Comparison of the effects of the pretreatment and treatment with RhIL-11 on acute liver failure induced by D-galactosamine


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Abstract. – OBJECTIVE: To compare the effects of the pretreatment and treatment with recombinant human interleukin-11 (rhIL-11) on acute liver failure induced by D-galactosamine (D-GalN).

METHODS: The Sprague Dawley (SD) male rats were randomly divided into five groups: control, model, pretreatment, treatment and repeated treatment groups. The acute liver failure model was established by intraperitoneal injections with D-GalN (1400 mg/kg). The pretreatment, treatment and repeated treatment groups were injected subcutaneously with rhIL-11 (500 µg/kg). The rats were killed 24, 48, or 72 h after the D-GalN injection. The symptoms and survival rate of the rats were analysed. Liver injury was assessed by serum ALT and AST levels and by histological analysis. The percentage of Proliferating Cell Nuclear Antigen (PCNA+) cells in the liver tissue was evaluated by immunohistochemistry.

RESULTS: Compared with the model group, the survival rate of the pretreatment group improved markedly, and these rats were protected from severe hepatic injury, as shown by the decreased serum ALT and AST levels and improved histological results. In the pretreatment group, the percentage of PCNA+ cells was significantly increased in the late stage. In contrast, the treatment and repeated treatment groups did not show improved survival rates or the prevention of severe hepatic injury, as shown by the absence of any decrease in the serum ALT and AST levels and the lack of any improvement in the histological results. The treatment and repeated treatment groups also have a significant increase in the percentage of PCNA+ cells in the late stage.

CONCLUSIONS: The pretreatment with rhIL-11 can reduce acute liver failure and protect the liver. In contrast, the treatment with rhIL-11 cannot reduce acute liver failure or protect the liver.

Key Words: Recombinant human interleukin-11, Galactosamine, Acute liver failure, Proliferating cell nuclear antigen.

Introduction

Liver failure is a syndrome marked clinically by jaundice, hepatic encephalopathy (HE), ascites and a disturbed blood clotting mechanism. It is caused by severe liver damage that leads to the decompensation of functions such as synthesis, detoxification, excretion and biotransformation. Liver failure is a common, severe clinical syndrome and there is no special treatment at present. Liver transplantation is currently the most effective therapeutic option for patients with liver failure. However, the application of liver transplantation is severely limited by the lack of liver donors and the high medical costs and, thus, the mortality of liver failure is very high, especially for acute liver failure. Therefore, alternative for effective and convenient therapy to treat liver failure remain urgently required.

IL-11, which is secreted by bone marrow stromal cells, is a pleiotropic cytokine with biological activities in many different cell types. It can raise peripheral platelet counts through its effects on both early and late progenitor cells to stimulate megakaryocyte differentiation and maturation. RhIL-11 has been approved for the treatment of chemotherapy-induced thrombocytopenia. RhIL-11 also exhibits anti-inflammatory effects in a variety of animal models of acute and chronic inflammation, including inflammatory bowel disease, mucositis, intestinal radiation injury, glomerulonephritis, diabetes mellitus, pneumonia, cardiac fibrosis, psoriasis and skin grafting2-10. In many animal models of hepatic damage, rhIL-11 can reduce inflammation, ameliorates liver function, protects the liver and reduces mortality though many methods11-15. However, rhIL-11 has only been used as a pretreatment in the animal models of hepatic damage. The effects of
treatment with rhIL-11 after the liver damage are unknown. Therefore, we compared the effects of the pretreatment and treatment with rhIL-11 on acute liver failure induced by D-GalN, and explored its potential mechanisms.

**Materials and Methods**

**Materials**

D-GalN was obtained from Boyuan Pharmatech Co., Ltd. (Nanjing, China). RhIL-11 was obtained from Amoytop Biotech Co., Ltd. (Xiamen, China). The rabbit monoclonal antibody against PCNA was obtained from Abcam Ltd. (Hong Kong). The HRP-conjugated secondary antibody as well as the DAB Kit were obtained from Zhongshan Goldenbridge Biotechnology Co., Ltd. (Beijing, China).

**Animals**

Healthy male Sprague-Dawley (SD) rats (clean grade), weighing 180 to 220 g, were purchased from the Vital River Experimental Animal Company (Beijing, China). The animals were sub-caged in a conventional clean facility at room temperature and allowed food and water ad libitum. After a 1-week acclimatization period, the experiment was performed on these animals. All the animals were treated humanly and procedures were performed with the approval of the Institutional Animal Care and Use Committee.

