Effects of the Japanese herbal medicine ‘Inchinko-to’ (TJ-135) on concanavalin A-induced hepatitis in mice

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ABSTRACT

Inchinko-to (TJ-135) is a herbal medicine consisting of three kinds of crude drugs, and in Japan it is administered mainly to patients with cholestasis. The present study evaluated the effects of TJ-135 on concanavalin A (con A)-induced hepatitis in mice in vivo and con A-induced cytokine production in vitro. When mice were pretreated with oral TJ-135 for 1 week before intravenous con A injection, the activities of serum aspartate aminotransferase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) were significantly decreased 8 h after con A administration (-82%, -96% and -66% respectively). In histological investigations, submassive hepatic necrosis accompanying inflammatory cell infiltration was not observed in mice pretreated with TJ-135. Serum levels of interleukin-12 (IL-12), interferon-γ (IFN-γ) and IL-2 were significantly lower in mice pretreated with TJ-135 compared with controls, while IL-10 levels were higher in these mice. Intrasplenic IL-12 levels were significantly lower in mice pretreated with TJ-135, while intrasplenic IL-10 levels were higher in these mice.

In vitro, IL-10 production by splenocytes was increased by the addition of TJ-135 to the culture medium, whereas the production of IL-12 and IFN-γ was inhibited. These results suggest that con A-induced hepatitis was ameliorated by pretreatment with TJ-135. With regard to the mechanism of these effects of TJ-135, we speculate that TJ-135 inhibits the production of inflammatory cytokine and enhances the production of anti-inflammatory cytokines. Therefore administration of TJ-135 may be useful in patients with severe acute hepatitis accompanying cholestasis or in those with autoimmune hepatitis.

INTRODUCTION

Treatment methods using herbal medicines were established in China in the 1st century B.C., and were introduced into Japan in the 6th century A.D. Thereafter these treatment methods were individually improved in Japan. Since the use of herbal medicines was approved by the Japanese Ministry of Health and Welfare approximately 20 years ago, herbal medicines have been used by physicians practising Western medicine. Therefore the usefulness of herbal medicine is gradually being supported in clinical settings. Of these herbal medicines,
Sho-saiko-to (TJ-9) has been most frequently used; long-term administration of this medicine has been used to treat approx. one million patients with chronic viral liver diseases, in order to improve liver dysfunction and to prolong life expectancy [1–5].

TJ-9 consists of seven crude drugs (bupleurum root, pinellia tuber, scutellaris root, jujube fruit, ginger rhizome, ginseng root and glycyrrhiza root). Many basic and experimental studies on the effects of TJ-9 have been reported [1–19]. Oka et al. [5] followed 260 patients with hepatic cirrhosis for 5 years and demonstrated that the development of liver cancer was significantly prevented in patients with non-B hepatic cirrhosis after administration of TJ-9, and TJ-9 treatment significantly prolonged the life expectancy of these patients. We reported previously that TJ-9 is useful in the treatment of chronic viral liver diseases, as it appears to facilitate the gradual recovery of a deteriorated biological defence mechanism [14].

Since ancient times, Inchinko-to (TJ-135; Yin-Chen-Hao-Tang in Chinese) has been recognized as another herbal medicine that is effective in treating liver diseases. At present, TJ-135 is used primarily to treat patients with cholestasis in China. In Japan, however, TJ-135 is sometimes used to treat patients with primary biliary cirrhosis [20] or hepatitis C [21]. The usefulness of TJ-135 has not yet been internationally acknowledged.

