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• *BASIC RESEARCH* •

Effects of emodin and double blood supplies on liver regeneration of reduced size graft liver in rat model

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Abstract

AIM: To study the influences of emodin and reconstruction of double blood supplies on liver regeneration of reduced size graft liver in rat model.

METHODS: A total of 45 SD-SD rat reduced size liver transplantation models were randomly divided into three groups (A-C). The conventional reduced size liver transplantation was performed on rats in group A, while the hepatic artery blood supply was restored in groups B and C. The emodin (1.5 mg/kg/d) was given by intraperitoneal route in group C only. The recipients were killed on the seventh day after the operation. The proliferative cell nuclear antigen (PCNA), TBil and ALT of serum were detected, and the pathological changes of liver cell were observed.

RESULTS: The numbers of the rats that survived in A, B, and C group on the seventh day after operation were 14, 13, 13, respectively. The levels of TBil (31.5±5.2 μmol/L, 23.2±3.1 μmol/L *vs* 38.6±6.8 μmol/L), and ALT (5 351± 1 050 nKat, 1300±900 nKat *vs* 5779±1202 nKat) in serum in groups B and C were lower than those in group A ($P<0.05$), while the expression of PCNA in groups B or C was higher than that in group A (22.0±3.5%, 28.2±4.2% *vs* 18.6±3.2%, $P<0.05$). The deeper staining nuclei, double nuclei, multi-nuclei and much glycogen were observed in liver cells of groups B and C, especially in group C, while fewer were found in liver cells of group A.

CONCLUSION: The reconstruction of arterial blood supply is very important for rat liver regeneration after reduced size liver transplantation. Emodin has the effect of promoting liver regeneration and improving liver function in rats after reduced size transplantation. The possible mechanism is improving proliferation of liver cell and protecting liver cells from injury.

Key words: Emodin; Reduced size transplantation; Hepatic artery; Regeneration

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INTRODUCTION

It is well-known recently that clinic liver transplantation has been the most effective method to treat liver disease in terminal stage. However, due to the deficiency of the donor, more surgical methods were applied for liver transplantation, for example, vivid liver transplantation and resized liver transplantation. In vivid liver transplantation and resized liver transplantation, it is very important that the donor and recipient match each other, and liver regeneration of the new liver is also a focus of study. Liver regeneration may be affected co-operatively not only by humoral factors such as hormones, growth factors and growth inhibitory factors, but also by the immune system[1-7].

Emodin is extracted from Chinese herb and more properties of it are being found[8,9]. The reported biological effects of emodin include antitumor, antibacterial and anti-inflammatory activities[10-13]. Experiments have demonstrated that the hepatic lesion induced by CCl_4 could be decreased by emodin by ameliorating cellular regeneration activities and protecting liver function[14,15].

In the present study we established the rat resized liver transplantation model with restored arterial blood supply to evaluate the effects of emodin and double blood supplies on liver regeneration.

MATERIALS AND METHODS

Animals and reagents

Emodin was presented by Huaian Reilei preparation Co., LTD (Jiangshu, China), dissolved and sterilized in dimethyl sulfoxide (DMSO) first and then diluted to the required working concentrations in RPMI 1640 (Gibco, USA) containing 100 mL/L calf serum (Sijiqing Co., Hangzhou, China). Male SD rats, 9-10 wk old, weighing 220±280 g, were purchased from the Animal Center of Xi'an Jiaotong University. Proliferative cell nuclear antigen (PCNA) ABC ultra-sensitive immunostaining kit was purchased from Boshide Biotechnology Developing Co. (Wuhan, China).

Effect of emodin and double blood supplies on liver regeneration in rat model

All the 45 SD rats were fasted for 24 h before operation, but had free access to tap water. Then they were divided

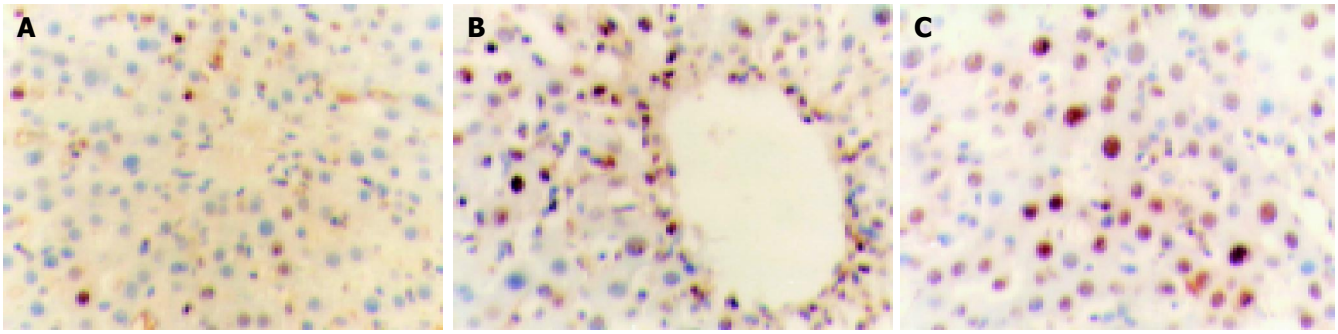

Figure 1 PCNA in transplanted rat liver SABC, ×400. Group **A:** 18.6±3.2% *vs* group **B:** 22±3.5% *vs* group **C:** 28.2±4.2%.

