Effects of Syô-saiko-tô (Xiao-Chai-Hu-Tang) on the level of cytosol estradiol receptors

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Abstract

Sex hormones are known to affect the immune system. Asymptomatic hepatitis B virus (HBV) carriers are thought to be immunologically tolerant to HBV, but in some HBV carriers, the immune response to HBV is induced, especially in females during their sexually-mature periods. This may be caused by the effects of estrogens on the immune system. In this study, we found that the level of estradiol receptors in the cytosol of peripheral blood mononuclear cells was significantly lower in asymptomatic HBV carriers and patients with chronic hepatitis B compared to normal controls. However, when the mononuclear cells from normal controls were pretreated with Syô-saiko-tô, which is clinically known to induce seroconversion in symptomatic HBV carriers, the level of cytosol estradiol receptors significantly increased. These results suggest that in asymptomatic HBV carriers and patients with chronic hepatitis B, the response of peripheral blood mononuclear cells to estradiol was lower compared to that of normal controls as a result of the low level of cytosol estradiol receptors, and that the pretreatment of the mononuclear cells with Syô-saiko-tô increased the levels of cytosol estradiol receptors.

Key words Syô-saiko-tô, estradiol receptor, hepatitis B virus, chronic hepatitis B.

Abbreviations EDTA, ethylenediaminetetraacetic acid ; ELISA, enzyme-linked immunosolvent assay ; HBV, hepatitis B virus ; HBe, hepatitis Be ; ME, mercaptoethanol ; MEM, minimum essential medium ; TEMG buffer, buffer containing 10 mM Tris-HCl, 1.5 mM EDTA-Na_2, 2 mM β-ME and 10% glycerol ; Syô-saiko-tô (Xiao-Chai-Hu-Tang), 小柴胡湯.

Introduction

The immune responses differ according to sex, and it has been reported that serum immunoglobulin levels of mammals including humans are higher in females than in males.[1] Antibody production induced by stimulation with a specific antigen is also higher in females than in males,[2] and autoimmune diseases are generally more frequently seen in females, especially in sexually-mature females.[3] These results indicate that sex hormones affect the immune system. It has been reported that in male mice, antibody production increases with the administration of estradiol[3] and decreases with the administration of male hormones.[4] Similarly, in hepatitis B virus (HBV) carriers, virus elimination and induction of the immune responses to viral antigens seem to differ between males and females. Studies on various virus markers in the serum of chronic HBV carriers have shown that seroconversion (the ap-

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pearance of anti-HBe antibody and disappearance of HBe antigen) is more frequently seen in females, especially in sexually-mature females.[5] That is, sex hormones also affect the immune responses in HBV carriers. Most asymptomatic HBV carriers are infected during the perinatal and infantile periods, and they are thought to be immunologically tolerant to HBV. An examination of their serum shows that they are positive to HBs antigen but have normal liver function. However, in some cases, the immune response to HBV is induced at puberty or sexually-mature periods, and they develop chronic hepatitis. This also indicates that the immune system of asymptomatic HBV carriers is influenced by female sex hormones. In order to analyze the response of the immune system of asymptomatic HBV carriers to female sex hormones, the level of estradiol receptors in the cytosol of peripheral blood mononuclear cells, which play an important part in expressing the effects of estradiol,[6] was measured in asymptomatic HBV carriers and patients with chronic hepatitis B. Since Syô-saiko-tô is recently being used in the treatment of chronic hepatitis B, the effects of Syô-saiko-tô treatment on estradiol receptors were also studied.

Subjects and Methods

Subjects : Fifteen HBs antigen-positive asymptomatic HBV carriers who were normal by the liver function test, liver scintigraphy and abdominal ultrasonic examination, 22 patients with chronic hepatitis B who were histologically diagnosed by liver biopsy and 24 normal controls were selected for this study.

Preparation of the cytosol of peripheral blood mononuclear cells : Heparinized peripheral blood was obtained from the patients and normal controls, and the mononuclear cells were separated by Ficoll-Conray density gradient centrifugation. They were suspended in TEMG buffer [10 mM Tris-HCl, 1.5 mM ethylenediaminetetraacetic acid (EDTA)-Na_2, 2 mM β-mercaptoethanol (ME), 10% glycerol, pH 7.4] to make a cell suspension of 2×10^7 cells/ml. This suspension was homogenized using an ultrasonic cell disruptor (Branson Sonifier) and centrifuged at 105,000 *g* for one hour. The supernatant containing cytosol was obtained and used for the assay.