TJ-135 is a hot-water extract of three herbs, namely Inchinko (Artemisiae capillariae spica), San-shishi (Gardeniae fructus) and Daio (Rhei rhizoma). The daily dose of TJ-135 (dried extract) is usually 1.5 g, and the medicine is made from 4 g of Inchinko, 3 g of San-shishi and 1 g of Daio. Although basic and experimental studies with TJ-135 have not yet been performed to the same extent as those for TJ-9, it was demonstrated previously in an animal model that Inchinko [22] and San-shishi [23] promote bile secretion. In our previous evaluations in vitro using human peripheral blood mononuclear cells, we reported that TJ-135 inhibited concanavalin A (con A)-induced interferon-γ (IFN-γ) production [24,25]. In addition, it was also reported that TJ-135 inhibits liver cell apoptosis induced by transforming growth factor β1 [26] or anti-Fas antibody [27]. Therefore, if TJ-135 inhibits liver cell apoptosis and controls IFN-γ production in the human body as well as improving cholestasis, it may be useful for treating severe acute hepatitis accompanying prolonged jaundice or autoimmune hepatitis.

Carbon tetrachloride has been used to induce acute liver failure in mice, to provide an experimental hepatopathy model [28,29]. In addition, d-galactosamine/lipopolysaccharide (LPS) [30–34] or Propionibacterium acnes/LPS [35–37] have been used frequently to induce acute liver failure experimentally. Tumour necrosis factor α (TNF-α), superoxide and platelet-activating factor play important roles in d-galactosamine/LPS-induced liver injury. On the other hand, cytokines such as IFN-γ, interleukin-12 (IL-12) and IL-18 play an important role in P. acnes/LPS-induced liver injury. In recent years, con A has been used frequently to induce hepatitis in hepatopathy models [38–52]. Since the development of hepatopathy is prevented by pretreatment with immunosuppressants such as dexamethasone, FK506 and cyclosporin A, the aetiology of hepatopathy in this animal model is similar to that of human autoimmune hepatitis via helper T cells [38]. In subsequent evaluations focusing on cytokine kinetics, the development of experimental hepatopathy in this animal model was prevented by pretreatment with anti-TNF-α or anti-IFN-γ monoclonal antibodies [38,42,43]. These studies demonstrated that both of these cytokines are essential for the development of hepatopathy in this animal model. Moreover, hepatopathy was exacerbated by pretreatment with anti-IL-10 monoclonal antibody, while the development of hepatopathy was prevented by pretreatment with recombinant IL-10. Therefore it was suggested that IL-10 is useful for treating hepatopathy [44].

In the present study, we evaluated the immunological influence of TJ-135 on con A-induced hepatitis in mice both in vivo and in vitro.

METHODS

Mice

Male ICR mice (5 weeks old) were purchased from Charles River Japan Inc. (Tokyo, Japan) and acclimatized for 1 week before use in experiments. All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

Reagents

Con A and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and Tween 80 was from Tokyo Kasei Co. (Tokyo, Japan). Extract of TJ-135 was kindly provided by Tsumura Co. (Tokyo, Japan). Activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were measured using kits for measurement of enzyme activities (Wako Pure Chemical Industries Ltd, Osaka, Japan) and an automatic analyser, model 7070 (Hitachi Ltd, Tokyo, Japan). Various cytokine levels in sera or supernatants of culture media were determined using kits for cytokine determination, manufactured by Biosource International Inc. (Camarillo, CA, U.S.A.).

Protocol of in vivo experiments

Mice were divided into six groups (Table 1). Once a day for 1 week, TJ-135 (0.5, 1.0 or 2.0 g/kg) or dexamethasone (20 µg/kg), diluted in 1% Tween 80 solution, was
Effects of TJ-135 on concanavalin A-induced hepatitis

Table 1  The six treatment groups for examining the effects of TJ-135 on con A-induced hepatitis in mice in vivo

<table>
<thead>
<tr>
<th>Group</th>
<th>Oral administration for 7 days</th>
<th>Intravenous administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1% Tween 80 solution</td>
<td>PBS</td>
</tr>
<tr>
<td>II</td>
<td>1% Tween 80 solution</td>
<td>Con A</td>
</tr>
<tr>
<td>III</td>
<td>TJ-135 (0.5 g/kg)</td>
<td>Con A</td>
</tr>
<tr>
<td>IV</td>
<td>TJ-135 (1 g/kg)</td>
<td>Con A</td>
</tr>
<tr>
<td>V</td>
<td>TJ-135 (2 g/kg)</td>
<td>Con A</td>
</tr>
<tr>
<td>VI</td>
<td>Dexamethasone (20 μg/kg)</td>
<td>Con A</td>
</tr>
</tbody>
</table>

Drugs diluted with 1% Tween 80 were administered orally once daily for 7 days. Con A (14 mg/kg) dissolved in PBS was injected intravenously 1 h after the last drug administration. Mice were killed at 2 or 8 h after PBS or con A injection.

