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***Basic Study***

**Magnesium isoglycyrrhizinate inhibits inflammatory response through STAT3 pathway to protect remnant liver function**

Tang GH *et al.* MgIG protects remnant liver function

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**Abstract**

**AIM:** To investigate the protective effect of magnesium isoglycyrrhizinate (MgIG) on excessive hepatectomy animal modal and its possible mechanism.

**METHODS:** We used the standard 90% hepatectomy model in Sprague-Dawley rats modified from Emond method, in which the left, middle, right upper, and right lower lobe of the liver were removed. Rats with 90% liver resection were divided into three groups, and were injected intraperitoneally with 3 mL saline (control group), 30 mg/kg (low-dose group) and 60 mg/kg (high-dose group) of MgIG, respectively. Animal was sacrificed at various time points and blood was drawn from the vena cava. Biochemical tests were performed with automatic biochemical analyzer for the following items: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyl endopeptidase, total bilirubin (TBil), direct bilirubin (DBil), total protein, albumin, blood glucose (Glu), hyper-sensitivity C-reactive protein, prothrombin time (PT), and thrombin time (TT). Postoperative survival time was observed hourly until death. Hepatocyte regeneration was analyzed by immunohistochemistry. Serum inflammatory cytokines (IL-1, IL-6, IL-10, and iNOS) was analyzed by ELISA. STAT3 protein and mRNA were analyzed by Western blot and quantitative reverse-transcription PCR, respectively.

**RESULTS:** The high dose group demonstrated a significantly prolonged survival time, compared with both the control and the low-dose group (22.0 ± 4.7 h *vs* 8.9 ± 2.0 *vs* 10.3 ± 3.3 h, *P* = 0.018). There were significant differences among the groups in ALT, Glu and PT levels starting from 6h after surgery. The ALT levels were significantly lower in the MgIG treated groups than that in the control group. Both Glu and PT levels were significantly higher in the MgIG treated groups than the control. At 12 h time point, ALT, AST, TBil, DBil and TT levels showed significant difference between the MgIG treated groups and the control group. No significant differences in hepatocyte regeneration were found. Compared to the control group, the high-dose group showed significantly increases in serum inflammatory cytokines IL-1 and IL-10, and decrease in IL-6. Both STAT3 protein and mRNA levels were significant lower in the MgIG treated groups than that in the control at 6h, 12 h, and 18 h after surgery.

**CONCLUSION:** High-dose MgIG can extend survival time in rat after excessive hepatectomy. This hepatoprotective effect was by inhibiting the inflammatory response through inhibition of STAT3 pathway.

**Key words:**Magnesium isoglycyrrhizinate; Excessive liver resection; Inflammation; Liver regeneration; STAT3

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**Core tip:** Magnesium isoglycyrrhizinate (MgIG), a hepatocyte protective agent, has been shown to have the effect of anti-inflammation, liver cell membrane protection, and liver function improvement. We designed this study, by using the standard 90% hepatectomy model in rats to clarify the MgIG of liver protection function and its mechanism. We have researched postoperative survival time, hepatocyte regeneration, liver function, serum inflammatory cytokines, STAT3 protein and mRNA expression. The protective effect of MgIG in standard 90% hepatectomy is limited which can prolong the survival time. This hepatoprotective effect was not by increase hepatocyte regeneration but rather by inhibiting the inflammatory response through inhibition of STAT3 pathway.

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**INTRODUCTION**

Excessive liver resection is the only hope of cure for extra-large occupying liver lesions. Most patients with liver occupying lesions often associated with other liver abnormalities such as cirrhosis. High surgical risk could be leading to fulminant hepatic failure that has a mortality rate up to 70% to 90[[1](#_ENREF_1),2]. Protection of the remnant liver function in the first 48h after surgery remains great challenge to the surgeons[[3](#_ENREF_1" \o "Yu, 2004 #11)]. The efficacy of various existing measures, including the artificial liver and other supporting methods, are limited. Our previous study in rat found that liver regeneration reaches to the highest level at 72 h after resection. Animal will most likely survive if the regeneration could compensate for the first 48 h[[4](#_ENREF_1" \o "Yu, 2004 #11)]. Therefore, to identify appropriate and effective measures to help the remnant liver to sustain through this risky period is particularly important.

