JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 681–688
© 2001 The Authors
Received May 17, 2000
Accepted November 28, 2000
ISSN 0022-3573

Ruscogenin glycoside (Lm-3) isolated from *Liriope muscari* improves liver injury by dysfunctioning liver-infiltrating lymphocytes

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Abstract

The effects of ruscogenin 1-O-[β-D-glucopyranosyl(1 $\rightarrow$ 2)] [β-D-xylopyranosyl(1 $\rightarrow$ 3)]-β-D-fucopyranoside (Lm-3) and its aglycone, ruscogenin, on liver injury induced in mice by delayed-type hypersensitivity to picryl chloride have been investigated. Lm-3 and ruscogenin significantly decreased liver injury when given during the effector phase of the delayed-type hypersensitivity reaction. The pretreatment of nonparenchymal cells, but not hepatocytes, with Lm-3 or ruscogenin in-vitro caused a concentration- and time-dependent inhibition against the damage. Lm-3 showed a stronger inhibition against the damage than ruscogenin (IC50: Lm-3 6.3×10^{-10} M, ruscogenin 3.9×10^{-7} M). However, neither Lm-3 nor ruscogenin blocked the hepatotoxic potential of CCl_4, when used to pretreat hepatocytes. Moreover, Lm-3 and ruscogenin inhibited concanavalin A-induced lymphocyte proliferation only at high concentrations. These results suggested that Lm-3 and ruscogenin improved the immunological liver injury by selectively causing dysfunction of the liver-infiltrating cells rather than by protecting hepatocyte membranes. Such characteristics would be significant for treating immunologically related liver diseases as well as for developing new drugs.

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Funding: This work was supported in part by National Natural Science Fund for Distinguished Young Scholars to Qiang Xu (no. 39925041) and a grant of young outstanding researcher from Fuk Ying Tung Educational Foundation, Hong Kong, awarded to Boyang Yu.

Introduction

It is well known that T cell-mediated immunity plays an important role in the development of a variety of hepatic diseases (Ferrari et al 1987; Milich et al 1990; Lau & Wright 1993; Chisari & Ferrari 1995). Such immune responses usually include class I-restricted $CD8^+$ cytotoxic T lymphocytes (Zinkernagel et al 1986; Barnaba et al 1990; Moriyama et al 1990; Peters et al 1991; Nelson et al 1998), and class II-restricted $CD4^+$ T cells or delayed-type hypersensitivity (DTH) reaction (Lohr et al 1996; Napoli et al 1996). In the pathogenesis of immunologically-induced hepatic damage, liver infiltration by lymphocytes may be one of the important events. For example, liver-infiltrating $CD4^+$ T lymphocytes from the periphery mediated the pathogenesis of hepatitis C virus-induced chronic liver disease through releasing a high level of interferon-γ and tumour necrosis factor-α (Lohr et al 1996). These findings suggested that there might exist an interaction between infiltrated T lymphocytes and liver parenchymal cells. So far, however, efforts to try to interfere with hepatocyte damage have usually been made by protecting hepatocytes from the damage, such as using an in-vitro or in-vivo acute hepatic injury induced by CCl_4 or D-galactosamine. The efficacy of drugs developed

by such assay is usually preventive and passive for defending the damage, and in the clinical treatment for hepatitis there is a frequency of relapse after drug withdrawal. Nevertheless, little has been tried to eliminate or decrease the hepatotoxic causes, especially the immunological attack.