Pathological evaluation

Livers were fixed with 10% formalin, then sectioned after paraffin embedding. Liver sections were stained with haematoxylin/eosin and observed under a microscope. In order to obtain an objective assessment of the degree of liver cell necrosis and inflammatory cell infiltration in the left and right lobes of the liver, the identity of the group was concealed.

Spleen homogenates

A buffer containing 10 mM Tris/HCl (Sigma), 1 mM EDTA, 2 μg/ml aprotinin, 2 μg/ml leupeptin and 1 μg/ml pepstatin A (Sigma) was prepared. The spleen was promptly suspended in 2 ml of this Tris/HCl buffer solution and homogenized for 15 s using a physcotron-type homogenizer (Nihon Medical Supply Co., Funabashi, Japan) on ice, and then centrifuged for 30 min (105000 g, 4 °C). The supernatant was stored at −80 °C until analysis.

Evaluation in vitro using splenocytes

For cell culture, RPMI 1640 medium containing 2 mM glutamine was purchased from Gibco Laboratories (Grand Island, NY, U.S.A.). The culture medium was prepared by supplementing the RPMI medium with penicillin (50 units/ml), streptomycin (50 μg/ml) (both from Flow Laboratories, Irvine, Scotland, U.K.), 2-mercaptoethanol (50 μM) (Sigma) and heat-inactivated fetal bovine serum (10%, v/v) (Gibco Laboratories). Extract of TJ-135 was dissolved in RPMI medium by stirring at 37 °C for 2 h, and then centrifuged twice (900 g for 10 min) to remove the precipitate. Subsequently the supernatant of extract of TJ-135 was sterilized by filtering twice through a filter unit (pore sizes: 1, 0.45 μm; 2, 0.22 μm) (Millipore Products Division, Bedford, MA, U.S.A.) before dilution with the culture medium.

Mice were killed by dislocation of the cervical vertebrae, and the spleen was removed and suspended in culture medium as above. Subsequently the cell fraction...
was obtained by mashing the spleen with a stainless steel mesh. After centrifugation (100 g for 5 min), the erythrocyte fraction was removed by inducing haemolysis after suspending the cell fraction in NH$_4$Cl/Tris buffer solution (Wako Pure Chemical Industries). The remaining splenocyte fraction was washed three times with culture medium, then resuspended at a concentration of $1 \times 10^7$ cells/ml. On the basis of the reagent added, splenocytes were divided into seven groups (A–G). Thus 0.1 ml of splenocyte suspension was injected initially into the wells of a 96-well culture plate (Becton Dickinson Labware, Lincoln Park, NJ, U.S.A.), then 100 µl of the culture medium was added to group A, 100 µl of TJ-135 (final concentration 200 µg/ml) was added to group B and 80 µl of con A (final concentration 5 µg/ml) was added to groups C–G. Then splenocytes of all groups were cultured at 37 °C in an incubator filled with 5% CO$_2$. After 1 h, 20 µl of culture medium was added to group C, while 20 µl of the various concentrations of TJ-135 (final concentrations 12.5, 50 and 200 µg/ml) was added to groups D, E and F respectively. In addition, 20 µl of dexamethasone (final concentration 1 ng/ml) was added to group G. Then all splenocytes were cultured for another 47 h. After culturing, all splenocytes were centrifuged (2500 g for 10 min) to obtain the supernatant, which was stored at –80 °C until analysis. Before starting the experiment, TJ-135 (final concentration 200 µg/ml) was added to the splenocyte fraction followed by culture for 7 days; cell viability as evaluated by Trypan Blue staining was > 90%.

