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Effects of Recombinant Growth Hormone Preconditioning on Ischemia-Reperfusion Injury in Rat Liver

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Abstract: To investigate the effects of recombinant Growth Hormone (rGH) preconditioning on rat liver ischemia-reperfusion injury. After rat hepatic ischemia-reperfusion model was established, animals were divided into experimental group, control group and sham group (n = 10 each group). The experimental group was subcutaneously injected with rGH (1 U/kg/day) for 1 week while the control group was given the same volume of saline. Perfusion was recovered at 40 min after portal triad clamping in both experimental and control groups. The serum levels of Tumor Necrosis Factor α (TNF-α) and Interleukin 1β (IL-1β) and Super Oxygen Dehydrogenises (SOD) and Methane Dicarboxylic Aldehyde (MDA) in liver homogenate were examined 2 and 24 h after reperfusion. Liver ultrastructural changes were observed under electron microscopy and Apoptosis Indexes (AIs) were determined by terminal-deoxynucleotidyl Transferase Mediated Nick End Labeling (TUNEL) staining. TNF-α levels were lower in the experimental group compared to the control group ($p<0.05$) while there was no significant difference in IL-1β level between the 2 groups. MDA levels in the experimental group were significantly lower than in the control group at 2 and 24 h after reperfusion ($p<0.05$) SOD levels between the groups were not different. The AI of hepatic cells in the control group was significantly higher than in the experimental group ($p<0.05$). Liver mitochondria of the control group were in disorder and apoptotic bodies had formed; these phenomena were not found in the experimental group. Recombinant growth hormone preconditioning can protect against ischemia-reperfusion injury in rat liver.

Key words: Recombinant growth hormone, ischemia-reperfusion injury, liver, MDA, SOD

INTRODUCTION

During liver transplantation there is a period of warm liver ischemia and restoring the blood supply can aggravate the liver damage induced by ischemia. After liver transplantation, hepatic ischemia-reperfusion injury is one of the important causes of allograft non-function and delayed liver function recovery (Busuttil and Tanka, 2003). Growth Hormone (GH) is a polypeptide consisting of 191 amino acids secreted by anterior pituitary cells. After binding to its receptor, through a series of signal transductions GH induces the transcription of target genes and promotes hepatic synthesis and secretion of Insulin-Like Growth Factor 1 (IGF-1) (Salvatori, 2004). Recent studies have shown that IGF-1 can protect some organs from an ischemia-reperfusion injury, significantly reduce apoptosis after myocardial ischemia-reperfusion injury and promote mitochondrial ATP synthesis (Lisa *et al*., 2011). Studies have found that the application of GH can significantly reduce the quantity of translocated bacteria in small mesenteric lymph nodes and parenteral organs such as the lung, liver and kidney, reduce TNF-α levels and enhance the intestinal barrier function (Arii *et al*., 2003; Yapicioglu *et al*., 2006). In this study, a rat model of warm ischemia-reperfusion liver injury was established to observe the effect of recombinant Growth Hormone (rGH) preconditioning on rat liver ischemia-reperfusion injury.

MATERIALS AND METHODS

Experimental animals and grouping: Thirty healthy and clean male Sprague-Dawley (SD) rats weighing about 280-300 g were provided by the Animal Laboratory of People's Hospital of Peking University. All animals were treated according to standard guidelines for the ethical use of laboratory animals. The rats were randomly divided into an experimental group, a control group and a sham group (n = 10 each group). The experimental group was subcutaneously injected with 1 U/kg/day of rGH (Saizen, Merck Serono, Swiss) for 7 consecutive days before surgery. The control group was injected with the same volume of saline. In the sham group, the abdomen was opened and the hepatic portal was identified but not

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blocked. At surgery, both experimental and control group were treated by blocking the hepatic pedicles for 40 min then allowing reperfusion.

Model preparation: Animals were fasted for 12 h before surgery and allowed drinking water. Ether inhalation combined with intramuscular injection of ketamine (100 mg kg^{-1}) anesthesia was carried out, a midline incision in the upper abdomen was made and the abdominal wall layers successively cut. The hepatic pedicles were exposed and the falciform ligament was transected. The hepatic pedicles were occluded with non-traumatic vascular clamps in the experimental and control groups and the clamps were released after 40 min. The animals were sacrificed to obtain samples for testing at 2 and 24 h after reperfusion.