Magnesium Isoglycyrrhizinate (MgIG), a hepatocyte protective agent, has been shown to have the effect of anti-inflammation, liver cell membrane protection, and liver function improvement. Efficacy studies showed that protective function of MgIG to the acute liver damage is induced by D-galactosamine. MgIG can decrease the serum level of transaminases, prevent liver cell degeneration, and reduce the incidence of necrosis and inflammatory cell infiltration. MgIG is especially effective in the treatment of chronic liver injury induced by carbon tetrachloride in rats, by reducing inflammation and fibrosis, lowering the nitro-monoxide levels, and improving liver function.

Most of the previous studies of MgIG have focused on chronic hepatitis, alcoholic cirrhosis, and drug-induced liver injury[[5](#_ENREF_1" \o "Yu, 2004 #11)]. A few researches mainly involved in surgical ischemia-reperfusion injury[[6](#_ENREF_1" \o "Yu, 2004 #11)] and liver regeneration[[7](#_ENREF_1)]. No publication was found for the anti-inflammatory effect of MgIG after liver resection. In this study, we verified the liver protective effect of MgIG in an animal model of hepatectomy and further investigated its mechanism.

**MATERIALS AND METHODS**

***Ethics statement***

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institute of Animal Care and Use Committee of Beijing Union Medical College (Permit No.: 2013050125). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

***Experimental animals***

Male Sprague-Dawley (SD) rats aged 8 wk (220-260 g) were provided by the Experimental Animal Center of Beijing Union Medical College Hospital.

***Reagents***

MgIG was provided by JCTT Pharmaceutical Ltd (Jiangsu Province, China). Experimental solution was prepared 3% (w/v) MgIG in saline.

***Establishment of rat model with 90% liver resection***

The rats were weighted, anesthetized with an intraperitonealinjection of pentobarbital sodium (60 mg/kg). After routine skin preparation and disinfection, an upper abdominal incision was made. A sequential isolation and resection were performed on the middle lobe, exterior lobe, right lower lobe, and right upper lobe. After being confirmed with no congestion on the caudate lobe, the abdomen opening was closed.

***Experimental design***

SD rats were randomly divided into three groups: control (C), low-dose (LD), and high-dose (HD) group. Hepatectomy was performed by resection of 90% liver tissue, followed by injection intraperitoneally with 3 mL saline (C group), 30 mg/kg (LD group), or 60 mg/kg (HD group) of MgIG on the same day of surgery, as well as once daily afterwards.

***Observation of survival time***

Starting from the closing of abdomen as 0 h, animal survival time was measured hourly until death. Meanwhile, the general condition of each animal was recorded. We used proper humane endpoints when the rats were in the moribund state or the signs of severe organ system dysfunction non-responsive to treatment appear and euthanized rats prior to the end of experiments.

***Liver function evaluation***

Animal was sacrificed at various time points and blood was drawn from the vena cava. Biochemical tests were performed with automatic biochemical analyzer (HITACHI, Japan) for the following items: serum alanine aminotransferase (Alt), aspartate aminotransferase (AST), glutamyl endopeptidase (GGt), total bilirubin (TBil), direct bilirubin (DBil), total protein (TP), albumin (Alb), blood glucose (Glu), hyper-sensitivity C-reactive protein (CRP), prothrombin time (PT), and thrombin time (TT).

***Measurement of liver regeneration***

The average proportion of liver weight against the body weight was established from 5 rats of same age. It was found that the liver weight is to be 3.96% ± 0.01% of the body weight. The remnant caudate lobe was removed from the animal model at various time points after surgery and weighed. Liver regeneration rate was calculated by the Okano T formula: Regeneration rate (R, %) = [C- (A-B)]/(A-B) × 100%, where in A is preoperative estimation of the rat liver weight, B is the weight of resected liver tissue, and C is the weight of the remnant caudate lobe[[8](#_ENREF_1)].

***Observation of liver damage and regeneration by HE and BrdU staining***

BrdU (Sigma, USA) (100 μg/g body weight) was administered intraperitoneally to animal 2 h prior to the removal of remnant caudate lobe. The remaining caudate lobe was removed at 0, 6, 12 and 18 h after surgery, fixed and sectioned, and undergone immunohistochemistry procedure or HE stained. BrdU positive cells were counted under microscope. At least 3 rats from each group, 3 slides from each rat, and 3 fields from each slide were counted, and the results were presented as the numbers of positive cells in every 500 total cells being counted.