To mimic the cellular immunological pathogenesis of human hepatitis in animals, we reported previously on a novel liver injury model induced in mice by delayed-type hypersensitivity reaction to picryl chloride (PCl-DTH). We demonstrated the usefulness of this model for immunopharmacological evaluation by using various drugs for the treatment of hepatitis (Xu et al 1997). Furthermore, we found evidence for the key role of $CD4^+$ T cells that mainly constituted the liver-infiltrating leucocytes (Xu et al 1993a, 1996, 1998). In an in-vitro co-culture, such CD4-predominant populations purified from liver-infiltrating cells caused significant damage to hepatocytes (Xu et al 1998). This assay was developed for the evaluation of drug efficacy, and by its use we found that astilbin, a flavanoid isolated from the rhizome of *Smilax glabra*, a Chinese herb, significantly improved the liver injury by selectively causing the apoptosis of liver-infiltrating T cells (Xu et al 1999). These investigations have raised the possibility of paving a new way for regulating the pathogenesis of liver damage by dysfunctioning infiltrated lymphocytes rather than by hepatoprotection.

To inhibit the function of T lymphocytes involved in immune responses, immunosuppressors such as glucocorticoids and cytotoxic agents are usually used in various immune disorders including hepatitis. However, these agents are known to cause serious side effects such as the inhibition of immune organs, and sometimes even to cause the exacerbation of the disorder. In the treatment of PCl-DTH liver injury by various drugs, we found that the consecutive administration of prednisolone (Xu et al 1997) resulted in more elevated serum transaminase levels and liver necrosis, when compared with control, suggesting a risk in steroid therapy for hepatitis. Whilst searching for novel immunosuppressors, our previous study (Xu et al 1993a) showed that a saponin, ruscogenin 1-O-[β-D-glucopyranosyl($1\rightarrow2$)][β-D-xylopyranosyl($1\rightarrow3$)]-β-D-fucopyranoside (Lm-3), isolated from *Liriope muscari* (Yu et al 1990) could inhibit the delayed-type hypersensitivity reaction occurring in the ear without inhibiting immune organs. In this study, therefore, we have examined the effect of this saponin and its aglycone, ruscogenin, on liver injury, and have attempted to elucidate the mechanisms involved.

Materials and Methods

Animals

The study complied with the current ethical regulations on animal research of this institute and all mice used in the experiment were treated humanely.

Female ICR mice (4–5-weeks old) and male BALB/c mice (8–9-weeks old) were purchased from the Experimental Animal House of China Pharmaceutical University (Nanjing, China) and Shizuoka Laboratory Animal Center, Japan, respectively. All mice were maintained with free access to pellet food and water in plastic cages at 21 ± 2°C and kept on a 12-h light/dark cycle.

Drugs and reagents

Ruscogenin 1-O-[β-D-glucopyranosyl($1\rightarrow2$)][β-D-xylopyranosyl($1\rightarrow3$)]-β-D-fucopyranoside (Lm-3) was isolated from the roots of *Liriope muscar*i (Decn.) Bailey (Fujian, China). Prednisolone and cyclophosphamide were purchased from the Shanghai 9th Pharmaceutical Factory and the 12th Pharmaceutical Factory, respectively (Shanghai, China).

Liver injury induced by PCl-DTH

Liver injury was induced as reported by Xu et al (1993a, 1996, 1998). Briefly, mice were sensitized twice by painting 0.1 mL 1% PCl in ethanol on the abdominal skin, with an interval of five days between paintings. Five days after the second sensitization, livers were injected with either 10 μL 0.2% (for BALB/c mice) or 0.5% PCl (for ICR mice) in olive oil.

Effect of administration of Lm-3 or ruscogenin during the effector phase on the serum levels of transaminases, and on liver and spleen weights in mice with PCl-DTH liver injury

ICR mice were administered Lm-3 (10 or 20 mg kg^{-1}, i.p.), ruscogenin (10 or 20 mg kg^{-1}, i.p.) cyclophosphamide (10 mg kg^{-1}, i.p.) or prednisolone (10 mg kg^{-1}, i.m.) three times (0, 5 and 10 h) after PCl challenge. Eighteen hours later the mice were exsanguinated and the livers removed. The serum was used for determining alanine transaminase (ALT) and aspartate transaminase (AST) activities as the parameters for indicating hepatic damage (Neuschwander-Teri 1995). The liver was used for pathological evaluation. The measurements for serum enzyme activities were made by standard methods using determination kits. Liver tissue sections were stained with haematoxylin–eosin. The histological

changes were read on a scale of 0 to 3 (0, no change; 1, mild; 2, moderate; and 3, severe) and expressed as an average score.

