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• BRIEF REPORT •

Prevention of grafted liver from reperfusive injury

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INTRODUCTION

The incidence of primary non-function (PNF) of grafted liver in the early postoperative stage is 2% - 23%[1-4], its main cause is the ischemic-reperfusive injury[5,6]. In this experiment, anisodamine was added into the preserving fluid and the grafted liver was rewarmed at different temperatures to protect the cell membrane and prevent ischemic-reperfusive injury.

METERIALS AND METHODS

Selection and grouping

Twenty male Wistar rats (270g - 330g in weight, 10 - 12 weeks in age) were used in the experiment. The rats were divided into 2 groups, 10 in each group, and the action of anisodamine was studied. In the experimental group, 40mg anisodamine was added into 1 liter of preserving fluid, no anisodamine was used in the control group. The rats were divided into 4 groups, 5 in each group, and the action of rewarming was studied. Before reperfusion, the 12℃, 20℃, 28℃ and 36℃ of gelofusine were injected into the portal vein respectively to rewarm the grafted liver.

Establishment of animal model[7-9]

Make a midline epigastric incision, dissociate the liver fully, incise the infrahepatic inferior vana cava (IVC), input a shaped three way stopcock, make its upper end 5mm higher than liver, ligate the both ends of IVC incision, and obstruct hepatic artery[10]. Cut off the portal vein, connect its distal end with one opening of the three-way stopcock, shunt the portal-cava vein provisionally, and reverse the blood of IVC and portal vein to the heart through the duct. Inject proximately 5 mL saline mixed with 1 mL heparin. Ligate the suprahepatic inferior vana cava provisionally, release the ligature above the IVC incision, wash the liver through portal vein, make the preserving fluid flow outside the duct. Wash the liver at low temperature for 4 hours within the body, maintain the pressure at 90 - 100 cm H_2O, and velocity at 8-12mL/min. The preserving fluid was the lactic Linger's fluid composed of 10mL dethomaxone, 100mg ATP and 100U/L insulin. After managing the experimental factors, take out the three-way stopcock, connect with the portal vein, release the occlusion of hepatic artery, repair the IVC incision, and restore the hepatic reperfusion[11].

Collection and test of samples

Liver tissue of 500mg was resected before the obstruction of blood and reperfusion, and 30min and 60min after reperfusion respectively. Superoxide dismutase (SOD)[12,13] and lipid peroxidase (LPO) were tested[14], and the morphologic changes were observed under microscopy and electric microscopy synchronically[15,16].

Statistical analysis

Data were presented as the mean ± SE. The *t* test was applied between two groups and variance analysis between multi-groups. $P<0.05$ values were regarded as significant.

RESULTS

Effect of anisodamine on the changes of oxygen-derived radicals (Table 1)

Table 1 Effect of anisodamine on change of oxygen-derived radicals

Groups	LPO(nmol/100mg)		SOD(nu/mg pr)	
	EG(10)	CG(10)	EG(10)	CG(10)
Pre-obstruction of blood	48.50±2.53	53.80±2.19	109.70±4.23	105.00±7.33
Pre-reperfusion	61.10±5.12	72.30±2.44	100.20±5.66	97.60±6.35
30' post-reperfusion	164.40±10.55	273.30±14.61[b]	72.50±5.60	55.10±6.47[b]
60' post-reperfusion	142.40±11.35[b]	242.40±11.92[b]	61.50±6.99[b]	43.10±6.61[b]

[b]$P<0.01$ *vs* control group.

Effect of rewarming on LPO and SOD of grafted liver (Tables 2 and 3)

Table 2 Effect of rewarming on LPO of grafted liver

Temperature of rewarming	*n*	Pre-obstruction	Post-rewarming	30'post-reperfusion[b]	60'post-reperfusion
12℃	5	51.25±536	71.00±14.72	245.00±44.63	195.25±38.14
20℃	5	51.00±6.92	68.00±11.95	211.25±37.49	192.25±10.08
28℃	5	55.50±11.24	70.00±13.01	206.25±38.80	180.25±38.54
36℃[a]	5	50.00±7.22	1.75±7.55	190.50±25.34	175.50±18.65

[a]$P<0.05$ *vs* the other groups; [b]$P<0.01$ *vs* the post-rewarming group.

Table 3 Effect of rewarming on SOD of grafted liver

Temperature of rewarming	*n*	Pre-obstruction	Post-rewarming	30'post-reperfusion[b]	60'post-reperfusion
12℃	5	105.00±10.02	87.25±14.00	52.75±13.90	44.50±10.74
20℃	5	103.20±13.64	90.75±10.46	64.50±8.21	55.50±7.35
28℃	5	108.23±6.89	92.50±5.98	65.50±4.50	56.50±4.65
36℃[a]	5	112.50±8.24	90.25±9.64	72.50±10.44	64.50±10.10

[a]$P<0.05$ *vs* the other groups; [b]$P<0.01$ *vs* the post-rewarming group.