**Statistical analysis**

Statistical analysis was performed using StatView software. All values are expressed as means ± S.E.M. Data from haematological and biochemical examinations, and blood and intrasplenic cytokine levels, were tested by one-way ANOVA. Subsequently the results were compared between group II and other groups using Fisher’s protected least significant difference test. For *in vitro* experiments, results for groups A and B were compared using Student’s *t* test, and those for groups C–G were compared using one-way ANOVA. Subsequently the results of *in vitro* experiments were compared between group C and groups D–G using Fisher’s protected LSD test. Moreover, correlations between the respective results were evaluated using Pearson’s correlation coefficient. A level of significance of < 5% was used.

**RESULTS**

**Biochemical measurements**

Biochemical measurements were performed using sera obtained from mice 8 h after con A administration. As shown in Figure 1, AST, ALT and LDH levels in mice from group I and groups III–VI were significantly lower than those in mice from group II.

**Histological examinations**

Inflammatory cell infiltration and liver cell necrosis were not observed in group I mice (results not shown).
Effects of TJ-135 on concanavalin A-induced hepatitis

Figure 3  Serum cytokine levels 2 h after con A injection
PBS was injected intravenously into ICR mice in group I, and con A solution was injected into mice in groups II–VI (see Table 1). Once a day for 1 week before injecting the above reagents, 1% Tween 80 was administered orally to groups I and II, various concentrations of TJ-135 (0.5, 1 or 2 g/kg) were administered orally to groups III–V, and dexamethasone solution was administered orally to group VI. Serum IL-2 levels were significantly lower in groups I, III, V and VI than in group II (**P < 0.01, *P < 0.05). Serum IL-4 levels in group I were significantly lower than those in group II, whereas those in group IV were significantly higher than those in group II. Serum IL-10 levels were higher in the three groups pretreated with TJ-135 than in group II. Serum TNF-α levels in group I were significantly lower than those in group II, while those in groups III–V (pretreated with TJ-135) were lower than those in group II.

However, sub-massive hepatic necrosis accompanying inflammatory cell infiltration was observed in group II (treated with con A) (Figure 2, left panel). TJ-135 (1 or 2 g/kg) or dexamethasone was administered to mice in groups IV, V and VI before con A administration. Normal histological findings were observed in mice from these groups, as observed in group I (Figure 2, right panel).

Figure 4  Serum cytokine levels 8 h after con A injection
PBS was injected intravenously into ICR mice in group I, and con A solution was injected into mice in groups II–VI (see Table 1). Once a day for 1 week before injecting the above reagents, 1% Tween 80 was administered orally to groups I and II, various concentrations of TJ-135 (0.5, 1 or 2 g/kg) were administered orally to groups III–V, and dexamethasone solution was administered orally to group VI. Serum IL-12 levels were significantly lower in groups I, V and VI than in group II (**P < 0.01). Serum IFN-γ levels were significantly lower in group I and groups III–VI than in group II (**P < 0.01, *P < 0.05). Values represent means ± S.E.M.

Serum levels of various cytokines
Serum levels of IL-2, IL-4, IL-10 and TNF-α were measured 2 h after con A administration. As shown in Figure 3, serum IL-2 levels were significantly lower in mice from groups I, III, V and VI than in those from group II. Serum IL-4 levels in group I were significantly lower than those in group II, whereas those in group IV were significantly higher than those in group II. Serum IL-10 levels were higher in the three groups pretreated with TJ-135 than in group II. Serum TNF-α levels in group I were significantly lower than those in group II, while those in groups III–V (pretreated with TJ-135) were lower than those in group II.

Serum levels of IL-12 and IFN-γ were measured 8 h after con A administration. As shown in Figure 4, serum IL-12 levels were significantly lower in mice from groups I, V and VI than in those from group II. Serum IFN-γ levels in group I and groups III–VI were significantly lower than those in group II.