Liver cell preparation

Parenchymal and nonparenchymal cells were isolated from PCl-DTH liver-injured BALB/c mice by a modified two-step perfusion method. In brief, mice were given 40 mg kg^{-1} pentobarbital intraperitoneally. The liver was first perfused via the portal vein with Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (HBSS) supplemented with 0.5 mM EGTA (ethylene glycol-bis-(β-amino ethyl)-N,N′-tetraacetic acid) (Dojindo Chemical Inst. Ltd, Kumamoto, Japan) and 25 mM HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid; Dojindo) (pH 7.4) at 37°C until the blood in the liver was completely removed. The solution was then exchanged with 0.1% collagenase (182 U mg^{-1}; Wako Pure Chemical Industries Ltd, Osaka, Japan) in HBSS (containing 4 mM $CaCl_2.2H_2O$ and 0.8 mM $MgSO_4.7H_2O$). After a few minutes of perfusion, the liver was excised rapidly from the body cavity and dispersed into cold HBSS. The cell suspension generated was filtered through a 100-gauze mesh, and parenchymal hepatocytes were separated from nonparenchymal cells by differential centrifugation at 50 *g* for 2 min. After being washed twice to remove dead cells and debris, the hepatocytes were suspended in William's Medium E (WE medium) containing 2 mM L-glutamine, 10% (v/v) foetal calf serum (Gibco BRL), 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. The supernatant obtained after the first centrifugation of slurry generated by perfusion with collagenase was used for preparation of nonparenchymal cells by centrifuging at 300 *g* for 10 min. The pellets of nonparenchymal cells were washed twice with WE medium. The hepatocytes and nonparenchymal cells were found to be approximately 90% viable as estimated by trypan blue exclusion and generally used immediately for culture.

Cell culture and transaminase-releasing assay

Hepatocytes thus obtained were suspended in WE medium at a density of 1×10^5 cells mL^{-1}. Portions (0.2 mL) were seeded onto 96-well microplates (Falcon) and cultured in a humidified incubator at 37°C with 5% CO_2/air. Three hours later, the hepatocyte monolayers were washed twice with WE medium, and then 8×10^4 nonparenchymal cells suspended in 0.2 mL WE medium were added to the wells. After a further 3 h of culture, the supernatant was collected and used for assaying ALT activity.

To observe the spontaneous ALT release from hepatocytes, the hepatocytes were cultured for 3 h without nonparenchymal cells. To observe the effects of Lm-3 or ruscogenin at various concentrations, nonparenchymal cells were treated respectively with medium (control) and either compound before their addition to the hepatocytes. The IC50 values were evaluated.

Effect of Lm-3 and ruscogenin on CCl_4-induced hepatocyte damage in-vitro

Hepatocytes (2×10^4 in 0.2 mL WE medium) were precultured in a 96-well microplate for 3 h. After two washings, they were pretreated with various concentrations of Lm-3 or ruscogenin for 2 h. After two further washings, the hepatocytes were exposed to 0.15% CCl_4 for 2 h. This was followed by collection of the supernatant to assay ALT.

Spleen cell preparation

Spleen cells were obtained from naive BALB/c mice. Briefly, the spleen was removed sterilely and the cells were dissociated in 5 mL RPMI-1640 medium (Gibco) containing 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin and 10% foetal calf serum (RPMI-1640 medium). The cell suspension was centrifuged at 700 rev min^{-1} for 7 min, and after removing the supernatant, 0.17 M Tris (hydroxymethyl aminomethane)-0.75% NH_4Cl solution was added to remove erythrocytes. After washing twice with RPMI-1640 medium, the cells were found to be approximately 98% viable, as estimated by trypan blue exclusion.