***Expression of PCNA***

Paraffin embedded sections of remnant liver tissue was studied with immunohistochemistry for Proliferating Cell Nuclear Antigen (PCNA) expression. PCNA antibody was obtained from Abcam USA. PCNA-positive cells were counted under the light microscope. At least 3 rats from each group, 3 slides from each rat, and 3 fields from each slide were counted, and the results were presented as the numbers of positive cells in every 500 total cells being counted.

***Western blot***

Remnant liver tissue was also homogenized and treated with RIPA lysis buffer (Dingguo, China); the extracted proteins were resolved by 4%-12% acrylamide gradient gel. After electrophoresis, sample was transferred to PVDF membrane using iBlot fast transfer electric transfer (Invitrogen, USA). Membrane was blocked at room temperature for 1 hour by 5% milk, and incubated with primary antibody again PCNA or STAT3 (1:1000, Abcam, USA) 4 °C overnight, followed by TBST washing three times, secondary antibody (1:8000, Abcam USA) incubation at room temperature for 2 h, TBST wash 3 times, and exposure to film with ECL kit (Pierce, USA). Densitometry analysis was performed with BandScan software.

***ELISA assay for inflammatory factor detection in serum***

Blood was drawn from vena cava at 0, 6, 12 and 18 h after surgery, and serum was separated and stored -80 °C. ELISA assays to determine IL-1, IL-6, IL-10, and iNOS expression levels were performed according to manufacturer’s instructions (Cusabio, Wuhan, China).

***Quantitative Real-time PCR***

Total RNA was extracted from the remnant liver tissue with Trizol method. TAKARA retroviral reverse transcriptase kit (TAKARA, Japan) was used to synthesize cDNA with the reaction conditions of 37 °C 60 min and 95 °C 3 min. Primers were designed as sense 5'-CACAACCTGCGAAGAATCAAG-3' and anti-sense 5'-GCTGCTTCTCCGTCACTAC-3' for STAT3 gene, and sense 5'-AACGGCTCCGGCATGTGCAA-3', antisense 5'-CTTCTGACCCATGCCCACCA-3 ' for β-actin.

Real-time PCR was performed with Applied Biosystems 7500 real-time quantitative PCR instrument (Applied Biosystems 7500, USA) at the following condition: 95 °C 20 s, 60 °C 30 s, 72 °C 30 s for 40 cycles[[8](#_ENREF_1)].

***Statistical analysis***

Statistical analysis was performed using SPSS13.0 software (Chicago, IL). Data were expressed as mean ± SD. The difference between groups was compared using one-way ANOVA, survival analysis was performed using the Kaplan-Meier method. Two-tailed *P* < 0.05 was considered statistically significant.

**RESULTS**

***Comparison of postoperative survival***

Seven out of fifteen (46.7%) rats in the control group did not recover from the anesthesia and resulted in death. The remaining rats in the control group exhibited poor condition even though they became awake from anesthesia. No active movement was observed; the hair was dry, and the breathing was slow and laborious. The response to external stimuli was weak, and there was no uptake of water. No animal from control group survived more than 24 h after surgery. 40% (6/15) of the rats in the low-dose MgIG treatment group died before waking up from anesthesia. The remaining rats showed better sign of life than the control group, in that the response to external stimuli was stronger, and some rats could uptake small volume of water. One of the animals survived longer than 24 h. 26.7% (4/15) of the rats in the high dose MgIG treatment group died shortly after surgery without woke up from anesthesia. The remaining animals showed slow active movement, uptake of water, and clean hair. Four rats survived longer than 24 h but none exceeded 60 h.

Survival time of the three groups was plotted using Kaplan-Meier survival curves, and the results were shown in Figure 1. Survival time of the control group was 8.9 ± 2.0 h with a median of 6.8 h, low-dose group was 10.3 ± 3.3 h with a median of 5.8 h, and high-dose group 22.0 ± 4.7 h with a median of 17.6 h. There were significant differences in survival time among the three groups, *P* = 0.018.

***Liver function assessment***

Liver function of the animal at various time points after hepatectomy was assessed by studying a variety of serum biomarkers including ALT, AST, GGT, TBIL, DBIL, TP, ALB, Glu, CRP, PT and TT.