Correlations among the results from sera obtained 8 h after con A administration were evaluated. Positive
PBS was injected intravenously into ICR mice in group I, and con A solution was injected into ICR mice in groups II–VI (see Table 1). Once a day for 1 week before injecting the above reagents, 1% Tween 80 was administered orally to groups I and II, various concentration of TJ-135 (0.5, 1 or 2 g/kg) were administered orally to groups III–V, and dexamethasone solution was administered orally to group VI. IL-2 levels were significantly lower in group I than in group II (***P < 0.01). IL-10 levels were higher in groups III–V than in group II. IL-12 levels were significantly lower in groups I and IV than in group II (***P < 0.01). Values represent means ± S.E.M.

Figure 5 Cytokine levels in homogenized spleen 2 h after con A injection

PBS was injected intravenously into ICR mice in group I, and con A solution was injected into ICR mice in groups II–VI (see Table 1). Once a day for 1 week before injecting the above reagents, 1% Tween 80 was administered orally to groups I and II, various concentration of TJ-135 (0.5, 1 or 2 g/kg) were administered orally to groups III–V, and dexamethasone solution was administered orally to group VI. IL-2 levels were significantly lower in group I than in group II (***P < 0.01). IL-10 levels were higher in groups III–V than in group II. IL-12 levels were significantly lower in groups I and IV than in group II (***P < 0.01). Values represent means ± S.E.M.

Correlations were observed between serum AST activity and serum IL-12 levels (n = 78, r = 0.262), between serum AST activity and serum IFN-γ levels (r = 0.252), between serum ALT activity and serum IL-12 levels (r = 0.281), and between serum ALT activity and serum IFN-γ levels (r = 0.260) (all P < 0.05). Positive correlations were also observed between serum IL-12 levels and serum IFN-γ levels (r = 0.547, P < 0.0001). However, significant correlations were not observed for other data.

Cytokine levels in homogenized spleen

There was a significant difference in IL-2 levels in the spleen between groups I and II (Figure 5). However, there were no significant differences in IL-2 levels between group II and groups III–VI. IL-4 levels were significantly lower in group I than in group II, but were slightly lower in groups III–V than in group II. IL-10 levels were higher in groups III–V (pretreated with TJ-135) than in group II. IL-12 levels were lower in group I and groups III–VI than in group II, and there were significant differences between group II and groups I and IV. There were no significant differences in IFN-γ levels between the various groups (results not shown). In addition, TNF-α levels were under the measurable limit (10 pg/ml) in all groups.

When correlations among the respective results from sera and spleen obtained 2 h after con A administration were evaluated, there were positive correlations between serum IL-2 levels and intrasplenic IL-2 levels (r = 0.517, P < 0.001) and between serum IL-4 levels and intrasplenic IL-4 levels (r = 0.774, P < 0.0001).

Evaluations in vitro

Levels of IL-2, IL-4, IL-10, IL-12, IFN-γ and TNF-α were determined in the supernatant of the splenocyte culture medium. When levels of each cytokine were compared between groups A and B (splenocytes were not treated with con A in either group), IL-4 and IL-12 production levels were significantly lower in group B than in group A (Figure 6). In contrast, IL-10 production levels were significantly higher in group B than in group A. However, there were no significant differences in IL-2 and IFN-γ production levels between groups A and B.
Effects of TJ-135 on concanavalin A-induced hepatitis

Figure 7 Effects of TJ-135 on con A-induced cytokine production in vitro using splenocytes from mice

Splenocytes of groups C–G were cultured with con A for 48 h. TJ-135 solution was added to splenocytes of groups D, E and F (final concentrations 12.5, 50 and 200 μg/ml respectively), and dexamethasone was added to splenocytes of group G, 1 h after con A addition. IL-10 production levels were significantly higher in group F than in group C (**P < 0.01). IL-12 production levels were significantly lower in groups F and G than in group C (*P < 0.05). IFN-γ levels were significantly lower in groups F and G than in group C (**P < 0.01). Values represent means ± S.E.M.