**Experimental Design**

The rats were randomly divided into five groups: the control group, the model group, the pretreatment group, the treatment group and the repeated treatment group. In each group except the control group, 10 rats were used to observe the survival rate 72 h after the D-GalN injection. Each experimental group was injected intraperitoneally with D-GalN (1400 mg/kg) to establish acute liver failure. The pretreatment group was injected subcutaneously with rhIL-11 (500 μg/kg) 2 h before the D-GalN injection. The treatment group was injected subcutaneously with rhIL-11 (500 μg/kg) 2 h after D-GalN injection. The repeated treatment group was injected subcutaneously with rhIL-11 (500 μg/kg) 2 and 24 h after the D-GalN injection. In the control group, the same volume of normal saline was subcutaneously injected at the same time points as the D-GalN. The symptoms in the animals, including appetite, behaviour, hair, urine and other statues, and the survival rate were observed and recorded. At 48 and 72 h after the D-GalN injection, 5 rats from the repeated treatment group were sacrificed by cervical dislocation, and at 24, 48 and 72 h after the D-GalN injection, 5 rats from the other experimental groups were sacrificed by cervical dislocation. Blood and liver samples were collected for further examination.

**Serum ALT and AST Activities**

Blood was obtained by extracting the eyeballs at 24, 48 and 72 h after the D-GalN injection. The sera were obtained by centrifugation (3000 rpm for 20 min) and stored at −20°C until analysis. The serum ALT and AST activities were determined with a Toshiba TBA120FR automatic analyser (Toshiba, Tokyo, Japan).

**Histopathological Analysis**

After the rats were killed, a portion of the liver was taken, fixed in 10% formalin, dehydrated in an ethanol gradient from low to high concentration and xylene, embedded in paraffin and sectioned. The injured condition of the liver tissue was observed under a microscope after HE staining.

**Immunohistochemistry Analysis**

The formalin-fixed tissues were embedded in paraffin and sectioned at a thickness of 4 μm. The sections were baked for 1 h at 60°C and deparaffinised in xylene and ethanol. The antigen retrieval was performed in a 0.01 M citric acid buffer solution (pH 6.0) in an autoclave for 2 min. The sections were incubated in 3% hydrogen peroxide for 30 min and incubated in 1% bovine serum albumin (BSA) for 20 min after washing with phosphate buffered saline (PBS). The sections were incubated overnight at 4°C with the primary antibody (1/250) and then incubated for 30 min at 37°C with a horseradish peroxidase (HRP)-conjugated secondary antibody. The sections were stained with 3,3-diaminobenzidine (DAB) and haematoxylin and then mounted with a coverslip. Cell with cell nuclei that were dyed a brown-yellow colour and showed a diffuse or granular pattern of varying intensity were considered to be positive. The percentage of cells positive for PCNA staining in five 400× fields was counted as the proliferation index using Imagepro-Plus5.1.

**Statistical Analysis**

The statistical analyses were performed with SPSS Version 17.0 (SPSS Inc., Chicago, IL, USA). The data were presented as the means ± SD. Multiple comparisons between the mean
variance analyses used one-way ANOVA. The LSD method was used to perform multiple comparisons for homoscedasticity. The Mann-Whitney U-test was used to perform multiple comparisons for heteroscedasticity. Survival curves were drawn using the Kaplan-Meier method and the statistical significance was estimated by the log-rank test. A $p$-value of less than 0.05 was considered statistically significant.

**Results**

**The Symptoms of the Animals**

The control rats had normal mental abilities, good activity levels, snow-white hair and a normal dietary intake. Some rats in the pretreatment group had mental malaise and poor activity levels, but most rats in the pretreatment group were similar to the control rats. At 24 h after the D-GalN injection, the rats in the model, treatment and repeated treatment groups showed mental malaise, lethargy, huddling, anorexia and hair fleeciness. Some of these rats showed restlessness, irritability, limb spasm, oliguria, deep yellow urine and hae-matemesis. The severely injured rats began to die.

**Survival Rate**

In all the groups, the death of most rats occurred within 3 days of the D-GalN injection. The survival rates in the model, treatment and repeated treatment groups were 50%, 50% and 30%, respectively, 72 h after the D-GalN injection. There was no significant difference among these three groups. The survival rate in the pretreatment group was 90%, which was significantly higher than those in the model, treatment and repeated treatment groups (Figure 1).