As shown in Table 1, there were significant differences among the groups in ALT, Glu and PT levels starting from 6 h after surgery. The ALT levels were significantly lower in the MgIG treated groups than that of the control group. Both Glu and PT levels were significantly higher in the MgIG treated groups than the control. At 12 h time point, ALT, AST, TBil, DBil and TT levels showed significant difference between the MgIG treated groups and the control group. *P* values were *P* < 0.05 for TBIL and *P* <0.01 for all the rests. We also tested serum ALB and CRP at various time points after hepatectomy and found no significant difference.

***Liver regeneration***

Figure 2 showed the liver regeneration status at various time points after surgery. Proportion of weight of the remnant liver was found to be higher immediately after hepatectomy surgery compared with the normal proportion of liver to the body weight in all three groups, and the weight of remnant liver tissue increased over time. In the control group, 2.71% ± 0.58% increase was found at the time 0 h, and 11.95% ± 1.14% at the time of 18h. However, there were no significant differences among the three groups (Figure 2).

Liver cell degeneration and necrosis was observed in HE staining of the remnant liver sections (microscope, magnificaiton × 100) in all animals.Fatty degeneration was found in animals of both low dose and high dose MgIG treatment groups, but was more prominontin the high dose group. The representative HE staining of liver sections of the animal in the high-dose group at the 0 h, 6 h, 12 h and 18 h time points were shown in Figure 3. Hepatocytes were shown being normal size and shape, no obvious damage was found in nucleus at 0 h (Figure 3A). At 6h, cells showed little change in size or shape. Cell number increased, but the arrangement is slightly disordered (Figure 3B). At 12 h, more increase in cells number as well as more disordered arrangements was observed. There was a small number of cells with fatty degeneration, but no obvious liver cell necrosis(Figure 3C). More fatty degeneration and necrosis were observed at 18 h. The number of cells decreased, and the arrangement became irregular (Figure 3D). No obvious generative nodule was observed under microscope. Obvious fatty degeneration and necrosis were observed in all three groups at 12 h and 18 h time points.

As a thymidine analogue, BrdU can substitute thymidine to incorporate into the double-stranded DNA during DNA synthesis. Therefore, cells that have gone through S phase (DNA synthesis phase) would become BrdU positive, an indication of cell proliferation. As shown in Figure 4, the number of BrdU-positive cells among the three groups showed no significant difference. No obvious change in BrdU-positive cells in the control group at time points of 0 h, 6 h and 12 h. Slightly increase in BrdU-positive cells at 18 h, but not statistically significant. Similar patterns were observed in both MgIG treated high-dose and low dose group.

PCNA is an auxiliary protein for DNA synthase δ whose expression is cell cycle dependent. Its expression starts at late G1 phase and reaches to peak level at S phase. PCNA has been used as an indicator for cell proliferation. In this study, we used both immunohistochemistry to detect the percentage of PCNA-positive cells in the remnant liver tissue, as well as western blot to assess the overall expression levels of PCNA in the liver. Figure 5 showed that there is no significant difference in the percentage of PCNA-positive cells among the three groups. Over the course of 18 h, the number of PCNA-positive cells in all groups remained at a relative low level. As shown in Figure 6, there were no significant differences in the overall expression levels of PCNA among three groups indicated by western blot analysis.

***Inflammatory cytokines***

Inflammatory cytokines IL-1, IL-6, IL-10 and iNOs in serum were detected by ELISA. As shown in Figure 7, IL-1 levels at 6h after surgery was significantly higher in the serum of the rats of the MgIG treated groups than that of the control group (*P <* 0.05, Figure 7A). IL-6 level at 12 h was significantly lower in the serum of the rats of low dose group than that of the control group, and that in high dose group was significantly lower than the low dose group (*P <* 0.05, Figure 7B). The level of IL-10 was found to be significantly higher in high dose group at 18h than the other groups (*P <* 0.05, Figure 7C). No significant difference was found among the groups in iNOs expression at any time (Figure 7D).

