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Effect of Kupffer cells on immune tolerance in liver transplantation

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ABSTRACT

Objective: To observe the effect of Kupffer cells on immune tolerance in liver transplantation. **Methods:** The rats were randomly divided into A, B and C groups. A group was sham operation group. The donor rats of group B had intraperitoneal injection of 1 nmol Kuppffer cells every other day for three days before liver transplantation. Rats of group C were injected with equal saline. The rat liver transplantation models were established by modified Kamada´s two-cuff technique. The rats were sacrificed after 24 hours. The concentrations of ALT and AST in serum were measured with the biochemical analyzer. The level of IL-2 and TNF- α in serum were measured by ELISA method. The apoptotic indexes were detected by immunohistochemical assay. **Results:** The concentration of ALT, AST, IL-1 and TNF- α in A, B and C groups were increased successively. The levels of group C were significantly higher than that of group B and A ($P<0.05$), and the levels of group B were significantly higher than that of group A ($P<0.05$). The apoptotic indexes of three groups were 3.40±0.37, 14.70±2.54 and 26.33±3.65, respectively, with significant difference among three groups ($P<0.05$). **Conclusions:** Pretreatment with Kupffer cells can reduce liver injury and raise liver transplantation immune tolerance.

1. Introduction

Liver transplantation is effective treatment for advanced liver diseases, but Immune rejection is major obstacle after transplantation. Immune tolerance to transplanted liver is important for liver transplant. Kupffer cells (KC) are settled in sinusoidal mononuclear macrophages, which account for 10%-15% of total liver cells and 80%-90% of all monocyte-macrophage cells. Liver KC can engulf and kill pathogenic microorganisms, rid of endotoxin. It has effects of antigen presentation, secretion of cytokine sand immune regulation[1]. Recently it has found that KC can induce T lymphocyte apoptosis and play an important role in the regulation of liver transplantation tolerance[2]. In this study, the donor rats were pretreated by liver KC to explore protective effect of KC on transplanted rat liver, discuss the relationship between immune tolerance the KC.

2. Materials and methods

2.1. Experimental animals and KC

A total of 24 male SD rats of the clean grade weighted 250-300 g were used for the test after adaptive feeding for 5 days. All rats were provided by Medical Animal Experimental Center of Guangdong Province. KC of rat liver were obtained by Collagenase isolated perfused method[3].

2.2. Experimental methods

The rats were randomly divided into three groups, with 8 rats in each group. A group was sham operation group, and the rats had abdominal surgery of free hepatic ligaments around. Group B served as experimental group (KC group). The donor rats had intraperitoneal injection of 1 nmol KC every other day for three days before transplantation. C group was control group. The donor rats were injected with equal saline. All rats had liver transplantation by modified Kamada's two-cuff technique[4], and were killed after 24 hour. Plasma was extracted and the concentrations of alanine

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aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by with Hitachi 7170 automatic biochemical analyzer, while iterleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α) were measured by ELISA method.

Liver pathological analysis was observed with immunohistochemical methods. Liver tissues were fixed in 4% paraformaldehyde, embedded in paraffin, then were sliced and HE stained. The apoptosis of liver tissue slice was measured by d-UTP nick end labeling staining method and apoptotic indexes were calculated. Pathological changes were observed under the optical microscope. Positive apoptotic cells showed brown staining in nucleus and part of the cytoplasm may be brown because of the escape of nuclear DNA fragmentation. Five high power optical fields were observed in each film. The number of positive cells were counted in 100 cells per field as apoptotic index[5].

2.3. Statistical analysis

SPSS11.0 statistical software was used. The data was analyzed by *t*-test and χ^2 test.

3. Results

3.1. Concentration f ALT and AST in serum

Concentrations of ALT and AST in A, B and C groups were increased successively. Group A was the lowest [(45.27±7.55) U/L and (86.62±7.43) U/L], while group C was the highest [(546.66±19.78 U/L and (656.91±22.56) U/L). The concentrations of ALT and AST of the group C were significantly higher than group B [(234.57±16.07) U/L and (318.68±21.69) U/L) and A (P<0.05). The difference between group A and B was also significant (P<0.05).