Lymphoproliferative response to mitogen

Splenocytes were first exposed in-vitro to various concentrations of Lm-3 or ruscogenin for 1 h. After two washings the cells were cultured in 96-well plates at a density of 2×10^5 cells/well in RPMI-1640 medium (0.2 mL). After two washings, splenocytes were stimulated with 5 μg mL^{-1} concanavalin A (Con A), which is an optimally mitogenic concentration. The cells were incubated for 96 h at 37°C in 5% CO_2/air. Cell growth was evaluated with modified MTT (3-(4,5-dimethylthiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; Sigma) assay (Sargent & Taylor 1989). Twenty microlitres of 5 mg mL^{-1} MTT in RPMI-1640 medium was added for a further 4-h incubation. After removing the supernatant, 175 μL dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The plate was shaken for 10 min, and then read on a microplate reader at 540 nm. The stimulation index was

Table 1 Effects of Lm-3 and ruscogenin, given during the effector phase, on the serum levels of transaminases and liver and spleen weights in mice with delayed-type hypersensitivity to picryl chloride liver injury.

Group	Dose (mg kg^{-1})	No. of mice	Serum ALT (Karmen unit)	Serum AST (Karmen unit)	Organ/body in weights (mg g^{-1})	
					Liver	Spleen
Normal	–	8	20.3 ± 29.9	348.3 ± 49.1	4.66 ± 0.4	5.15 ± 1.14
Control	–	8	582.1 ± 508.6$^{\#\#}$	711.3 ± 327.0$^{\#\#}$	4.58 ± 0.4	4.57 ± 0.54
Lm-3	10	10	159.6 ± 159.3*	500.2 ± 102.8	4.46 ± 0.37	4.34 ± 1.32
	20	6	151.3 ± 39.4*	533.2 ± 170.2	4.45 ± 0.35	3.91 ± 1.10
Ruscogenin	10	9	273.3 ± 358.9	652.9 ± 257.8	4.56 ± 0.36	4.02 ± 0.90
	20	10	446.8 ± 514.6	780.0 ± 355.9	4.83 ± 0.53	4.67 ± 1.49
Cyclophosphamide	10	8	283.9 ± 59.0*	477.0 ± 61.0*	–	–
Prednisolone	10	9	433.2 ± 324.4	789.3 ± 234.4	6.26 ± 0.7**	2.71 ± 0.72**

Each figure represents the mean ± s.d. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the normal group; $*P < 0.05$, $**P < 0.01$ compared with the control group. –, Not done.

Table 2 Effects of Lm-3 and ruscogenin, given during the effector phase, on the liver histological changes in mice with delayed-type hypersensitivity to picryl chloride liver injury.

Treatment	Dose (mg kg^{-1})	Hepatocyte granular degeneration	Adipose degeneration	Hepatocellular necrosis
Control	–	0	0.75	2.50
Lm-3	10	0.50	0	0.30
	20	0.00	0	0.83
Ruscogenin	10	1.00	0	0.56
	20	1.00	0	0.20
Cyclophosphamide	10	0	0	0
Prednisolone	10	0.60	0	2.30

Liver tissue sections were stained with haematoxylin–eosin. Histological changes were read on a scale of 0 to 3 (0, no change; 1, mild; 2, moderate; and 3, severe) and expressed as an average score.

calculated by using the ratio in absorbance of Con A-treated and non-treated.

Statistics

Data were expressed as mean ± s.d. and statistically evaluated using Student's *t*-test.

Results

Effect of Lm-3 or ruscogenin, given during the effector phase, on the acute liver injury induced by PCl-DTH in ICR mice

A significant elevation in serum ALT and AST levels was observed in the control group compared with normal animals (Table 1). Compared with the control, a significant decrease in ALT and a slight reduction in AST elevation were seen with 10 and 20 mg kg^{-1} Lm-3, respectively. Ruscogenin 10 mg kg^{-1} tended to decrease the ALT level compared with control.

