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Effect of Sho-saiko-to on interleukin 1 production by hepatic sinusoidal endothelial cells

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Abstract

We studied whether mouse hepatic sinusoidal endothelial cells produce interleukin 1 (IL 1) and if so, the effect of Sho-saiko-to on the production of IL 1 by these cells. As a result, we found that when the hepatic sinusoidal endothelial cells from mice were incubated with lipopolysaccharide (LPS), IL 1 was produced in a concentration-dependent manner. Moreover, Sho-saiko-to increased the production of IL 1 induced by stimulation with LPS in a dose-dependent manner. IL 1 was also produced when the cells were incubated with Sho-saiko-to alone in absence of LPS. These results suggested that Sho-saiko-to acts on hepatic sinusoidal endothelial cells to increase the production of IL 1, thereby affecting the interleukin cascade.

Key words Sho-saiko-to (Syô-saiko-tô), interleukin 1, hepatic sinusoidal endothelial cell.

Abbreviations Con A, concanavalin A ; EDTA, ethylenediaminetetraacetic acid ; GBS, Gey's balanced salt solution ; HBSS, Hanks' balanced salt solution ; HEPES, *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid ; IL, interleukin ; LPS, lipopolysaccharide ; ^{3}H-TdR, tritiated thymidine ; Sho-saiko-to (Xiao-Chai-Hu-Tang), 小柴胡湯.

Introduction

Sho-saiko-to has recently been reported to be effective in the treatment of chronic hepatitis.[1] Its pharmacological actions are known to include immunopotentiation such as activation of macrophages, enhancement of interleukin 1 (IL 1) production and enhancement of antibody production by B lymphocytes.[2,3] However, these studies on the pharmacological effect of Sho-saiko-to have been done using the peripheral blood or splenic cells, and there have been few studies on the sinusoidal cells of the liver itself. We have therefore been studying the effect of Sho-saiko-to on hepatic sinusoidal cells. In this study, we examined the hepatic sinusoidal endothelial cells which are a type of cells that comprise the sinusoidal wall, separating the hepatic chords from the sinusoids via Disse's spaces and surrounding the entire sinusoidal cavity. We investigated whether these hepatic sinusoidal endothelial cells produce IL 1 and if so, the effect of Sho-saiko-to on IL 1 production by these cells.

Materials and Methods

Materials : The mice used in the study were BALB/c mice (6-week-old males) and C3H/HeJ mice (6-week-old males) purchased from Clea Japan, Inc. Pronase E was purchased from Merck (Darm Stadt, F.R.G), collagenase type IV and ethylenediaminetetraacetic acid (EDTA) disodium salt from Wako Pure Chemical Indus-

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tries (Osaka, Japan), and *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES) and 2-[3-acetamido-5-*N*-methylacetamido-2,4,6-triiodobenzamido]-2-deoxy-D-glucose (metrizamide) from Sigma (St. Louis, MO) for use in isolating the mouse hepatic sinusoidal endothelial cells. Lipopolysaccharide (LPS) was of *Salmonella enteritidis* origin and purchased from Difco (Detroit, MI). Tritiated thymidine (^{3}H-TdR) was purchased from Amersham Int. (U.K.). The extract of Sho-saiko-to (Xiao-Chai-Hu-Tang) used was obtained from Tsumura & Co. (Tokyo, Japan) and prepared as previously reported.[4]

Isolation of mouse hepatic sinusoidal endothelial cells : Six-week-old BALB/c mice were anesthetized by an infusion of 1 mg of pentobarbital into the peritoneal cavity. After laparotomy, the portal vein was exposed and a catheter was placed. Through this catheter, 10 ml of Hanks' blanced salt solution (HBSS) to which Ca^{++}-free HBSS with 10 mM HEPES had been added was infused, followed by 10 ml of HBSS with 20 mM HEPES containing 0.05% collagenase and 10 ml of HBSS with 20 mM HEPES containing 0.2% pronase E, at a rate of 3 ml/min. Following perfusion, the liver was extracted and sliced using ophthalmic scissors, and these liver slices were enzymatically digested by shaking in 50 ml of HBSS solution with 20 mM HEPES containing 0.2% pronase E at 37℃ for 20 minutes. The undigested material was filtered with gauze and centrifuged (400 *g*, 4℃, 10 min) to isolate cell debris. The cell pellets obtained were suspended in 5 ml of Gey's balanced salt solution (GBS), mixed with 7 ml of 30% metrizamide solution and centrifuged (1,400 *g*, 4℃, 15 min). The non-parenchymal cell phase, which was the top layer, was removed, washed and adjusted to make a concentration of 1×10^8 cells/100 ml in HBSS solution.[5] Hepatic sinusoidal endothelial cells were isolated from this cell suspension according to the method of Knock[5] using a model SRP6Y Elutriator rotor (Hitachi Koki Co., Ltd., Tokyo, Japan). These sinusoidal endothelial cells were examined under an electron microscope and found to have a purity of approximately 95%.