3.2. IL-1 and TNF-α

The level of IL-1 and TNF-α of group C [(335.48±36.45) U/L, (205.82±24.50) U/L] were significantly higher than group B [(105.77±21.26) U/L, (76.23±10.63) U/L] and group A [(36.73±5.13) U/L, (5.96±1.36) U/L] (P <0.05). The difference between group A and B was also significant (P<0.05).

3.3. Apoptotic index

The apoptotic indexes of three groups were 3.40 ± 0.37, 14.70 ± 2.54 and 26.33 ± 3.65, respectively, which of group C was significantly higher than those of the group B and group A (P<0.05). The difference between group A and B was also significant (P<0.05). Positive apoptotic cells showed brown staining of the nucleus. The quantity of apoptosis cell of the group C was significantly increased and the group A was the least.

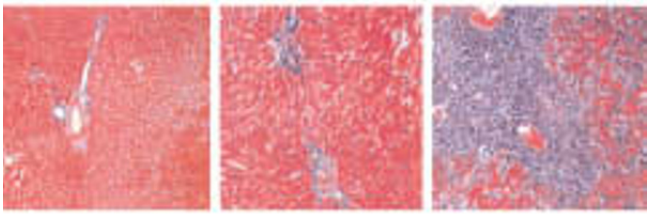

Figure 1. Expression of liver apoptotic cell (DAB×400).
A: Group A, B: Group B; C: Group C.

4. Discussion

Ischemia-reperfusion injury of donor liver away from the body in transplantation process is a main reason for primary nonfunction post-operative graft. Hepatic KCs participate in multiple aspects of warm ischemia-reperfusion injury. It can reduce inflammatory cytokine production, restore calcium homeostasis of self-regulating system and reduce mitochondrial damage, thereby reduce reperfusion injury[6]. Pretreated donor liver in the liver transplantation is a valuable method. KCs have protective effects on liver transplantation. They are involved in the immune response of the transplanted liver. The donor liver pretreated with KCs can raise HO-1 expression and reduce the stress response, protect the liver tissue[7].

ALT is mainly presented in the cytoplasm of liver cells, and is the most sensitive indicator of liver function tests. The concentration of AST in the myocardium is the highest, followed by the liver. As massive liver tissuenecrosis, AST was released from mitochondrial and AST in serum will be significantly increased[8]. In this experiment, the concentration of ALT and AST in group C are higher than group B and group A. ALT and AST concentrations of the control group is 2.33 and 2.06 times higher than that of group B (P <0.05). It showed that the liver transplantation pretreated with KC can reduce ALT and AST concentrations and suggested protective effects on the liver transplant.

IL-1 and TNF-α play an important role in the inflammatory process of okines[9]. TNF-α is one of the most important cytokines released by activated KCs and has a dual role inhepatic cells. It is involved in immune regulation, maintaining a certain level of TNF-α in the early injury. It can protect hepatic cells from apoptosis and itcan promote conducive liver regeneration and repair. But as TNF-α continued to increase, it can cause liver

damage. The experimental results showed the level of TNF-α of group B than the group A, and A, B, C group increased successively. It indicated that KCs pretreated liver transplantation can reduce TNF-α level to protect liver cells from apoptosis. IL-1 is the initiation factor for immune regulation and inflammatory response. It was significantly increased after graft reperfusion. The experimental results show that the level of IL-1 of group B was significantly lower than group A (P <0.05), suggesting that liver transplantation pretreated with KCs can reduce the level of IL-1, reduce reperfusion resistance, improve blood rheology, reduce microcirculation and liver tissue injury.

Apoptosis is the active process of the cells death. The prominent changes are endonuclease activation which leads to the chromosomal DNA of controlled degradation. In this study, the apoptotic cells from tissue sections were of nuclear dyed dark brown. Apoptosis amount in group A is at least and the highest in group C. The group B was significantly lower than group A (P <0.05). It is consistent with the results from Kobayashi *et al*, who showed after reperfusion in rat liver the live cells can cause protective effect [10].

In conclusion, the liver transplantation pretreated with KCs can reduce concentrations of ALT and AST, reduce the level of TNF-α and IL-1, which have protective effect on the liver cells. It can reduce hepatic apoptosis, enhance the immune tolerance of the transplanted liver and improve the survival rate of liver transplant.

Conflict of interest statement

We declare that we have no conflict of interest.

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