***STAT3 levels in mRNA and protein***

STAT3 is an important transcription factor that can be activated in response to a variety of cytokines and growth factors. Since we found the elevated serum levels of IL-1 and IL-10 as well as reduced level of IL-6 in the rats of the high dose group, suggesting that MgIG may modulating the inflammation response. To further clarify the mechanism, we studied the STAT3 protein expression in the remnant liver tissue with Western Blot. At least 6 rats from each group were studied, Figure 8 showed one representative western blot. Densitometry analysis was performed upon the western blot and relative protein expression levels were calculated based on the level of β-actin. There was no significant difference in STAT3 levels among all three treatment groups at 0 h. The expression level of STAT3 in the high dose group at 6h was found to be significantly lower than the those of the control group as well as the low-dose group (*P* < 0.05). At 12 h, the high-dose group still exhibited lower STAT3 level compared with the control and the low-dose group, but not statistically significant. At 18h, STAT3 expressions of low-dose and high-dose groups were lower than the control group. The expression of STAT3 in control group was found to be increasing gradually over the course of 18 hours after hepatectomy, and significant differences exist between each time points (*P* < 0.01). No statistical significant differences in STAT3 levels, however, were found among different time points in the low dose group. In high dose group, STAT3 protein was inhibited initially, followed by gradually increase, and reached 4.5 times at 18 h over 0h time. This may explained the suppression of inflammatory reaction of the high-dose group.

Quantitative Real-time PCR was used to detect the relative levels of STAT3 mRNA, and results were shown in Table 2. At 0 h, no significant differences in STAT3 mRNA levels were seen among three groups. At both 6 h and 12 h, the levels of STAT3 mRNA in high dose group were significantly lower than the control and low-dose group (*P* < 0.05). At 18 h, STAT3 mRNA levels in rat livers of low-dose and high-dose groups were lower than that of the control group, but not statistically significant.

**DISCUSSION**

Glycyrrhizin is commonly used clinically as a liver protection medicine，MgIG is the fourth generation of glycyrrhizin preparations. It has better affinity with target cell receptors, and stronger anti-inflammatory and anti-oxidation effect. It has been shown to stabilize hepatocyte membrane and improve liver function[[7](#_ENREF_1" \o "Yu, 2004 #11),9].MgIG has shown effect of lower the liver toxicity of free fatty acid by preventing mitochondria damage[[10,](#_ENREF_1)11] and protecting hepatocyte from ischemia and reperfusion induced injury[[12,](#_ENREF_1)13].

Employing an improved version of Emond method[[14](#_ENREF_1" \o "Yu, 2004 #11)], we generated an animal model of 90% hepatectomy with 100% mortality within 24 h[[15](#_ENREF_1)], for the purpose of evaluating appropriate treatments.

Our results showed significantly longer survival time of rats in the treatment group than the control group. And the survival time seemed to be MgIG dose-dependent. Also, general appearance in treated group was also superior to the control group. By examining liver functions, it was found that MgIG demonstrated liver protection effect resulting in low ALT, AST, TBil, and DBil in the treated group. This study found that postoperative ALT, AST, TBil, TBil gradually increased to reach the peak after 18 h, which may be due to direct physical injury and surgery factors both ischemia and reperfusion injury. In the postoperative 0 h, the liver function and coagulation parameters have no significant differences among the three groups, implying that MgIG protected the liver function, but cannot have an immediate impact. It was found that the magnitude of increase in transaminase of treatment group was significantly lower than that in the control group at postoperative time points of 6 h and 12 h, with the high dose group being more obvious. These findings demonstrated protective effect of MgIG to the remnant liver after hepatectomy. Compared serum biochemical markers of normal rats and early death rats, we found that a rapid liver cells deterioration and significant raise of liver enzymes. It reflected an excessive inflammatory response and a severe necrosis of the residual liver cells after 90% hepatectomy, and confirmed the correspondence between early death and excessive inflammation in rats.

Liver regeneration after hepatectomy is one of the major mechanisms for compensating liver volume loss, maintaining sufficient liver function[16-18]. In this study, however, we did not find obvious regeneration, even though the weight of remnant liver tissue did increase over time which could be caused by physical response towards surgery, such as edema and congestion, and short time period of the study. It thus implies that MgIG improve the survival time of the rats with 90% hepatectomy mainly through the decreased inflammatory response rather than regeneration.