Specimen collection and testing: After sacrifice of the animals at the designated time points, 5 mL of blood was taken from the inferior vena cava and was centrifuged at 2500 rpm for 10 min. The serum was then preserved at -80°C until testing. A specimen of liver (~100 mg) was obtained and stored in liquid nitrogen and 10% for malin. Another specimen of about 20 mg of liver tissue was also obtained and stored in 4% formaldehyde solution. The serum was subsequently assayed for determination of the levels of Tumor Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β) in accordance with the ELISA kit (RD, USA) manufacturer's instructions. The 100 mg liver tissue stored in liquid nitrogen was homogenized and assayed for Methane Dicarboxylic Aldehyde (MDA) and Superoxide Dismutase (SOD) in accordance with the kit (Jiancheng Bioengineering Institute, Nanjing) manufacturer's instructions. The second liver tissue specimen was fixed with 4% glutaraldehyde, dehydrated gradually, fixed with 1% osmic acid, embedded in epoxy resin, cut into thin slices with an ultramicrotome and doubly stained with uranium acetate and lead citrate. The ultramicrostructure was observed under transmission electron microscopy and the morphology and distribution of apoptotic cells were observed by the terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (In *Situ Cell* Death Detection kit, Boehringer Mannheim, Germany) method. Ten non-overlapping fields of view were observed under a 400x objective lens and the Apoptotic Index (AI) was obtained from the ratio of positive cells to total cell number.

Statistical analysis: Data were expressed as mean±standard deviation and compared using the independent samples t-test. All analyses were performed with the SPSS 12.0 Statistical Analysis Software. A value of $p<0.05$ was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Serum TNF-α and IL-1β levels: TNF-α levels in the experimental group were lower than in the control group at 2 and 24 h after reperfusion ($p = 0.049$, $p = 0.049$) whereas IL-1β levels at the 2 time points were not different (Table 1). IL-1β levels in both the experimental and control groups were higher than those in the sham group.

MDA and SOD levels in liver homogenates: MDA levels in the experimental group at 2 and 24 h after reperfusion were significantly lower than in the control group ($p = 0.049$, $p = 0.046$) whereas SOD levels were not different ($p = 0.135$, $p = 0.090$) (Table 2). SOD contents in control and experimental groups were significantly lower than those in the sham group ($p = 0.001$).

TUNEL staining of liver tissues: The results of TUNEL staining are shown in Fig. 1. Large amounts of visible brown particles appeared in hepatic nuclei in the control group at 24 h after hepatic ischemia-reperfusion while the normal liver tissues were damaged and infiltrated with a large number of inflammatory cells. The extent of liver damage in the experimental group was significantly less than in the control group the number of positive-stained nuclei was less than in the control group and the AI value was significantly lower ($p = 0.030$).

Electron microscopy: Electron microscopy (Fig. 2) showed that the liver mitochondria of the control group were in disorder, the mitochondria ridge had disappeared, the cell nucleus had broken and ruptured, chromosome fragments spilled over and apoptotic bodies had formed

Table 1: TNF-α and IL-1β levels in different groups

	TNF-α (pg mL^{-1})		IL-1β (pg mL^{-1})	
Groups	2 h after reperfusion	24 h after reperfusion	2 h after reperfusion	24 h after reperfusion
Experiment	230.92±62.37*	66.95±28.82*	4.14±1.29	1.07±0.58
Control	343.99±73.32	123.73±28.72	7.52±2.49	2.03±0.69
Sham	0	0	0	0

Table 2: MDA and SOD levels in liver homogenates of different groups

	MDA (nmol mg^{-1} port)		SOD (nmol mg^{-1} port)	
Groups	2 h after reperfusion	24 h after reperfusion	2 h after reperfusion	24 h after reperfusion
Experiment	7.59±0.55*	6.71±0.85*	181.90±32.8	209.25±17.1
Control	8.33±0.62	7.65±0.65	187.00±29.7	200.42±28.0
Sham	1.34±0.12	1.31±0.14	9.53±0.38	9.49±0.41

*$p<0.05$ compared with control group

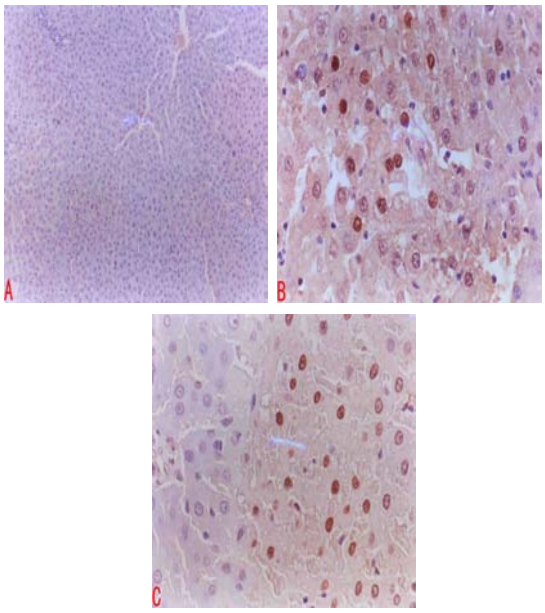

Fig. 1: A) TUNEL staining in sham group. Brown staining of apoptotic nuclei was not seen and the liver tissue structure was normal (100x); B) TUNEL staining at 24 h after hepatic ischemia-reperfusion in the control group. A large number of nuclei with yellow-brown staining are seen which consists of a single apoptotic nucleus or apoptotic body. Disorganized liver structure, unclear delineation of hepatic cords and sinusoids, liver cell degeneration, necrosis and inflammatory cell infiltration are noted (400x); C) TUNEL staining at 24 h after hepatic ischemia-reperfusion in the experimental group. The number of apoptotic bodies and single apoptotic nuclei was significantly less than that in the control group in the same field of view. The liver structure was relatively clear with mild hepatic edema and inflammatory cell infiltration (400x)