**Liver Histopathology**

The liver tissue in control group showed a normal lobular architecture and cell structure. Most liver specimens in the model group looked like that shown in Figure 2C, whereas others looked like that shown in Figure 2B. At 24 h after the D-GalN injection, the liver tissue in the model group showed a disordered arrangement of the liver cells and hepatic lobule structure, sinusoidal expansion, debris and flake necrosis, and inflammatory cell infiltration in the portal areas. At 48 h, the injury was the most serious. The liver tissue showed tissue structure collapse, massive necrosis, sinusoidal expansion and hyperaemia, and infiltration of a large number of inflammatory cells. At 72 h, the liver tissue showed swelling and disorganised hepatocytes, debris and flake necrosis and inflammatory cell infiltration in the portal areas. Most liver specimens in the treat-

![Figure 1. The cumulative survival curve of each group. The survival rate in the pretreatment group was significantly higher than those in the model, treatment and repeated treatment groups ($p < 0.05$).](image)

![Figure 2. Three types of gross specimens from the rats: A. The liver was ruddy, tender and similar in appearance to a normal liver. B. The liver was red and soft, and its surface had a large number of petechiae. C. The liver was white, smaller and softer.](image)
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Figure 3. Liver histopathology (HE staining, ×200) in each group at each time point: A. Control group. B-D. Model group at 24, 48 and 72 h, respectively, after the D-GalN injection. E-G. Pretreatment group at 24, 48 and 72 h, respectively, after the D-GalN injection. H-J. Treatment group at 24, 48 and 72 h, respectively, after the D-GalN injection. K-L. Repeated treatment group at 48 and 72 h, respectively, after the D-GalN injection.

Serum Levels of ALT and AST

The serum ALT and AST levels in each group (except the control group) increased at 24 h after the D-GalN injection and reached a peak at 48 h. The serum ALT and AST levels in the model group were significantly higher than those in the control group at each time point. The serum ALT and AST levels in the treatment and repeated treatment groups were also significantly higher than those in the control group at
each time point and showed no improvement compared with the model group. The serum ALT and AST levels in the pretreatment group were significantly lower than those in the model group, treatment and repeated treatment groups at each time point, but were not significantly different compared with the treatment group at 24 h (Table I).

**PCNA+ cells in the Liver Tissue**

The percentage of PCNA+ cells in the model group began to increase 24 h after the D-GaIN injection and reached a peak at 48 h. This percentage was significantly different compared with the control group. The percentage then decreased and returned to its normal level at 72 h. The percentage of PCNA+ cells in the pretreatment and treatment groups also began to increase after 24 h and was not significantly different compared with the model group at 48 h. The percentage of PCNA+ cells in the repeated treatment group was also not significantly different compared with the model group at 48 h. After 48 h, the percentage of PCNA+ cells in the pretreatment, treatment and repeated treatment groups did not decrease, but rather increased further, which was significantly different compared with the model group at 72 h (Figure 4, Table II).

### Table I. Serum levels of ALT and AST in each group at each time point (U/L, n=5)

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<thead>
<tr>
<th></th>
<th>ALT</th>
<th>AST</th>
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<tbody>
<tr>
<td><strong>Control group</strong></td>
<td>24h</td>
<td>58.4 ± 10.0</td>
</tr>
<tr>
<td><strong>Model group</strong></td>
<td>24h</td>
<td>3445.2 ± 1169.8*</td>
</tr>
<tr>
<td></td>
<td>48h</td>
<td>12236.6 ± 2094.2*</td>
</tr>
<tr>
<td></td>
<td>72h</td>
<td>3209.2 ± 1852.8*</td>
</tr>
<tr>
<td><strong>Pretreatment group</strong></td>
<td>24h</td>
<td>676.2 ± 497.1*</td>
</tr>
<tr>
<td></td>
<td>48h</td>
<td>1826.7 ± 1431.3*</td>
</tr>
<tr>
<td></td>
<td>72h</td>
<td>368.7 ± 222.5*</td>
</tr>
<tr>
<td><strong>Treatment group</strong></td>
<td>24h</td>
<td>1269.8 ± 1074.9*</td>
</tr>
<tr>
<td></td>
<td>48h</td>
<td>7301.6 ± 1729.7*</td>
</tr>
<tr>
<td></td>
<td>72h</td>
<td>7301.6 ± 1729.7*</td>
</tr>
<tr>
<td><strong>Repeated treatment group</strong></td>
<td>48h</td>
<td>9737.8 ± 3061.9*</td>
</tr>
<tr>
<td></td>
<td>72h</td>
<td>1487.3 ± 952.0**</td>
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*: p < 0.05 vs. control group; **: p < 0.05 vs. pretreatment group.