Measurement of estradiol receptors : The level of estradiol receptors in the cytosol was measured by enzyme-linked immunosolvent assay (ELISA; ER-EIA kit, Dainabot Co.) using anti-estradiol receptor monoclonal antibody.[7,8] In brief, 100 μl of each sample (prepared cytosol) was incubated with a bead, on which the anti-estradiol receptor monoclonal antibody was fixed, at 4℃ for 18 hours. Each bead was washed with distilled water and incubated with 200 μl of the peroxidase-conjugated anti-estradiol receptor monoclonal antibody at 37℃ for one hour. Each bead was washed again with distilled water and incubated with 300 μl of orthophenylanediamine solution at room temperature for 30 min. The reaction was stopped by adding 1 ml of 1 N HCl, and the absorbance was measured at 492 nm by a spectrophotometer. The level of estradiol receptors in each sample was calculated using the standard curve obtained from the standard estradiol receptor solution.

Pretreatment of peripheral blood mononuclear cells with Syô-saiko-tô : Peripheral blood mononuclear cells from the normal controls were suspended in Eagle minimum essential medium (MEM) containing 10% fetal calf serum to make a cell suspension of 5×10^6 cells/ml. Each mononuclear cell suspension was incubated with 10 to 200 μg/ml of Syô-saiko-tô at 37℃ for 24 hours. Syô-saiko-tô was obtained from Tsumura Juntendo Inc. (Tokyo) and prepared as previously reported.[9] For comparison, the mononuclear cell suspension was incubated without Syô-saiko-tô. After incubation, each suspension was washed with TEMG buffer and suspended in the same medium to make a cell suspension of 2×10^7 cells/ml. The level of cytosol estradiol receptors was measured as described before.

Statistical analysis : All results were expressed as mean±S.D. Statistical analysis was done by Student's *t*-test.

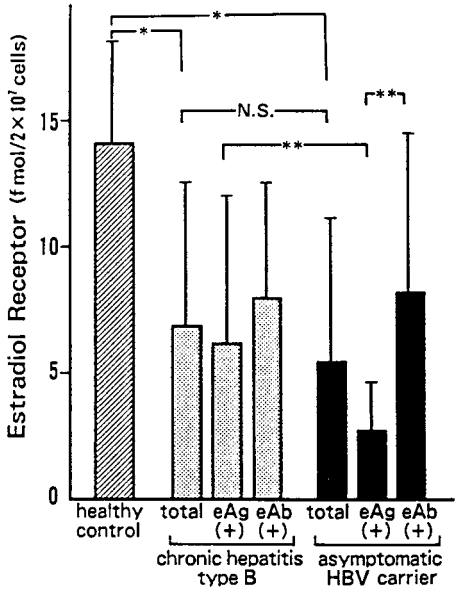

Fig. 1 Level of estradiol receptors in the cytosol of peripheral blood mononuclear cells from asymptomatic hepatitis B virus carriers and patients with chronic hepatitis B.
*$p<0.01$, **$p<0.05$.

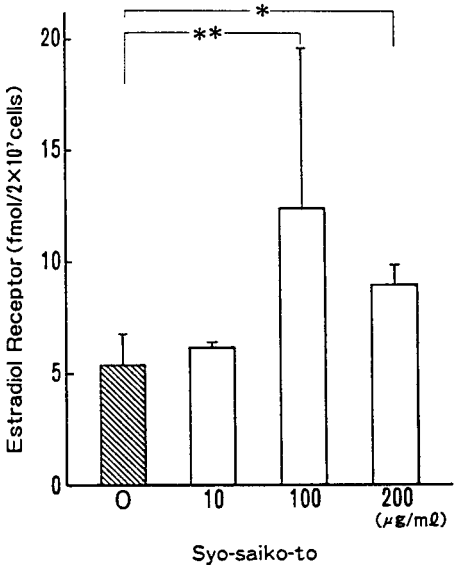

Fig. 2 Effects of Syô-saiko-tô on estradiol receptors in the cytosol of peripheral blood mononuclear cells (n=10).
*$p<0.05$, **$p<0.01$.

Results

Level of estradiol receptors in the cytosol of peripheral blood mononuclear cells

As shown in Fig. 1, the level of cytosol estradiol receptors per 2×10^7 of peripheral blood mononuclear cells was 14.14 ± 4.04 fmol in the normal controls. However, it was 5.53 ± 5.62 fmol in the asymptomatic HBV carriers and 6.87 ± 5.76 fmol in the patients with chronic hepatitis B and significantly lower than that of the normal controls ($p<0.01$). Among the asymptomatic HBV carriers, the level was 8.26 ± 6.29 fmol in the HBe antibody-positive patients, but it was significantly lower in the HBe antigen-positive patients at 2.80 ± 1.89 fmol ($p<0.05$). However, among the patients with chronic hepatitis B, there was no significant difference between the HBe antigen-positive patients and HBe antibody-positive patients.