DISCUSSION

Con A-induced hepatitis is an animal model of autoimmune active hepatitis that was developed by Tiegs et al. [38]. When con A is administered to mice, serum aminotransferase activity increases markedly within 8 h of the injection. Macrophage activation and subsequent T-lymphocyte activation are considered to play an important role during the development of con A-induced hepatitis. When mice are pretreated with dexamethasone, FK506 or cyclosporin A, the increase in serum ALT activity is completely inhibited. In the present study, the serum activities of AST, ALT and LDH were all significantly lower in mice pretreated with TJ-135 for 1 week before con A administration. TJ-135 also improved the histological appearance of the liver, and this effect was almost identical to that of dexamethasone, a control drug (results not shown). These results suggest that TJ-135 has anti-inflammatory action equivalent to that of previously characterized immunosuppressants.

TJ-135 consists of three crude drugs. Although several chemical components contained in these crude drugs have been analysed, this analysis is not yet complete. However, various pharmacological actions have been evaluated in animal models. *Artemisiae capillari spica* contains capillarin, capillin, capillen, capillarisin and scoparone. It has been reported that: (1) capillin, capillarisin and scoparone promote bile secretion [53]; (2) capillarisin relaxes the smooth muscle of the gall bladder and the narrow distal segment of the bile duct [54]; (3) a water extract of *Artemisiae capillari spica* inhibits β-glucuronidase activity (β-glucuronidase increases blood bilirubin levels) [55]; (4) capillarisin and scoparone alleviate experimental acute liver failure [56]; (5) a water extract of *Artemisiae capillari spica* inhibits β-glucuronidase activity (β-glucuronidase increases blood bilirubin levels) [55]; (6) capillarisin and scoparone promote bile secretion [23] and induce diarrhoea [61], and that genipin, geniposide and crocetin improve lipid metabolism [62,63]. Yamamoto et al. [27] found that liver cell apoptosis induced by anti-Fas antibody was inhibited by genipin. *Rhei rhizoma* contains sennosides A–F, rhatannin, rhein and lindleyin. It has been reported that

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sennosides A–F induce diarrhoea [64], rhatannin improves renal function [65], rhein exhibits anti-bacterial action [66], and lindleyin exhibits anti-inflammatory and analgesic actions [67].

The doses of TJ-135 used in the in vivo experiment in mice were 3–13 times higher than the clinical dose when expressed as g/kg. However, when the dose for humans is estimated from that used in animals, body surface area is considered more important than body weight [68]. From this estimation, the doses used in this experiment in mice are very similar to clinical doses used. Therefore the effects of TJ-135 observed in this experiment may be expected to occur in humans.

With regard to the aetiology of con A-induced hepatitis, various studies have been performed focusing on the analysis of cytokine production mechanisms. TNF-α and IFN-γ are important agents that induce inflammation, and IL-10 is also an important factor that controls inflammation [39,42–44]. In a preliminary study in which the time courses of levels of various serum cytokines were evaluated after drawing blood 2, 5, 8 and 12 h after con A administration, TNF-α reached a maximal level 2 h after administration, but decreased to below the measurable limit from 8 h (results not shown). IFN-γ levels continued to increase slowly at all times after con A administration. Serum IL-2, IL-4 and IL-12 reached their maximal levels between 2 and 5 h after con A administration, and then decreased slowly thereafter. However, serum IL-10 remained at a high level up to 12 h later. These results are almost identical with those reported by Louis et al. [44].