### Discussion

IL-11, an important potent anti-inflammatory cell factor, belongs to the IL-6 family of GPl30 receptor ligands. It possesses anti-inflammatory activity in different acute and chronic inflammation models. Additionally, IL-11 can reduce inflammation, ameliorates liver function, protects the liver tissue, improves the survival rate through many pathways in a variety of liver damage models. The anti-inflammatory mechanism is not yet clear and may include the following. (1) RhIL-11 can induce the synthesis of heat shock proteins, protects intestinal epithelial cells from ischaemia and immune-mediated inflammation, enhances intestinal repair and maintains intestinal barrier function. (2) RhIL-11 directly interacts with macrophages, modulates the synthesis of NF-KB, inhibits the production of proinflammatory cytokines production, such as TNF-α and NO, and

### Table II. Percentages of PCNA+ cells in each group at each time point (%, n=5)

<table>
<thead>
<tr>
<th></th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group</strong></td>
<td>10.2 ± 2.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Model group</strong></td>
<td>12.3 ± 3.89</td>
<td>34.2 ± 2.07*</td>
<td>13.6 ± 7.60</td>
</tr>
<tr>
<td><strong>Pretreatment group</strong></td>
<td>13.2 ± 1.34</td>
<td>32.7 ± 3.60*</td>
<td>52.0 ± 6.81**</td>
</tr>
<tr>
<td><strong>Treatment group</strong></td>
<td>11.2 ± 1.92</td>
<td>31.1 ± 3.27*</td>
<td>55.1 ± 7.08**</td>
</tr>
<tr>
<td><strong>Repeated treatment group</strong></td>
<td>32.6 ± 2.28*</td>
<td>57.2 ± 6.18**</td>
<td></td>
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</table>

*: p < 0.05 vs. control group; **: p < 0.05 vs. pretreatment group.
then reduces the inflammatory response. However, rhIL-11 cannot render the macrophages insensitive to endotoxins or induces the production of endogenous IL-10, TGF-β1 or IL-6. RhIL-11 can interact with T cells in T cell-mediated diseases, modulates the Th1/Th2 balance and reduces the inflammatory response by enhancing the Th2 response and reducing the Th1 response. RhIL-11 has been used as a pretreatment in these liver damage models. Because we are unable to predict liver failure in the clinic, it is necessary to find a treatment that is effective when administered after liver failure has occurred. So we wanted to determine the effects of the treatment with rhIL-11 and whether rhIL-11 can be used as a treatment for liver failure in the clinic in the fu-
tured. Therefore, we compared the effects of the pretreatment and treatment with rhIL-11 on acute liver failure induced by D-GalN and explored its potential mechanisms.

The animal model of liver damage induced by D-GalN is one of the classical animal models. The morphology change in the liver induced by D-GalN is similar with the acute viral hepatitis, such as acidophilic degeneration, ballooning degeneration and diffuse massive necrosis. D-GalN only induces the liver damage, does not affect others organs, and the damage induced by D-GalN can be well repeated. So D-GalN is always used to induce the acute hepatitis or the acute liver failure and determine the effect of drugs. Previous research assayed the LD₅₀ of D-GalN in rats with modified Spearman-Kärber method and showed that 1400 mg/kg was the upper limit of 95% CI of LD₅₀, and D-GalN of 1400 mg/kg used to induce acute liver failure in the rats can be find in others papers. So we used 1400 mg/kg in this experiment, and also found that the clinical manifestations, serological and histological changes and survival rate were the closest to those seen in acute liver failure.