into three groups randomly: groups A-C. In group A, reduced size liver transplantation was performed on the rats by using cuff method[8], with the left lobe of liver resected which accounts for the 30% of total liver weight; in groups B and C, reduced size liver transplantation was performed on the rats and hepatic arteries were anastomosed by sleeve method[16], while only group C was given emodin (1.5 mg/kg/d) by intraperitoneal route for 7 d. All the recipient rats were killed on the 7th d after transplantation and a 3-5-mL blood sample was obtained from the right ventricles. These blood samples were centrifuged immediately at 3 000 r/min at room temperature for 5 min and the serum samples were assayed by Olympus AV800 auto-analyses instrument for the detection of serum total bilirubin (TBil) and alanine aminotransferase (ALT).

PCNA expression in rat's allograft liver tissue

The allograft liver tissues taken from group A, B and C were 100 mL/L formalin-fixed, paraffin-embedded and cut into 4 μm thick sections for staining. PBS of 0.01 mol/L was used to substitute for primary antibody for negative control, while a breast cancerous tissue expressing PCNA was used for positive control. The working concentration of antibody was 1∶100. The staining procedures used were as described in ABC immunostaining kit. The sections were examined twice on different days by the same pathologist and the distribution of positively stained cells was evaluated semi-quantitatively by calculating the percentage of positive cells in 100 cells in five nonoverlapping microscopic high-power fields.

Histopathologic and ultra structural examination

The allograft liver tissues taken from group A-C were 100 mL/L formalin-fixed, paraffin-embedded and cut into 4 μm thick sections for HE staining, while some samples were cut into small pieces of 1 mm^3 on ice and fixed in glutaral for electron microscopic observation.

Statistical analysis

All values were expressed in the mean±SD.SPSS statistics software was used to evaluate the statistical significance of the differences among the three groups with Student's *t* test. When $P<0.05$, it is considered significant.

RESULTS

Survivals and liver function

The numbers of survivals on the 7th d after transplantation in groups A-C were 14, 13, and 13, respectively. The levels of TBil and ALT in serum in groups B and C were lower than those in group A ($P<0.05$), and the levels of TBil and ALT in serum in group C was still lower than those in group B ($P<0.05$, Table 1).

Table 1 Levels of ALT and TBIL in the rat's serum on the 7th d after transplantation

Liver transplantation	*n*	ALT (nKat/L)	TBil (μmol/L)
A: reduced size	14	5 779±1 202	38.6±6.8
B: reduced size +double blood supplies	13	5 351±1 050[a]	31.5±5.2[a]
C: reduced size +double blood supplies +emodin	13	1 300±900[a,c]	23.2±3.1[a,c]

[a]$P<0.05$ *vs* group A; [c]$P<0.05$ *vs* group B.

PCNA expression in rat's allograft liver tissue

The expression of PACN in B or C groups was higher than that in A group ($P<0.05$), and the expression of PACN in C group was higher than that in B groups ($P<0.05$). The positive cells percentage in the groups A-C was 18.6±3.2%, 22±3.5%, and 28.2±4.2%, respectively (Figure 1).

Histopathologic and ultra structural examination

The deeper staining nuclei, double nuclei, multi-nuclei and much glycogen were observed in liver cells of groups B and C, especially in group C, while fewer were found in liver cells of group A (Figures 2 and 3).

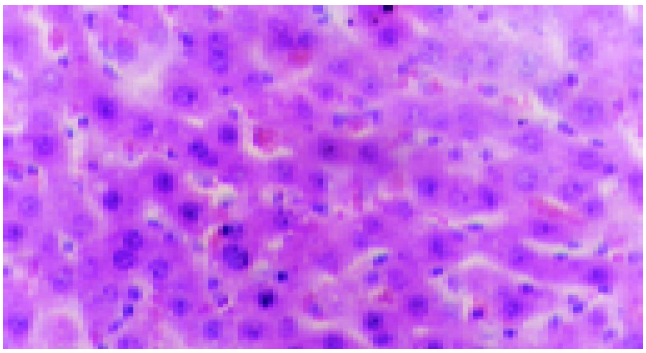

Figure 2 HE staining: the deeper staining nuclei and double nuclei in group C, HE ×400.