these phenomena were not found in the experimental group. Mitochondrial swelling was only found at 24 h after ischemia-reperfusion in the experimental group but the mitochondrial structure was basically normal a small amount of hepatocyte nuclear chromatin was noted as a marginal phenomenon but no nucleus fracture or rupture was observed.

Hepatic ischemia-reperfusion injury is a pathological process in liver transplantation (De Rougemont *et al*., 2010) and is an inflammatory response that is mediated by a series of pro-inflammatory cytokines and oxygen free radicals (Ohkohchi, 2002). The exact pathogenesis is not

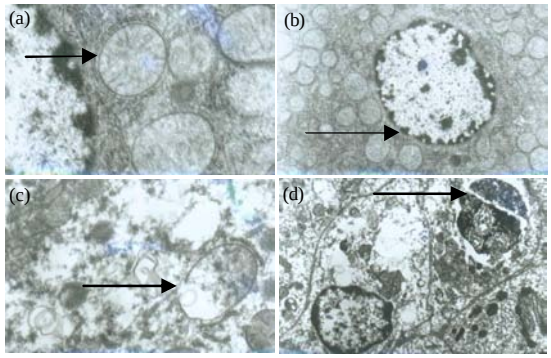

Fig. 2: a, b) Electron micrographs at 24 h after liver ischemia-reperfusion in the experimental group. Liver cytoplasmic edema and mitochondrial swelling (a, arrow), vacuolization of mitochondria and dilation and vesiculation of the endoplasmic reticulum can be partially observed. b) Approximately normal liver nuclei with the chromatin concentrated in the edge of the nucleus (arrow). c, d) Electron micrographs at 24 h after liver ischemia-reperfusion in the control group. c) Severe hepatic edema, dilation and vesiculation of the endoplasmic reticulum and ridge disappearance (arrow) are noted. d) Broken nuclei (arrow), spillover of chromosome fragments and formation of apoptotic bodies are seen

well understood. TNF-α and IL-1β are cytokines that play important roles in the inflammatory response (Abu-Amara *et al*., 2010). Oxygen free radicals can attack polyunsaturated fatty acids in biological membranes and induce lipid peroxidation to produce lipid peroxides such as MDA which cause cell injury. The amount of MDA often reflects the degree of lipid peroxidation in the body and indirectly reflects the degree of cell damage. SOD is an endogenous antioxidant produced by the body (Bhogal *et al*., 2011). Researchers established a rat warm liver ischemia-reperfusion injury model to evaluate the effects of rGH on liver injury by observing changes of TNF-α, IL-1β, SOD, MDA and hepatic AI all of which can reflect the degree of liver injury. It was observed that MDA levels in the experimental group were significantly lower than those in the control group while there was no significant difference in SOD levels between the 2 groups. The protective effects of GH might be achieved by reducing the generation of oxygen free radicals and alleviating lipid peroxidation but not by increasing the SOD level. TNF-α in hepatic ischemia-reperfusion injury is mainly produced by activated Kupffer cells. It can lead directly to swelling of the liver sinusoidal endothelial cells

and also can cause liver microcirculation disorder through the interaction of neutrophils and endothelial cells (Perry *et al*., 2011). TNF-α levels in the experimental group were significantly lower than those in the control group, indicating that GH could inhibit the production of TNF-α. (Basoglu *et al*., 2002) speculated that GH might reduce the production of TNF-α by binding to cell surface receptors, inducing tyrosine phosphorylation and activating the intracellular tyrosine protein kinase thus, changing the phosphorylation levels in the cytoplasm and nucleoproteins. In addition, GH can protect the mucosal barrier against intestinal ischemia-reperfusion injury, reduce the rate of intestinal bacterial translocation and lower serum levels of endotoxins (Haglind *et al*., 1998) thereby reducing the activation of Kupffer cells and thus, indirectly reducing TNF-α levels. IL-1β is one of the important inflammatory mediators in Systemic Inflammatory Response Syndrome (SIRS) and it can induce the production of peroxide, induce monocytes and polymorphonuclear cells to move chemotactically to the area of inflammation and mediate the formation of local inflammation (Wanderer, 2009). Blocking the IL-1β receptor can reduce hepatic ischemia-reperfusion injury (Koyano *et al*., 1997) but no change of IL-1β was observed in the experiment. This suggests that rGH does not reduced hepatic ischemia-reperfusion injury by reducing the generation of IL-1β; the specific mechanism requires further study. The liver is a target organ of GH action and GH promotes hepatic synthesis of IGF-1 (Takahashi, 2012). Recent data has indicated that IGF-1 can significantly reduce apoptosis after ischemia-reperfusion injury enhance, the expression of anti-apoptotic gene *bcl-2* and promote mitochondrial ATP synthesis (Rizk *et al*., 2007). In the experiment, damage of the cell ultrastructure in the experimental group was significantly reduced compared to that in the control group and the AI was significantly lower than that in the control group which indicates that rGH inhibits cell apoptosis during hepatic ischemia-reperfusion injury and affords a protective role.