This experiment compared the effects of pretreatment and treatment with rhIL-11 on acute liver failure though the symptoms, survival rate, liver histopathology and serum ALT and AST levels. The results showed that D-GalN induced acute liver failure, as demonstrated by a decrease in daily activity, increases in serum ALT and AST levels, induction of large areas of necrosis, and decreased survival rate. After the pretreatment with rhIL-11, the results were consistent with previous reports. Compared with the model group, rhIL-11 could ameliorate the liver failure induced by D-GalN, as shown by the improved symptoms, increased survival rate, decreased serum ALT and AST levels and reduced liver injury. In contrast, the treatment with rhIL-11, regardless of whether one or two injections was used, resulted in no change compared with the model group, as shown by the decreased daily activity, increased serum ALT and AST levels, induction of large areas of necrosis, and decreased survival rate. Therefore, this experiment demonstrated that pretreatment with rhIL-11 can significantly reduce the inflammation of acute liver failure, ameliorate the liver function and protect the liver tissue, and that treatment with rhIL-11 has no effect on acute liver failure.

Previous research showed that IL-6 could bind to IL-6R expressed in hepatocytes, which then activates the STAT3 signalling pathway mediated by gp130 and finally promotes cell proliferation and differentiation. IL-11, a member of the IL-6 family, binds its specific receptor α-subunit, followed by the activation of the common receptor subunit, gp130. Thus, IL-11 should also promote liver regeneration in theory, but there are few relevant reports. Therefore, our experiment explored whether rhIL-11 promoted liver regeneration by observing the PCNA+ cells in the liver tissue. The results showed that the percentage of PCNA+ cells increased 24h after the D-GalN injection in the model group, reached a peak after 48 h, and then decreased to its normal level after 72 h. The percentage of PCNA+ cells in the pretreatment, treatment and repeated treatment groups were not different at each time point; when the majority of rats were dying and the liver tissue was seriously damaged at 48 h after the D-GalN injection, the percentage of PCNA+ in those three groups was the same as that in the model group. The percentage of PCNA+ cells in pretreatment, treatment and repeated treatment groups continued to increase after 48 h and was significantly higher than that in the model group at 72 h, but at that time point, the mortality decreased and the liver injury was ameliorated. Thus, we believe that both pretreatment and treatment with rhIL-11 can bind to the IL-11R expressed in hepatocytes, activate the STAT3 signalling pathway mediated by gp130, and then promote liver regeneration. However, this process may have effect during the later stage of liver failure, but has no effect during the early stage of liver failure. Because our experiment showed that pretreatment with rhIL-11 can reduce the liver damage and protect the liver tissue in the early stage, its positive effect occurs though another pathway.

Regardless of whether one or two injections were used, the effect of the treatment with rhIL-11 on liver failure was much weaker than that of pretreatment with rhIL-11. There must be some mechanism responsible for this difference. If we can identify the mechanism and use that information to ensure that treatment with rhIL-11 has the same effects as pretreatment with rhIL-11 by making some adjustments, then our ability to treat liver failure in the clinic will be improved. Although this experiment failed to determine the responsible mechanism, we can make some hypotheses. (1) Previous studies showed that macrophages played an important role in the model induced by D-GalN. Gut-derived endotox-
in or antigen was released after the D-GalN treatment, which then activated the macrophages. The activated macrophages caused the expression of proinflammatory cytokines, such as TNF-α, resulting in an inflammatory response and liver injury. The pretreatment with rhIL-11 directly interacted with macrophages, modulated the synthesis of NF-KB, inhibited the expression of proinflammatory cytokines, such as TNF-α and NO, and reduced the inflammatory response. The hypothesis is that the pretreatment with rhIL-11 can inhibit the expression of proinflammatory cytokines; however, if proinflammatory cytokines begin to be expressed, the treatment with rhIL-11 cannot prevent their further expression and inhibit their function even though rhIL-11 can directly interact with macrophages, activate certain signalling pathways and reverse the liver failure. These hypotheses require further experimental confirmation.

Conclusions

We demonstrated that the pretreatment with rhIL-11 can reduce acute liver failure and protect the liver. In contrast, the treatment with rhIL-11 cannot reduce acute liver failure or protect the liver. The mechanism responsible for this difference needs further experimental confirmation.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

References


11) BOZZA M, BLISS JL, MAYLOR R, ERICKSON J, DONNELLY L, BOUCHARD P, DORNER AJ, TREPICCHIO WL. Inter-


25) LAM SP, LAM JM, MAN K, NG KT, CHEUNG CK, ROSE-JOHN S, LO CH. Activation of interleukin-6-induced glycoprotein 130/signal transducer and activator of transcription 3 pathway in mesenchymal stem cells enhances hepatic differentiation, proliferation, and liver regeneration. Liver Transpl 2010; 16: 1195-1206.