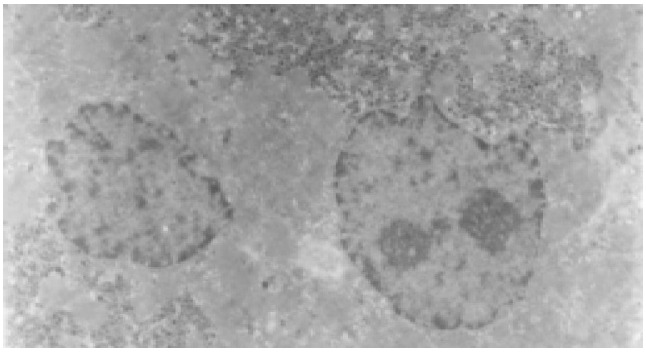

Figure 3 Double nuclei and much glycogen of hepatocyte in group C, TCM×1 500.

DISCUSSION

It is well known that the activity of liver regeneration is very strong. The synthesis of DNA of the liver cell could be found in 12-16 h after 2/3 liver was resected, and this regeneration process could be completed in 6-8 d[17]. Liver regeneration is a special and complicated process, which could be affected by many factors, for example, insulin, estrogen, and thyroxine. There are many problems in the liver regeneration after transplantation remained to be explored, and rat liver transplantation model, especially reduced size liver transplantation, is an ideal model for the study of these problems. Recent studies have shown that the liver of rat demonstrated strong activity of regeneration after liver transplantation or partial liver resection. Compared with liver after partial liver resection, the proliferation peak of liver cells after liver transplantation or partial liver transplantation occurred late, but the proliferation cycle is longer. The possible reasons are the injuries due to operative procedure or ischemia-reperfusion, or the modulation of cytokines and hormones produced by recipient's immune system[4].

At present, the most common orthotopic rat liver transplantation model was performed by the cuff technique without hepatic artery reconstruction. However, the effects of hepatic artery on the regeneration of liver cells are obvious. Therefore, the liver transplantation model with double blood supplies mostly accords with the physiological demand of experimental animals. In our study the rat reduced size liver transplantation model was applied with double blood supplies to study the liver cell regeneration. The advantages of the model are as follows: (1) There are double blood supplies in the grafted liver, whose micro-environment is more similar to that of human liver graft than the traditional liver transplantation model; (2) Because the transplantation is a homogenic transplantation, there is no more involvement of immunity factors, so the factors of research were easy to be controlled.

Proliferating cell nuclear antigen (PCNA) is a popular marker to assess the activities of liver cell regeneration. The molecular mass of PCNA is 36 ku and PCNA consisted of 261 amino acids. PCNA exists and is synthesized in the nucleus, and it is required for DNA replication and repair. In quiescent cells the level of PCNA is very low, while it is higher in proliferated and transformed cells[18-29]. In this study we found that PCNA exists in the liver of all three groups. This means that the cell proliferation after reduced size liver transplantation is active. Compared with group A, the expressions of PCNA in groups B and C are much higher ($P<0.05$), and compared with group B, the expression of PCNA in group C is still much higher too ($P<0.05$). The histopathologic and electron microscopic examination showed that the deeper staining nuclei, double nuclei, multi-nuclei and much glycogen were observed in liver cells of groups B and C, especially in group C, while fewer was found in liver cells of group A. Besides, the levels of ALT and TBil in the rat's serum on the 7th d after transplantation in groups B and C were lower than that in group A ($P<0.05$), and the levels of TBil and ALT in serum in group C was still lower than those in group B ($P<0.05$). All these results showed that restoring liver artery is very important in liver cell regeneration.

Emodin, whose molecular mass is 270.23 ku, is extracted from Chinese herb and more properties of it are being found[8,9]. The reported biological effects of emodin include antitumor, antibacterial and anti-inflammatory activities[10-13]. Experiments have demonstrated that the hepatic lesion induced by CCl_4 could be decreased by emodin by ameliorating cellular regeneration activities and protecting liver function[14,15]. In our experiment, the lower levels of TBil and ALT in serum in group C, and more cell regeneration in the group C than those in groups A and B were found ($P<0.05$). These results are consistent with others which support that emodin has the effects of promoting cellular regeneration and restoring liver function.

On the whole, the reconstruction of arterial blood supply is very important to rat liver regeneration after reduced size transplantation. Emodin has the effect of promoting liver regeneration and improving liver function in rat after reduced size transplantation. However, the molecular mechanism of emodin still remains to be studied.

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