Morphologic change of liver cells

Observation under microscopy No obvious changes in HE stain between the post-rewarming groups, the hepatic tissue swelled when rewarmed at 4℃ at 30min and 60min post-reperfusion. Light red granules could be seen in the cellular plasm, no obvious changes after the rewarming at 28℃ and 36℃. At 60min post-reperfusion, the effect was better in anisodamine group than in the other groups.

Observation under electric microscopy The chrodrosome of hepatic cells swelled slightly after rewarming and the structure was roughly normal. At 30min post-reperfusion, the chrodrosomes of hepatic cells swelled, being destroyed partially and impaired in structure and the endoplasmic reticulums dilated in the 4℃, 12℃ and 20℃ rewarming groups. The injury was more serious at 60min post-reperfusion. Accasionally, the chrodrosomes swelled slightly and the ridges decreased. At 60min post-reperfusion, the chrodrosomes of hepatic cells swelled, and were impaired obviously, and the endoplasmic reticulums dilated in the non-anisodamine group. The results of anisodamine group were better evidently than the other groups. The injury of hepatic cells was the most slight in the 36℃ rewarming group.

DISCUSSION

Oxygen-derived radical and malmicrocirculation were the main causes of postoperative primary nonfunction of grafted liver[15,17]. Resent studies found that anisodamine can stabilize cell membrane and resist oxygen-derived radical[18-21], thus protecting cells from injury. Up to now, there has been no report about application of anisodamine in liver transplantation. This study deals with the protective action of anisodamine during the low temperature preserving period. The results showed that anisodamine had no obvious influence on LPO and SOD during the low temperature preserving period, yet it may reduce the production of LPO and stop the decrease of SOD after reperfusion[22]. At the time of ischemia-reperfusion, the increase of intracellular Ca^{2+} activates Ca^{2+} dependent proteinase, which can change xanthine dehydrogenase into xanthine oxidase (XOD). Rich oxygen supply accompanying with reperfusion oxidates xanthine and hypoxanthine into uric acid under the action of XOD, meanwhile produces- lots of oxygen-derived radicals[23,24]. Anisodamine is the antagonist of Ca^{2+}, it may inhibit the change of xanthine dehydrogenase into xanthine oxidase, thereby the anti-oxygen-derived radical action of anisodamine may reduce the peroxide injury of lipid of cell membrane, and relieve the reperfusive injury of grafted liver[25].

The study found that the production of LPO and decrease of SOD occurred chiefly after reperfusion. With the increase of LPO, SOD decreased gradually, indicating that SOD may antagonize LPO[26]. Pathologic observation verified that the injury of hepatic cells became more serious with the lasting of reperfusion, indicating that peroxide action of lipid caused by oxygen-derived radicals mainly occurred after reperfusion. Oxygen-derived radicals may lead to peroxide reaction of lipid, and the lipid radicals cava cause further decrease of mobility and increase of the permeability of cell membrane, swelling of the chrodrosome, release of lysosome, and serious injury of tissues[27]. We reckoned that the oxygen-derived radicals after reperfusion may damage the grafted liver, which is a chief cause of post-operative primary non-function of grafted liver.

In 36℃ rewarming group, the level of LPO was obviously lower and the activity of SOD higher than that in other groups. There was no evident morphologic change under microscopy in the 28℃ and 36℃ rewarming groups, and the change under electric microscopy was slight. It indicated that rewarming to grafted liver preserved in the low temperature fluid reduced the production of oxygen-derived radicals, and relieved the injury of grafted liver. Low temperature may decrease the activity of ATPase and the function of K^+ Na^+ and Ca^{2+} pumps in cell membrane, impair the electrolytes[28,29]. Reperfusion may lead to anomaly of Ca^{2+} and production of oxygen-derived radicals. Rewarming may improve the activity of ATPase and restore the function of pumps, therefore decreasing the intracellular concentration of Ca^{2+} and inhibiting the production of oxygen-derived radicals[30], and protecting the cells of grafted liver[31]. This study showed that morphologic change of hepatic cells was slighter in the 28℃ and 36℃ rewarming groups than in other groups. There was no significant difference between the 28℃ and 36℃ groups. Less oxygen-derived radical was produced in the 36℃ group than in other groups. Therefore, we think that it is a favorable choice for liver transplantation to apply anisodamine during the low temperature preserving period and

rewarm the grafted liver before reperfusion at 36℃[32].

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