CONCLUSION

Results of this study indicate that rGH may reduce hepatic ischemia-reperfusion injury and provides the foundation for a new treatment method to improve the prognosis of critically ill patients after liver transplantation.

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REFERENCES

Abu-Amara, M., S.Y. Yang, N. Tapuria, B. Fuller, B. Davidson and A. Seifalian, 2010. Liver ischemia/reperfusion injury: Processes in inflammatory networks-a review. Liver Transpl., 16: 1016-1032.

Arii, S., K. Teramoto and T. Kawamura, 2003. Current progress in the understanding of and therapeutic strategies for ischemia and reperfusion injury of the liver. J. Hepatobiliary Pancreatic Surgery, 10: 189-194.

Basoglu, M., A. Kiziltunc, M.I. Yildirgan, K. Gumustekin, M. Gumus, A. Yildirim and S.S. Atamanalp, 2002. Recombinant human growth hormone modulates the hepatic acute-phase response and P-selectin in burned rats. Burns, 28: 760-764.

Bhogal, R.H., R. Sutaria and S.C. Afford, 2011. Hepatic liver ischemia/reperfusion injury: Processes in inflammatory networks-a review. Liver Transpl, Vol. 17, 10.1002/lt.22205.

Busuttil, R.W. and K. Tanaka, 2003. The utility of marginal donors in liver transplantation. Liver Transplantation, 9: 651-663.

De Rougemont, O., P. Dutkowski and P.A. Clavien, 2010. Biological modulation of liver ischemia-reperfusion injury. Curr. Opin. Organ Transplantation, 15: 183-189.

Haglind, E., K. Malmlof, J. Fan and C.H. Lang, 1998. Insulin-like growth factor-I and growth hormone administration in intestinal ischemia shock in the rat. Shock, 10: 62-68.

Koyano, T., T. Takahashi, I. Takeyoshi, Y. Hasegawa and Y. Sato *et al*., 1997. Effect of TNF-α and IL-1β production inhibitor, FR167653 on reperfusion injuries-experiment with preserved heart transplantation. Nihon Geka Gakkai Zasshi, 98: 795-795.

Lisa, M., N. Haleagrahara and S. Chakravarthi, 2011. Insulin-Like Growth Factor-1 (IGF-1) Reduces ischemic changes and increases circulating angiogenic factors in experimentally-induced myocardial infarction in rats. Vasc Cell, Vol. 3.

Ohkohchi, N., 2002. Mechanisms of preservation and ischemic/reperfusion injury in liver transplantation. Transplant Proc, 34: 2670-2673.

Perry, B.C., D. Soltys, A.H. Toledo and L.H. Toledo-Pereyra, 2011. Tumor necrosis factor-α in liver ischemia/reperfusion injury. J. Invest. Surg., 24: 178-188.

Rizk, N. N., J. Myatt-Jones, J. Rafols and J.C. Dunbar, 2007. Insulin like growth factor-1 (IGF-1) decreases ischemia-reperfusion induced apoptosis and necrosis in diabetic rats. Endocrine, 31: 66-71.

Salvatori, R., 2004. Growth hormone and IGF-1. Rev. Endocr. Metab. Disord., 5: 15-23.

Takahashi, Y., 2012. Essential roles of Growth Hormone (GH) and Insulin-like Growth Factor-I (IGF-I) in the liver. Endocr. J., 59: 962-962.

Wanderer, A.A., 2009. Rationale for IL-1beta targeted therapy for ischemia-reperfusion induced pulmonary and other complications in sickle cell disease. J. Pediatr. Hematol. Oncol., 31: 537-538.

Yapicioglu, H., M. Satar, N. Canacankatan, E. Tutak, Y. Sertdemir, E. Antmen and N. Narli, 2006. The effect of human growth hormone on superoxide dismutase activity, glutathione and malondialdehyde levels of hypoxic-ischemic newborn rat brain. Biol. Neonate, 90: 168-173.