Effects of Syô-saiko-tô on estradiol receptors in the cytosol of peripheral blood mononuclear cells

As shown in Fig. 2, when peripheral blood mononuclear cells from the normal controls were incubated without Syô-saiko-tô, the level of cytosol estradiol receptors was 5.37 ± 1.30 fmol. However, when the mononuclear cells were pretreated with 10, 100 and 200 μg/ml of Syô-saiko-tô, the levels significantly increased to 6.08 ± 0.16, 12.36 ± 7.32 and 9.02 ± 0.82 fmol, respectively.

Discussion

Most asymptomatic HBV carriers are infected during the perinatal and infantile periods, and they are thought to be immunologically tolerant to HBV. It has been reported that about half of the patients with chronic hepatitis B show a response, while none of asymptomatic HBV carriers are responsive to the lymphocyte transformation test using HBs antigen, suggesting that asymptomatic HBV carriers completely lack sensitized lymphocytes against HBV.[10] Sex hormones are known to affect various immune mechanisms including the immune responses and virus

elimination. In HBV carriers, there are more male HBe antigen-positive patients, while seroconversion is more frequently seen in females, especially at puberty or sexually-mature periods.[5] We have previously shown *in vitro* that antibody production is not induced by estrogen in asymptomatic HBV carriers,[11] and that this may be due to the low estradiol binding capacity of peripheral blood mononuclear cells.[12] This reduced estradiol binding capacity is recovered by pretreating the mononuclear cells with interleukin 1 and interleukin 2.[13]

In this study, we investigated the level of estradiol receptors in the cytosol of peripheral blood mononuclear cells in asymptomatic HBV carriers and patients with chronic hepatitis B. Since Syô-saiko-tô is recently being used in the treatment of chronic hepatitis B, the effects of Syô-saiko-tô on cytosol estradiol receptors were also studied.[14] As a result, the level of cytosol estradiol receptors was significantly lower in asymptomatic HBV carriers and patients with chronic hepatitis B compared to normal controls. In addition, Syô-saiko-tô increased the level of estradiol receptors.

Although the mechanism of sex steroid hormones in the cells is not clear, the following steps are known : Estrogen enters the target cells through the cell membrane, binds with receptors in the cytoplasm and activates them. It then enters the nucleus, binds with acceptors and shows its effects.[15] Furthermore, it has recently been reported that inhibitors are present in the cytoplasm along with the receptors, and that these inhibitors allosterically inhibit the binding of estrogen with receptors.[15,16] There have also been reports of a molecule that becomes an inhibitor at each stage, when estrogen binds with the receptors, when it enters the nucleus and when it binds with the acceptors.[15,17]

We have reported that one effect of estrogen on the immune system is that it adjusts antibody production in normal controls. This effect of estrogen on the immune system is thought to be shown by following the steps described previously. Therefore, the low level of estradiol receptors in the cytosol of peripheral blood mononuclrear cells in asymptomatic HBV carriers which we found in the present study may be the cause of the reduced immunoenhancing effects of female hormones in asymptomatic HBV carriers. The fact that HBe antigen-positive asymptomatic HBV carriers, who completely lack the immune response to the virus, had a lower level of cytosol estradiol receptors than patients with chronic hepatitis B, who have the immune response to the virus, indicated that the decrease in the immunoenhancing effects of female hormones has some kind of effect in the impairment of virus elimination in asymptomatic HBV carriers. Furthermore, we found that Syô-saiko-tô increased the level of cytosol estradiol receptors. This suggested that Syô-saiko-tô not only enhances the immune responses but also induces the immunoenhancing effects of female hormones. In future, further studies should be made to elucidate how HBV infection reduces the level of estradiol receptors in the cytosol of peripheral blood mononuclear cells and how Syô-saiko-tô increases the level of estradiol receptors.

和文抄録

女性ホルモンは多くの場合，免疫系に対して促進的に作用することが多くの研究で示されており，無症候性B型肝炎ウィルスキャリア患者ではウィルスに対し寛容状態にあるが，思春期から性成熟期の女性に seroconversion が比較的高頻度に認められることも，女性ホルモンの免疫系への作用を示唆する。一方，小柴胡湯は，B型肝炎ウィルスキャリアの seroconversion を促進することが臨床的に確認されている。そこで，B型肝炎ウィルスキャリアの末梢血単核細胞のサイトゾール・エストラジオールレセプターおよび小柴胡湯のレセプターにおよぼす影響を検討した。その結果，無症候性B型肝炎ウィルスキャリアおよび慢性B型肝炎患者の末梢血単核細胞のサイトゾール・エストラジオールレセプターは健常ヒトに比して著明に減少しており，小柴胡湯はレセプターを増加させることが明らかとなった。

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