Excessive inflammatory reaction plays an important role in remaining liver damage in the postoperative outcome[19-21]. Inhibition of inflammatory reaction would be beneficial to the hepatocyte regeneration[[22-25](#_ENREF_1" \o "Yu, 2004 #11)]. MgIG may prolong the survival time after hepatectomy by enhancing liver regeneration and/or suppressing excessive inflammatory response[21,26,27]. We found that comparing to the control groups, the serum IL-10 levels were significantly increased in the MgIG treated groups, while IL-6 levels were significantly decreased. This indicated that MgIG may modulate inflammatory response in rats after hepatectomy, in which the inflammatory response in the MgIG treated group was inhibited. This may explain the reason that the MgIG treated groups had a prolonged survival time after hepatectomy.

JAK/STAT3 pathway, which plays a critical role in the inflammation response, can be activated by various cytokines including IL-6[28-30]. We found that the expression levels of STAT3 in remnant livers of the animals treated with MgIG was decreased comparing to the ones treated with saline. This could be one of the possible mechanisms for the inflammation inhibition. Further study is required to assess STAT3 function, as well as other related protein expression and function.

More fatty degeneration was found in the MgIG high dose treatment group with visible necrosis, indicating that MgIG might affect the fatty acid metabolism in severely injured liver. It is not known whether high dose of MgIG could have any side effect, since this is not observed in the MgIG low dose treatment group. The clinical significance of the fatty degeneration remains unclear. The prolonged survival time that the high dose group demonstrated is a collective result of the administration of the MgIG; however, since no animals survived longer than 60 h in this study, it is not clear whether there is any drawback of the use of the drug in the long run.

There were certain limitations in this study. The animal model was generated to an extreme of 90% resection, which probably will never occur in human being. Therefore, it would be hard to relate our result to clinical practice. Excessive hepatectomy might also limit the effect of MgIG. In addition, the dose-effect relationship is not clear, besides the high dose resulted in longer survival time. This creates more questions than answers such as: whether MgIG works through receptor binding on cell surface or directly on cellular proteins; what is the mechanisms for the decreased levels of ALT, AST, TBil, and DBil resulted from MgIG treatment.

In conclusion, MgIG application in excessive hepatectomy animal model resulted in prolonged survival time, reduced transaminases, total bilirubin, as well as the inflammation response. STAT3 pathway was inhibited in a way that the expression of STAT3 protein was decreased. The prolonged survival time could be potentially critical and lifesaving which created a valuable time window for other treatment application.

**COMMENTS**

***Background***

Excessive liver resection is the only hope of cure for large occupying liver lesions. Protection of the remnant liver function in first 48 h after surgery remains great challenge to the liver surgeons. The efficacy of various existing measures, including the artificial liver and other supporting methods, are often limited. Anti-overwhelming-inflammation in the remnant liver after hepatectomy may be of benefit for the patients.

***Research frontiers***

Previous study in rat found that liver regeneration reaches to the highest level at 72 h after resection. Animal will most likely survive if the regeneration could compensate for the first 48 h. Therefore, to identify appropriate and effective measures to help the remnant liver to sustain through this risky period is particularly important. Magnesium Isoglycyrrhizinate (MgIG), a hepatocyte protective agent, has been shown to have the effect of anti-inflammation, liver cell membrane protection, and liver function improvement. Most of the previous studies of MgIG have focused on chronic hepatitis, alcoholic cirrhosis, and drug-induced liver injury. A few researches mainly involved in surgical ischemia-reperfusion injury and liver regeneration. This study was designed to investigate the protective effect of MgIG on excessive hepatectomy animal modal and its possible mechanism.

***Innovations and breakthroughs***

This is the first to report the anti-inflammatory effect of MgIG after liver resection. The protective effect of MgIG has been shown to prolong the survival time following standard 90% hepatectomy. This hepatoprotective effect was not *via* an increase in hepatocyte regeneration, rather through inhibition in the inflammatory response through *via* STAT3 pathway.

***Applications***

The protective effect of MgIG in standard 90% hepatectomy can prolong the survival time. This study provides experimental evidence with potential benefits for the further mechanism researches or clinical studies.

***Terminology***

Liver regeneration rate was calculated by the Okano T formula: Regeneration rate (R, %) = [C- (A-B)]/(A-B) × 100%, where A is preoperative estimation of the rat liver weight, B is the weight of resected liver tissue, and C is the weight of the remnant caudate lobe.