The histological changes are shown in Table 2. Lm-3 and ruscogenin decreased the hepatocelluar necrosis and adipose degeneration compared with control. Cyclophosphamide showed a significant lowering in serum ALT and AST levels and almost normal histological findings. However, prednisolone showed neither a lowering in serum transaminase levels nor provided improvement on the pathological changes, but significantly increased liver weight and decreased spleen weight compared with control (Tables 1 and 2).

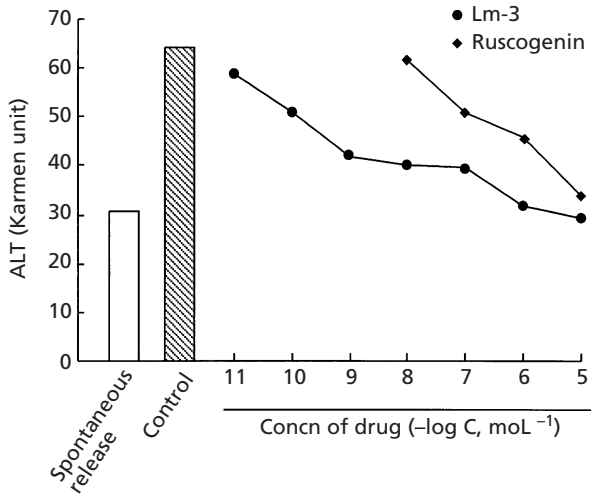

Figure 1 Effects of Lm-3 and ruscogenin on the potential of nonparenchymal cells to cause the release of alanine transaminase (ALT) from hepatocytes in-vitro. Nonparenchymal cells and hepatocytes were isolated from BALB/c mice with liver injury induced by delayed-type hypersensitivity 12 h after the challenge with picryl chloride. Hepatocytes (2×10^4 in 0.2 mL WE medium) were precultured in a 96-well microplate for 5 h. After two washings, they were co-cultured with 8×10^4 nonparenchymal cells at 37°C for a further 3 h, followed by collecting the supernatant and assaying alanine transaminase activity. Drug treatments were performed for nonparenchymal cells at various concentrations for 1 h before co-culture. Each point represents the mean of three experiments and each experiment included triplicate sets. Spontaneous release: spontaneous release of ALT from hepatocytes. Control: ALT release from hepatocytes co-cultured with nonparenchymal cells.

Effect of Lm-3 or ruscogenin on the potential of nonparenchymal cells to release transaminase from hepatocytes

A 3-h co-culture of nonparenchymal cells with hepatocytes resulted in a marked elevation of the release of ALT in the culture supernatant. The treatment of nonparenchymal cells with Lm-3 or ruscogenin at various concentrations, before their addition to the hepatocytes, dose-dependently blocked the release of ALT. The IC50 value was 6.3×10^{-10} M for Lm-3, 619-fold stronger than for ruscogenin (3.9×10^{-7} M; Figure 1).

Figure 2 shows the kinetics for the time-dependent inhibition by Lm-3 and ruscogenin of the hepatotoxic potential of nonparenchymal cells. The incubation with Lm-3 or its aglycone for more than 30 min completely blocked the potential of nonparenchymal cells to release alanine transaminase from hepatocytes. However, no effect was observed on the release when the drug pretreatment was performed for hepatocytes, even at the high dose of 10^{-5} M for more than 1 h (Figure 3).

Effect of Lm-3 or ruscogenin on CCl_4-induced hepatocyte damage in-vitro

Lm-3 or its aglycone at concentrations of 10^{-8}–10^{-5} M had no effect on the CCl_4-induced ALT release from hepatocytes (Figure 4).