We evaluated the serum levels of IL-2, IL-4, IL-10 and TNF-α 2 h after con A administration, as well as serum IL-12 and IFN-γ levels after 8 h. After con A administration, serum levels of cytokines other than IL-10 were significantly increased. When the results were compared between the various groups treated with con A, serum IL-2 and TNF-α levels were lower in groups pretreated with TJ-135, and there were significant differences in serum IL-2 levels between these groups. Decreases in IL-2 and TNF-α levels were more marked in the group pretreated with dexamethasone. Serum IL-12 and IFN-γ levels were also significantly lower in groups pretreated with TJ-135, and these decreases were identical with those in the group pretreated with dexamethasone. In contrast, serum IL-10 levels were higher in groups pretreated with TJ-135. Serum IL-4 levels were significantly higher only in the group pretreated with 1 g/kg TJ-135. There were no changes in serum IL-4 or IL-10 levels in mice pretreated with dexamethasone. These results suggest that excessive production of IL-2, IL-12 and IFN-γ may be controlled by pretreatment with TJ-135.

Similar to the effects of pretreatment with dexamethasone, inflammatory reactions induced by T cells may be inhibited by pretreatment with TJ-135. Dexamethasone directly inhibits the inflammatory activity of the macrophages. However, TJ-135 inhibits the inflammatory reaction in a manner different from that of dexamethasone. For example, TJ-135 inhibits the inflammatory reaction by inducing IL-10, an anti-inflammatory cytokine. In addition, positive correlations were observed between serum levels of AST and IL-12, AST and IFN-γ, ALT and IL-12, and ALT and IFN-γ. Moreover, there was a strong positive correlation between IL-12 and IFN-γ levels. Therefore it was confirmed that both IL-12 and IFN-γ are important cytokines in the induction of inflammation in con A-induced hepatitis.

In humans, most immunological roles of the spleen remain unclear. In mice, however, splenocytes have been reported to play an important role in immunological defence reactions [69–72]. The present study using splenocytes has provided important information. In the present study, the spleen was removed from mice 2 h after con A administration, and homogenized in order to determine the cytokine levels in the supernatant. Levels of IL-2, IL-4 and IL-12 were markedly increased after con A administration. However, pretreatment with TJ-135 did not influence IL-2 levels. IL-4 levels were increased by pretreatment with TJ-135, and IL-4 levels in the group pretreated with 0.5 g/kg TJ-135 were significantly higher than in the controls. Increases in IL-12 levels were inhibited by pretreatment with TJ-135, and IL-12 levels in the group given 1.0 g/kg TJ-135 were significantly lower than in the controls. IL-10 levels were higher in groups pretreated with TJ-135. However, the levels of cytokines were not markedly influenced by pretreatment with dexamethasone. Thus intrasplenic and blood levels of each cytokine were not identical. However, intrasplenic levels of IL-10 and IL-12 closely reflected serum levels of these cytokines. Moreover, there were positive correlations between intrasplenic and serum levels for both IL-2 and IL-4. Therefore it is suggested that splenocytes play an important role in the inflammatory reaction in mice as immunomodulatory cells.

When measuring blood levels of cytokines, it is necessary to consider both production and consumption, because higher levels may not always represent accelerated production, while lower levels may not always represent decreased production. For example, when large amounts of inflammatory cytokines are produced to control an inflammatory reaction, higher blood levels of these cytokines may not persist for a prolonged period, since they may be instantly or rapidly consumed. Therefore it is difficult to confirm the time at which large amounts of cytokines are produced. Based on the results of the present study in vivo, it was speculated that TJ-135 inhibited the production of inflammatory cytokines (IL-12 and IFN-γ), but promoted the production of IL-10, an anti-inflammatory cytokine, thus ameliorating hepatopathy. Therefore, to confirm the
above hypothesis, we evaluated the action of TJ-135 in vitro using splenocytes. Compared with splenocytes cultured in conventional culture medium alone, levels of production of IL-2, IL-4, IL-12 and IFN-γ were all lower in splenocytes cultured in media supplemented with TJ-135. In contrast, production levels of IL-10 were increased by approx. 120% when splenocytes were treated with TJ-135. Subsequently, when production of each cytokine was induced in splenocytes by treatment with con A, similar results were obtained after supplementing the culture medium with TJ-135. In particular, IL-10 levels were increased by approx. 550% when splenocytes were treated with TJ-135. In contrast, IL-12 and IFN-γ levels were inhibited by more than 70%. Supplementing the culture medium with TJ-135 did not apparently influence IL-4 production by splenocytes. However, it was speculated that TJ-135 is an IL-10 inducer, as well as a regulator of IL-12 and IFN-γ production. Thus the results of in vitro experiments strongly supported the data obtained in vivo.