Induction of IL 1 production by hepatic sinusoidal endothelial cells : The hepatic sinusoidal endothelial cells prepared as described above were washed twice with HBSS solution and adjusted to a concentration of 5×10^5 cells/ml using RPMI 1640 solution containing 10% fetal calf serum. One ml samples of this cell suspension were placed in plastic culture dishes (Falcon 3047) and incubated with various concentrations of LPS (final concentrations : 0 to 50 μg/ml) for 18 hours. After incubation, the suspensions were centrifuged (400 *g*, 4℃, 10 min), and the supernatants were used for IL 1 measurement. The samples were frozen and stored at −80℃ until use.

Effect of Sho-saiko-to on production of IL 1 : The hepatic sinusoidal endothelial cells (5×10^5 cells/ml) were incubated with various concentrations of Sho-saiko-to for fixed periods of time. After incubation, they were incubated with LPS for another 18 hours, and IL 1 production in the culture supernatants was measured by the method described below.

Measurement of IL 1 production : IL 1 production was measured according to the method of Simon *et al.*[6] as follows. Thymus cells were extracted from C3H/HeJ mice, and a cell suspension of 5×10^5 cells/ml was prepared using RPMI 1640 culture medium containing 5% fetal calf serum and 5×10^{-5} M 2-mercaptoethanol. Aliquots of 100 μl of this suspension were placed in each well of a 96-well plate (Falcon 3047), and concanavalin A (Con A) was added to make a final concentration of 0.75 μg/ml. One hundred μl of LPS-stimulated hepatic sinusoidal endothelial cell culture supernatant was added to each well, and the plate was incubated at 37℃ and 5% CO_2 for 44 hours. The plate was then incubated with 0.5 μCi/10 μl of ^{3}H-TdR for an additional 24 hours. After incubation, the cells were collected on a filter using a cell harvester, and ^{3}H-TdR uptake of the cells was measured using a liquid scintillation counter. A culture medium containing no sample was also counted as a control.

Statistical analysis : All experimantal data were expressed as mean±S.E.M., and statistical analysis was done using the F test.

Results

IL 1 production by hepatic sinusoidal endothelial cells

The hepatic sinusoidal endothelial cells (5×10^5 cells/ml) were incubated with various concentrations of LPS for 18 hours in order to induce the production of IL 1. As shown in Fig. 1, this resulted in a concentration-dependent increase in the amount of IL 1 produced by the hepatic sinusoidal endothelial cells, and IL 1 production was $24,380\pm1,620$ dpm, when 10 μg/ml of LPS was added (n=5 per group). Based on this result, the subsequent experiments were conducted using 10 μg/ml of LPS.