Inhibition of the in-vitro lymphoproliferation by Lm-3 or ruscogenin

When spleen cells were pre-treated with various concentrations of Lm-3 or ruscogenin, only the high concentrations (Lm-3 10^{-5}–10^{-4} M, ruscogenin 10^{-4} M) showed an inhibition on Con A-induced lymphoproliferation (Figure 5).

Discussion

Delayed-type hypersensitivity is a typical cellular immune response that can be divided into two stages, induction and effector phases. The induction phase represents the formation of antigen-specific T lymphocytes and the effector phase includes the release of cytokines from effector T cells against the antigen challenge and the consequent inflammatory reaction with an infiltration of monocytes and lymphocytes. This study first examined the improvement in the activity of Lm-3 or ruscogenin on the liver injury induced in mice by delayed-type hypersensitivity to picryl chloride. Compared with the control, Lm-3 showed a significant inhibition and ruscogenin a slight inhibition against elevation in the levels of serum ALT and AST when administered during the effector phase of delayed-type hypersensitivity (Table 1). Histological examination (Table 2) revealed that Lm-3 and ruscogenin decreased the hepatocellular necrosis and adipose degeneration also. Cyclophosphamide significantly lowered serum transaminase levels compared with control and its administration resulted in almost normal histological findings. Prednisolone showed no improvement in the activity on liver injury, and it increased liver weight and decreased spleen weight significantly compared with the control (Table 1). Moreover, histological examination showed that prednisolone did not decrease the hepatocyte necrosis but increased hydropic degeneration (average score 2.4). Previously, we reported (Xu et al 1997b) that the consecutive administration of prednisolone in the induction phase and sustaining process of liver injury conversely caused a more severe liver damage with PCl-DTH-liver injury. Prednisolone-induced liver injury has been used for pharmacological evaluation (Liu et al 1979). We reported also (Xu et al 1993b) that Lm-3

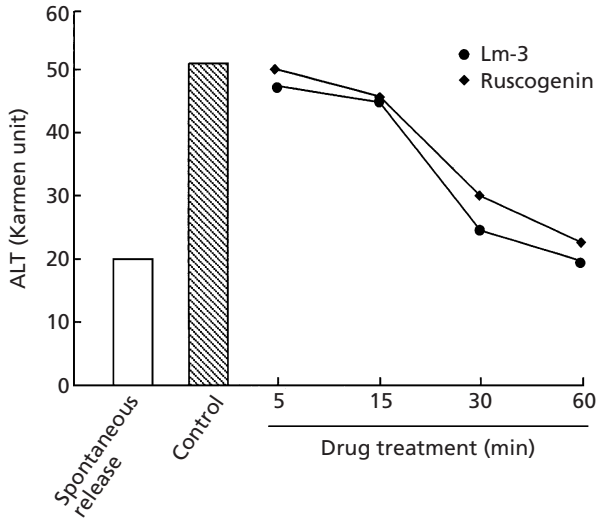

Figure 2 Time-dependent inhibition of the alanine transaminase (ALT) release from hepatocytes by the treatment of nonparenchymal cells with 10^{-6} M Lm-3 or ruscogenin for 5, 15, 30 and 60 min, incubated at 37°C. Spontaneous release: spontaneous release of ALT from hepatocytes. Control: ALT release from hepatocytes co-cultured with nonparenchymal cells. For other details, see legend to Figure 1.