It has been reported that con A-induced hepatitis was suppressed by pretreatment with anti-IFN-γ monoclonal antibodies [43,51] and in IFN-γ knockout mice [46]. Thus it was concluded that con A-induced hepatitis is a Th1-mediated immunorespense. Recently, however, Nishikage et al. [52] reported that LPS suppressed con A-induced hepatitis by inhibiting IL-4 synthesis, and that both Th2 and Th1 were involved in this model. In our studies, TJ-135 inhibited IFN-γ and IL-12 production, and increased IL-4 levels in serum and spleen. Thus it is considered that TJ-135 suppresses con A-induced hepatitis mainly by the inhibition of the Th1 system.

IFN-γ-induced apoptosis has recently been reported as a factor in the aetiology of liver cell apoptosis that occurs in con A-induced hepatitis [44,50,72]. Several theories exist to account for liver cell apoptosis, such as apoptosis via the Fas/Fas-ligand system or via perforin/granzyme. In the present study, TJ-135 inhibited the development of experimental hepatopathy. As one of its actions, TJ-135 may inhibit IFN-γ production, thus inhibiting cell death. We also speculate that chemical components contained in TJ-135 (genipin) directly influence the Fas/Fas-ligand system and inhibit cell death [27].

Although TJ-135 consists of three crude drugs, it remains unclear which of these are responsible for amelioration of con A-induced hepatitis in mice. In previous studies using human peripheral lymphocytes, Artemisiae capillari spica and Rhei rhizoma induced IL-10 production and decreased IFN-γ production (M. Yamashiki, unpublished work). These crude drugs probably control the inflammatory reaction in mice. Further studies are required in order to determine which chemical components are responsible for the pharmacological effects of TJ-135.

Herbal medicine is characterized by limited side effects. However, although rare, TJ-9 has been reported to induce interstitial pneumonia [73]. Therefore attention should also be paid to the effects of TJ-135 administration. In China, TJ-135 is frequently used as a primary drug to treat cholestasis. In Japan, TJ-135 is used to treat patients with primary biliary cirrhosis. In the future, the application of TJ-135 may be extended to patients with autoimmune hepatitis, acute hepatitis accompanying prolonged jaundice or severe acute hepatitis. At present, however, long-term administration of TJ-135 should be avoided in cirrhotic patients at risk of complication by hepatoma, because TJ-135 inhibits not only the apoptosis of liver cells in mice in vivo, but also the apoptosis of human hepatocellular carcinoma cell lines [26,27].

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REFERENCES

7 Fujiiwara, K. and Ogihara, Y. (1986) Pharmacological effect of oral saikosaponin may differ depending on conditions of the gastrointestinal tract. Life Sci. 4, 297–301


Yano, H., Mizoguchi, A., Fukuda, K. et al. (1994) The herbal medicine Sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at the G0/G1 phase. Cancer Res. 54, 448–454


Matsui, K., Yoshimoto, T., Tsutsumi, H. et al. (1997) Propionibacterium acnes treatment diminishes CD4+ NK1.1+ T cells but induces Type 1 T cells in the liver by induction of IL-12 and IL-18 production from Kupffer cells. J. Immunol. 159, 97–106


Nishikage, T., Seki, S., Tovabe, S. et al. (1999) Inhibition of concanavalin A-induced hepatic injury of mice by bacterial lipopolysaccharide via the induction of IL-6 and the subsequent reduction of IL-4: the cytokine milieu of concanavalin A hepatitis. J. Hepatol. 31, 18–26


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