***Peer-review***

This is an interesting study which was designed to investigate the magnesium isoglycyrrhizinate inhibits inflammatory response through STAT3 pathway to protect remnant liver function. In this study, SD rats with 90% liver resection were divided into three groups. The postoperative survival time, hepatocyte regeneration, liver function, serum inflammatory cytokines and STAT3 protein were analyzed. They found that high-dose MgIG can extend survival time in rat after excessive hepatectomy. And the hepatoprotective effect was not by increase hepatocyte regeneration but rather by inhibiting the inflammatory response through inhibition of STAT3 pathway. The study is well designed and conducted, and the results are reliable and interesting.

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Fig1.tif

**Figure 1 Kaplan-Meier survival curves of the three experimental animal groups.**

**Fig2.tif**

**Figure 2 Regeneration rate over varies time points in three groups.**

Fig3.tif

**Figure 3 Hematoxylin-eosin staining in the high-dose group at the 0 h, 6 h, 12 h and 18 h time points (magnification is × 100).**

Fig4.tif

**Figure 4 BrdU positive rate of the caudate lobe hepatocyte at varies time points in three groups**

Fig5.tif

**Figure 5 PCNA positive rate of the caudate lobe hepatocyte at varies time points in three groups.**

Fig6.tif

**Figure 6 Expression of PCNA in caudate lobe detected by Western blot at varies time points in three groups.**

Fig7.tif

**Figure 7 Expression of cytokines IL-1, IL-6, IL-10 and iNOS at varies time points in three groups.**

Fig8.tif

**Figure 8 Expression of STAT3 in caudate lobe detected by Western blot at varies time points in three groups.**

**Table 1 Serological test results of experimental animals after excessive liver resection**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **Time points (mean ± SD)** | | | | |
| **Item** | **Group** | **0 h** | **6 h** | **12 h** | **18 h** | ***P* value1** |
|  | Control | 165.8 ± 118.0 | 864.0 ± 471.7 | 1927.5 ± 1079.0 | 1969.6 ± 1201.6 | 0.000 |
| ALT (U/L) | Low dose | 86.4 ± 23.4 | 357.2 ± 119.8 | 705.1 ± 341.5 | 1597.7 ± 998.6 | 0.000 |
|  | High dose | 145.6 ± 106.8 | 524.7 ± 182.5 | 1234.8 ± 784.2 | 1321.2 ± 797.4 | 0.000 |
|  | *P* value2 | 0.133 | 0.002 | 0.003 | 0.300 |  |
|  | Control | 228.4 ± 110.4 | 929.3 ± 477.7 | 1779.5 ± 495.9 | 1673.2 ± 668.8 | 0.000 |
| AST (U/L) | Low dose | 192.6 ± 37.5 | 664.7 ± 171.5 | 1027.5 ± 396.9 | 1904.3 ± 794.7 | 0.000 |
|  | High dose | 193.1 ± 99.5 | 694.6 ± 278.7 | 1370.6 ± 502.7 | 1355.2 ± 671.5 | 0.000 |
|  | *P* value | 0.556 | 0.158 | 0.001 | 0.192 |  |
|  | Control | 1.11 ± 0.93 | 1.50 ± 0.85 | 2.14 ± 1.51 | 1.55 ± 0.93 | 0.197 |
| GGT (U/L) | Low dose | 0.70 ± 0.48 | 0.88 ± 0.35 | 1.27 ± 1.27 | 2.89 ± 2.42 | 0.008 |