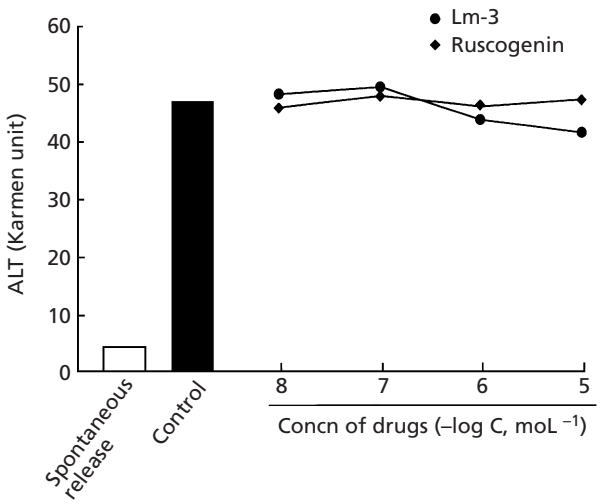

Figure 4 Effects of Lm-3 and ruscogenin pretreatment of hepatocytes on CCl_4-induced hepatocyte damage in-vitro. Each point represents the mean of three experiments and each experiment included triplicate sets. Spontaneous release: spontaneous release of ALT from hepatocytes. Control: ALT release from hepatocytes co-cultured with nonparenchymal cells.

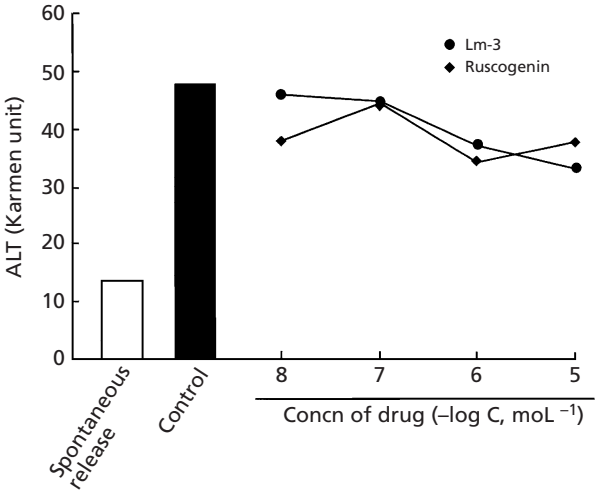

Figure 3 Effects of Lm-3 and ruscogenin pretreatment of hepatocytes on the potential of nonparenchymal cells to cause the release of alanine transaminase (ALT) from hepatocytes. Hepatocytes (2×10^4 in 0.2 mL WE medium) were pre-cultured in a 96-well microplate for 3 h. After two washings, they were pretreated with various concentrations of ruscogenin or Lm-3 for 2 h. After two further washings, the hepatocytes were exposed to 8×10^4 nonparenchymal cells for a further 3 h followed by collection of the supernatant to assay ALT. Each point represents the mean of three experiments and each experiment included triplicate sets. Spontaneous release: spontaneous release of ALT from hepatocytes. Control: ALT release from hepatocytes co-cultured with nonparenchymal cells.

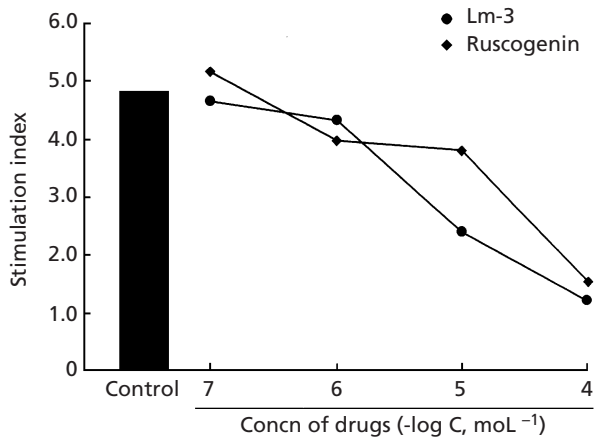

Figure 5 Effects of Lm-3 and ruscogenin on in-vitro lymphoproliferation in response to concanavalin A. The stimulation index was calculated by using the ratio in absorbance of concanavalin A-treated and non-treated cells. Each point represents the mean of three experiments and each experiment included triplicate sets. Control: concanavalin-A induced lymphoproliferation without Lm-3 or rusogenin treatment.

possessed strong immunopharmacological activities and the suppression to delayed type hypersensitivity was displayed mainly through affecting the formation of effector T cells and the inflammatory action without affecting the immune organs. This characteristic is quite different from prednisolone as one of the strongest inhibitors of delayed-type hypersensitivity reaction, and

may be very important for the treatment of immunologically-related diseases, including hepatitis.

