectious lung diseases. The pharmacological action of SST has been studied mainly in regard to modulation of immunological and inflammatory response. In studying the immunomodulatory action of SST, special attention has been directed to the increased nonspecific resistance of the host to pathogenic microorganisms due to stimulation of the immune system. This immunopotentiation includes activation of macrophages, enhancement of IL-1, TNF-α and IFN-γproduction, and increased antibody production as well as lymphokine activating killer cell activity.[1-6]

The results obtained in this study indicate the oral administration of SST to stimulate the production of TNF-α and IL-1β in the lung, especially when given along with IFN α/β. IFNs are endogenously produced in various infectious diseases, and are indispensable to host defense against viruses and parasites.[15,16] TNF-α and IL 1β are major proinflammatory cytokines characteristically produced at sites of inflammation by macrophages / monocytes.[13,14] TNF-α and IL-1β are considered to help eliminate microbial invaders.[17-19] The self defence system is thus shown by the present study to be strengthened by SST in combination with endogenous IFNs and the effectiveness of SST for treating pulmonary infectious diseases in which IFNs are likely produced endogenously at sites of infection has been confirmed.

An attempt was also made to elucidate the mechanisms for the onset of interstitial pneumonitis induced by SST and exogenous IFN α/β.[10,11] IFN α/β and SST are used to treat chronic viral heptitis,[7,8,20,21] and the effectiveness of their combined administration was previously demonstrated toward chronic viral hepatitis in Japan. The therapeutic potential of this combination for viral hepatitis has recently been denied since interstitial pneumonitis occasionally develops when SST is administered with exogenous IFNs in excess.[10,11] The mechanism for the onset of interstitial pneumonitis by these drugs still remains little understood. Allergic reactions to the drugs may possibly be involved in this mechanism. Furthermore synergistic or additive effects of SST and IFNs have been suggested in the pathogenesis of the adverse effect by several investigators. For example, SST was reported to enhance the expression of HLA class II antigens as well as IFN-α-induced HLA class I antigens in human peripheral mononuclear cells *in vitro*.[22] We previously reported that SST and IFN α/β act synergistically in nitric oxide generation *in vivo*.[12]

TNF-α and IL-1β stimulate fibroblast proliferation and collagen production with consequent fibrosis in interstitial tissue.[13,14] Recent studies clearly demonstrate TNF-α to be importantly involved in the pathogenesis of pulmonary fibrosis.[23,24] Excessive focal immune response in the lung due to the excessive production of proinflammatory cytokines caused by the additive effects of these drugs should thus be able to bring about the onset of interstitial pneumonitis.

SST exerts anti-inflammatory and anti-allergic action, and even the effectiveness of SST as treatment for interstitial pneumonitis has been reported.[9] However, it should be remembered that adverse side effects occasionally developed when SST is administered with much exogenous IFN α/β or in some pathological conditions with immunological disorders. Effects of SST and IFNs on cytokine production in lung tissue should be studied further, especially in regard to their doses and duration of the drug administration. A more detailed study of the pathogenesis and identification of active components contributing to the additive effect with IFNs should be pursued in the future.

和文抄録

肺組織の Tumor Necrosis Factor α (TNF-α) と Interleukin-1β (IL-1β) の産生に対する小柴胡湯 (SST) とインターフェロン α/β (IFN α/β) の作用をマウスを用いて検討した。飲水に混じた SST (0.92 g/kg 体重) を経口的に 3 週間連続投与し，IFN α/β (10^5 unit/kg 体重) は週 2 回 (計 7 回) 腹腔内投与を行った後，肺組織の TNF-α と IL-1β のレベルを測定した。肺組織中の TNF-α と IL-1β 量は SST あるいは IFN α/β の投与により増加し，併用により相加的に増加した。TNF-α と IL-1β はサイトカインのネットワークの中心として生体防御機構で重要な役割をはたすと同時に，組織の炎症や線維化の増悪にも関与している。従って今回の実験結果は，内因性の IFN が産生される感染症に対する SST の有効性の作用機序を示唆すると同時に，大量の IFN 投与との併用や内因性の IFN が過剰に産生されるような病態では，組織の炎症増悪や線維化などの副作用が起こり得る可能性をも示唆している。

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