|  | High dose | 0.72 ± 0.47 | 1.00 ± 0.71 | 2.36 ± 1.21 | 2.00 ± 1.08 | 0.001 |
|  | *P* value | 0.316 | 0.154 | 0.147 | 0.158 |  |
|  | Control | 50.7 ± 4.9 | 47.3 ± 3.6 | 46.3 ± 4.8 | 45.5 ± 3.6 | 0.031 |
| TP (g/L) | Low dose | 48.3 ± 2.9 | 45.0 ± 2.9 | 44.9 ± 3.2 | 44.6 ± 3.8 | 0.039 |
|  | High dose | 51.6 ± 3.3 | 45.5 ± 2.4 | 48.5 ± 3.6 | 45.6 ± 5.8 | 0.001 |
|  | *P* value | 0.101 | 0.212 | 0.094 | 0.849 |  |
|  | Control | 1.95 ± 0.62 | 6.68 ± 1.32 | 16.59 ± 2.16 | 20.13 ± 4.19 | 0.000 |
| TBil (U/L) | Low dose | 1.20 ± 0.30 | 4.35 ± 0.77 | 7.45 ± 4.66 | 14.82 ± 2.06 | 0.000 |
|  | High dose | 1.67 ± 0.43 | 6.23 ± 2.22 | 14.31 ± 2.16 | 12.81 ± 2.29 | 0.000 |
|  | *P* value | 0.539 | 0.532 | 0.011 | 0.206 |  |
|  | Control | 1.22 ± 0.37 | 4.27 ± 0.64 | 13.56 ± 1.83 | 16.33 ± 3.46 | 0.000 |
| DBil (U/L) | Low dose | 0.65 ± 0.15 | 2.88 ± 0.47 | 5.25 ± 1.01 | 11.15 ± 1.72 | 0.000 |
|  | High dose | 0.99 ± 0.26 | 4.62 ± 1.82 | 10.95 ± 1.59 | 9.72 ± 1.80 | 0.000 |
|  | *P* value | 0.353 | 0.527 | 0.004 | 0.140 |  |
|  | Control | 7.41 ± 1.27 | 4.92 ± 2.54 | 6.83 ± 2.33 | 4.21 ± 1.40 | 0.001 |
| Glu (mmol/L) | Low dose | 6.50 ± 1.21 | 6.30 ± 1.62 | 6.09 ± 1.84 | 4.11 ± 2.13 | 0.012 |
|  | High dose | 7.12 ± 2.05 | 8.54 ± 3.69 | 5.25 ± 2.19 | 5.21 ± 1.02 | 0.002 |
|  | *P* value | 0.395 | 0.016 | 0.162 | 0.149 |  |
|  | Control | 42.7 ± 6.1 | 42.0 ± 4.6 | 47.8 ± 10.1 | 41.2 ± 10.0 | 0.754 |
| TT (s) | Low dose | 48.9 ± 3.5 | 49.7 ± 6.6 | 49.5 ± 5.3 | 48.4 ± 0.4 | 0.984 |
|  | High dose | 38.2 ± 6.1 | 46.2 ± 2.0 | 11.5 ± 1.1 | 43.2 ± 1.1 | 0.000 |
|  | *P* value | 0.127 | 0.226 | 0.001 | 0.360 |  |
|  | Control | 8.9 ± 0.6 | 10.3 ± 0.3 | 11.7 ± 1.1 | 12.4 ± 0.4 | 0.001 |
| PT (sec) | Low dose | 9.9 ± 0.6 | 10.9 ± 0.5 | 12.6 ± 1.9 | 14.2 ± 1.6 | 0.016 |
|  | High dose | 9.1 ± 0.1 | 12.6 ± 0.7 | 11.5 ± 1.1 | 14.8 ± 0.8 | 0.000 |
|  | *P* value | 0.077 | 0.003 | 0.618 | 0.076 |  |

1Comparison among time points of same group; 2Comparison among groups at same time point. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PT: Prothrombin time; TT: Thrombin time; TBil: Total bilirubin; Glu: Glucose; DBil: Direct bilirubin.

**Table 2 STAT3 mRNA levels at varies time points after excessive liver resection in rats**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **STAT3 mRNA expression (mean ± SD)** | | | | | | | | ***P* value1** |
| **Group** | 0 h | *n* | 6 h | *n* | 12 h | *n* | 18 h | *n* |
| Control | 0.33 ± 0.11 | 3 | 0.43 ± 0.21 | 3 | 0.57 ± 0.24 | 3 | 1.07 ± 0.47 | 3 | 0.021 |
| Low dose | 0.41 ± 0.15 | 3 | 0.44 ± 0.19 | 3 | 0.78 ± 0.34 | 3 | 0.89 ± 0.45 | 3 | 0.043 |
| High dose | 0.38 ± 0.24 | 3 | 0.11 ± 0.07 | 3 | 0.30 ± 0.14 | 3 | 0.87 ± 0.36 | 3 | 0.025 |
| *P* value2 | 0.401 | | 0.012 | | 0.044 | | 0.849 | |  |

1Comparison among time points of same group; 2Comparison among groups at same time point.