We investigated the effect of Lm-3 and ruscogenin on the hepatotoxic activity of liver-infiltrating cells by using a co-culture assay with nonparenchymal cells and hepatocytes. This assay was described previously (Xu et al 1998), where we showed that spleen cells and nonparenchymal cells from liver-injured mice could induce a marked release of ALT and AST from hepatocytes in-vitro. The peak release was caused by the nonparenchymal cells 12 h after the elicitation of liver injury in parallel with the peak expression of LFA-1 (lymphocyte function associated antigen 1) on nonparenchymal cells and ICAM-1 (intracellular adhesion molecule 1) on hepatocytes. The main populations in nonparenchymal cells are $CD4^+$ T cells infiltrated from the periphery. We analysed the cell populations contained in nonparenchymal cells by flow cytometric analysis, and a great change in cell composition of nonparenchymal cells was found from 32–36 % at 0 h to 72.6–78 % LFA-1^+ cells at 12 h after the challenge with picryl chloride in liver. The latter included 47.9 % of $CD4^+$ and 23.2 % of $CD8^+$ T cells. As well correlated to the change in hepatotoxic potential of isolated parenchymal cells, the serum transaminase levels reached their peak at 18 h. Such a 6-h delay suggested that the lymphocytes against the antigen invasion finished their liver-infiltration at approximately 12 h and caused the hepatocyte damage at approximately 18 h. Therefore, in this study, nonparenchymal cells and hepatocytes isolated at 12-h liver injury were used to evaluate the interaction between nonparenchymal cells and hepatocytes. A dose-dependent inhibition of the enzyme release was observed with Lm-3 and ruscogenin, with Lm-3 showing an approximately 619-fold stronger activity than its aglycone (Figure 1). This result, taken together with the finding of in-vivo improvement on liver injury, suggested that the sugar parts in the structure of Lm-3 were very important for displaying their activity. Indeed, further study revealed that the synthesized ruscogenin 1-O-β-D-glucopyranoside and ruscogenin 1-O-β-D-glucopyranosyl $(1 \rightarrow 2)$-β-D-xylopyranoside, which has two sugars, showed a lower activity than Lm-3 with its three sugars (data not shown). A detailed mechanism on the role of the sugar parts in the activity of Lm-3 needs further investigation.

Time-dependent behaviour in the dysfunctioning of nonparenchymal cells was confirmed using Lm-3 and ruscogenin. More than 30-min treatment at a concentration of 10^{-6} M led to a complete blockade for the hepatotoxicity of nonparenchymal cells (Figure 2). When Lm-3 or ruscogenin were used to treat hepatocytes before co-culture with nonparenchymal cells, however, no protection was observed from the attack of nonparenchymal cells or CCl_4 (Figure 3 and 4). These results suggested that the improvement by Lm-3 and its aglycone of delayed-type hypersensitivity- but not CCl_4-induced liver injury may have been due to the selective dysfunction of immune cells that caused hepatocyte damage rather than the protection of hepatocyte membranes. On the other hand, this study revealed that Lm-3 and ruscogenin inhibited Con A-induced lymphocyte proliferation only at high concentrations (Figure 5). In addition, the treatment with Lm-3 or ruscogenin did not mask the LFA-1 expression (data not shown), suggesting a mechanism other than an influence on the LFA-1/ICAM-1 interaction. Taken together, Lm-3 and ruscogenin may be effective for improving the immuno-inflammatory liver injury, mainly through producing dysfunction of liver-infiltrating cells rather than through protecting the hepatocyte membrane. These results will be of significance for the development of new drugs as well as for the therapy of immunologically-related liver diseases. Further investigation is necessary to detail the mechanisms for producing the dysfunction of liver-infiltrating cells by Lm-3 and its aglycone.

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