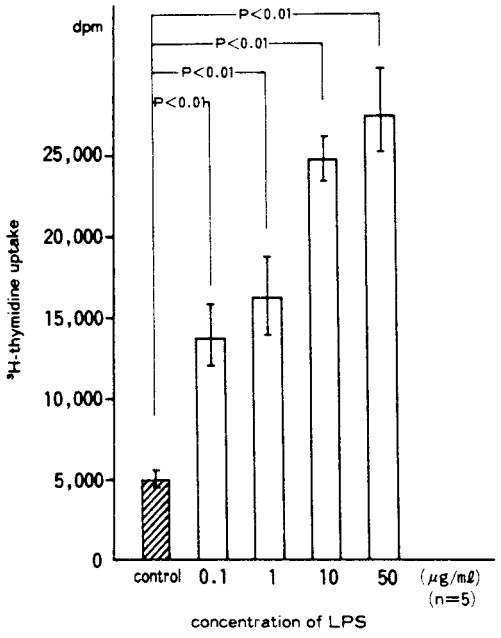

Fig. 1 IL 1 production by hepatic sinusoidal endothelial cells.
Hepatic sinusoidal endothelial cells (5×10^5 cells/ml) were incubated with various concentrations of LPS for 18 hours. The LPS-stimulated hepatic sinusoidal endothelial cell culture supernatant obtained was incubated with Con A and thymus cells for 44 hours. This was then incubated with ^{3}H-TdR for an additional 24 hours, and ^{3}H-TdR uptake of the cells was measured using a liquid scintillation counter.

IL 1 production by various plating densities of hepatic sinusoidal endothelial cells

Various plating densities of the hepatic sinusoidal endothelial cells were incubated with 10 μg/ml of LPS for 18 hours in order to induce the production of IL 1. As shown in Fig. 2, this resulted in a density-dependent increase in the amount of IL 1 produced by the hepatic sinusoidal endothelial cells, and there was a significant increase at 5×10^5 hepatic sinusoidal endothelial cells/ml or more (n=5 per group). Therefore, the subsequent experiments were conducted using 5×10^5 hepatic sinusoidal endothelial cells/ml.

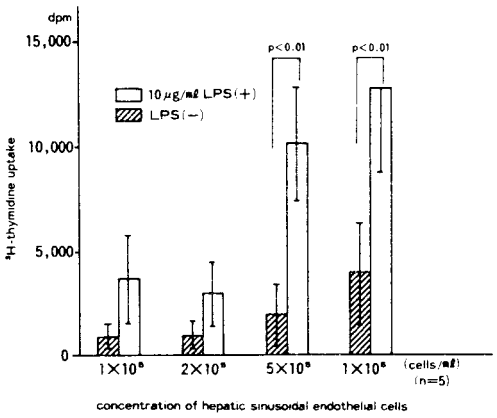

Fig. 2 IL 1 production by various plating densities of hepatic sinusoidal endothelial cells.
Various plating densities of hepatic sinusoidal endothelial cells were incubated with 10 μg/ml of LPS for 18 hours. The LPS-stimulated hepatic sinusoidal endothelial cell culture supernatant obtained was incubated with Con A and thymus cells for 44 hours. This was then incubated with ^{3}H-TdR for an additional 24 hours, and ^{3}H-TdR uptake of the cells was measured using a liquid scintillation counter.

Effect of Sho-saiko-to on production of IL 1

The hepatic sinusoidal endothelial cells (5×10^5 cells/ml) were incubated with various concentrations of Sho-saiko-to for 30 minutes. After incubation, the cells were incubated with 10 μg/ml of LPS for an additional 18 hours, and IL 1 production was determined. As shown in Fig. 3, the amount of IL 1 produced by the cells increased in a concentration-dependent manner, and there

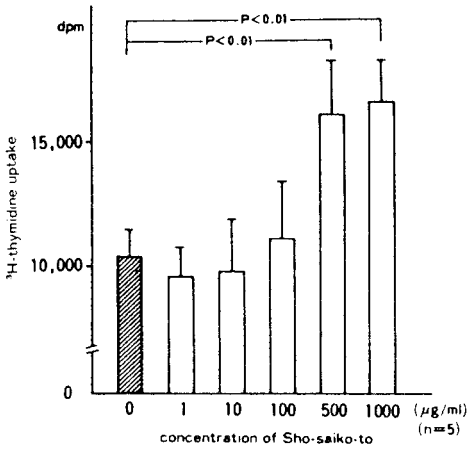

Fig. 3 Effect of Sho-saiko-to on production of IL 1 by hepatic sinusoidal endothelial cells stimulated with 10 μg/ml of LPS.

Hepatic sinusoidal endothelial cells (5×10^5 cells/ml) were incubated with various concentrations of Sho-saiko-to for 30 min, followed by incubation with 10 μg/ml of LPS for 18 hours. The LPS-stimulated hepatic sinusoidal endothelial cell culture supernatant obtained was incubated with Con A and thymus cells for 44 hours. This was then incubated with ^{3}H-TdR for an additional 24 hours, and ^{3}H-TdR uptake of the cells was measured using a liquid scintillation counter.

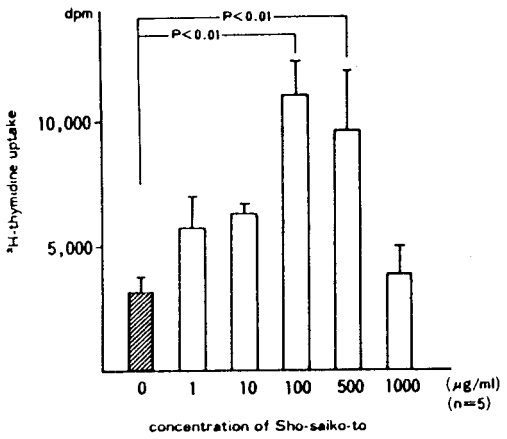

Fig. 4 Effect of Sho-saiko-to on the production of IL 1 by hepatic sinusoidal endothelial cells in absence of LPS.

Hepatic sinusoidal endothelial cells (5×10^5 cells/ml) were incubated with various concentrations of Sho-saiko-to in absence of LPS for 18 hours. The hepatic sinusoidal endothelial cell culture supernatant obtained was incubated with Con A and thymus cells for 44 hours. This was then incubated with ^{3}H-TdR for an additional 24 hours, and ^{3}H-TdR uptake of the cells was measured using a liquid scintillation counter.

was a significant increase at Sho-saiko-to concentrations of 500 μg/ml or more ($p<0.01$, n=5).

Next, the hepatic sinusoidal endothelial cells (5×10^5 cells/ml) were incubated with various concentrations of Sho-saiko-to in absence of LPS for 18 hours, and IL 1 production was determined. As shown in Fig. 4, the amount of IL 1 produced by the cells significantly increased when 100 μg/ml of Sho-saiko-to alone was added ($p<0.01$, n=5). However, the production of IL 1 was inhibited when a concentration of more than 500 μg/ml was added.

Discussion

Sho-saiko-to has been used in the treatment of chronic liver diseases with favorable results. For example, we have conducted a multiple-center open study on patients with HBe antigen-positive chronic hepatitis and noted significant seroconversion and remarkable decreases in serum transaminase levels.[1] In addition, because an annual average of about 7% of patients with liver cirrhosis develop liver cancer, the immunopotentiating effects of Sho-saiko-to have been applied in the prevention of liver cancer in these patients with liver cirrhosis.[7] These reports have led us to study how and why Sho-saiko-to is effective for the treatment of liver diseases. In particular, we have been studying the pharmacological effects of Sho-saiko-to on the liver itself using hepatic sinusoidal cells. In this study, in order to find out whether hepatic sinusoidal endothelial cells are involved in the immune responses, we investigated whether these cells produce IL 1 and if so, the effect of Sho-saiko-to on IL 1 production by these cells. As a result, IL 1 was produced by hepatic sinusoidal endothelial cells when stimulated with LPS. In addition, not only did Sho-saiko-to increase IL 1 production by hepatic sinusoidal endothelial cells induced by stimulation with LPS, but also IL 1 was produced

by addition of Sho-saiko-to alone in absence of LPS.

Prostaglandins are known to be produced from hepatic sinusoidal endothelial cells.[8] Therefore, our results suggested that hepatic sinusoidal endothelial cells may affect the immune responses and inflammatory responses in the liver by regulating both the IL cascade including the production of IL 1 and the arachidonic acid cascade including the production of prostaglandins. In this study, high concentrations of Sho-saiko-to of 100 to 500 μg/ml were added. However, we have previuosly reported *in vitro* that the optimum concentration of Sho-saiko-to for the activation of macrophages, induction of IL production by macrophages and induction of antibody production by pokeweed mitogen is 100 to 500 μg/ml.[2,3] Therefore, the high concentrations of Sho-saiko-to in our study may not matter in the *in vitro* experimental model of Sho-saiko-to. In any case, our results suggested that Sho-saiko-to acts on hepatic sinusoidal endothelial cells to increase the production of IL 1, thereby affecting the immune response in the liver. In order to elucidate the pathogenesis of hepatic cell injury and the effects of various drugs in the treatment of liver diseases, the study of hepatic sinusoidal cells including hepatic sinusoidal endothelial cells may be important.

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