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Effect of Nutritional Status on Hypothermic Liver Perfusion

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Abstract

The effect of nutritional status on livers preserved either by simple cold storage or by hypothermic perfusion was studied using isolated rat liver perfusion model. Livers either form fed or fasted rats were preserved for 18 hours by simple cold storage procedure with UW solution, or continuously perfused for 12 hours at 5 or 20°C. Each liver was assessed by one hour normothermic reperfusion following preservation period. Fasted livers in each preservation procedure demonstrated deterioration of hepatocytes more than fed livers assessed by AST, ALT and LDH liberation into perfusate. PNP in the perfusion procedures showed no difference between fasted and fed livers. Slight sinusoidal lining cells changes and vacuolization in hepatocytes were preferential in all groups. Patchy areas of hepatocytic discoloration were often seen in fasted group in each preservation procedure. The nutritional status of hepatic graft is important in both simple storage and continuous perfusion preservation method.

Introduction

Liver plays an important role in energy homeostasis. More attention should be paid to nutritional status of preserved hepatic grafts which might affect the outcome of liver transplantation. Nutritional status has obvious effect on livers on which warm ischemic insults imposed[6], while a debate exists whether viability of simple cold preserved livers is affected by hepatic nutritional status or not[1,2,8]. In addition, no report is available about the influence of nutritional status on livers preserved with continuous hypothermic perfusion procedure, a possible future choice. The effect of nutritional status on livers preserved either by simple cold storage or by hypothermic perfusion was studied using isolated rat liver perfusion model.

Key words: Perfusion, Nutrition, Liver, Preservation, Transplantation.
索引語: 灌流, 栄養, 肝, 保存, 移植

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; LDH, lactate dehydrogenase; PNP, purine nucleoside phosphorylase.

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Materials and Methods

Male Lewis rats, weighting 180–270 g were used in all experiments. Rats were either fed ad libitum or fasted for 36 hours before use depending on experimental purpose.

Following anesthesia with 50 mg/kg sodium pentobarbital (Abbott Laboratories, N. Chicago. IL), abdominal cavity was opened by a cruciform incision. The common bile duct was cannulated with a 22 G teflon catheter and 3 mg heparin sodium was injected into the penile vein. Through a 18 G teflon catheter inserted into the portal vein, the liver was perfused with 60 ml cooled UW solution (simple storage group) or Krebs-Henseleit bicarbonate buffer solution (perfusion groups). Perfusion pressure was 10 cm H_2O. The liver was decompressed by cutting and cannulating (14 G teflon catheter) the inferior vena cava (IVC) just below the liver. The suprahepatic IVC was cut allowing the liver to be gently removed and transferred to the perfusion apparatus after weighing.

In simple storage group, each six livers obtained from fed or fasted rats were immersed in UW solution and stored for 18 hours a 0–2°C. In perfusion groups, each six livers obtained from fed or fasted rats were perfused at a flow rate of 3.0–3.5 ml/g liver/min for 12 hours at 5°C or 20°C. The hypothermic perfusion system was described elsewhere[3]. The recirculating perfusion medium was 300 ml Krebs-Henseleit bicarbonate (KHB) solution with 5.6 mM glucose containing 12 mg sulfamethoxazole and 2.4 mg trimethoprim. The KHB perfusate was equilibrated with a gas mixture of 95% O_2–5% CO_2 via oxygenator at a flow rate of 2 L/min. The entire apparatus except pump were placed in an Olympus experimental organ preservation system (XOPS-R10, Olympus Co., Tokyo, Japan). After hypothermic preservation, livers were weighed and transferred to the normothermic perfusion system.

The normothermic perfusion system was described elsewhere[7]. Livers were perfused for one hour following 15 minutes equilibrium time with 250 ml oxygenate KHB solution at 37°C at a flow rate of 3.0–3.5 mg/g liver/min.

Perfusate samples were collected from the reservoir (hypothermic perfusion) or from the outflow (nnormothermic perfusion) at the end of each perfusion. They were used for analysis of potassiu, glucose, AST, LDH and purine nucleoside phosphorylase (PNP). Amount of bile production was measured during normothermic reperfusion period. At the end of one hour perfusion, tissue samples were taken for histological study.

AST, LDH and glucose in the perfusate were determined by a Technicon RA-500 analyzer (Technicon Instrument Co., Tarrytown, NY) using commerciall available kits (Technicon).

PNP levels in the effluent were measured by the methods of Hoffee et al[4] and used as a specific marker of sinudoidal endothlia cell damages[10].

Potassium concentration in the perfusate was determined by an ion specific electrode of NOVA 6 (NOVA Biomedical, Newton, MA).

Tissues for histopathological examination were fixed with Bouin's solution for 24 hours, followed by 80% alcohol and stained with Hematoxylin-Eosin. Severity of histological damage was scored as none, slight, moderate or severe changes of round configuration or denudation in sinusoidal lining cells and patchy area of discoloration or vacuolization in hepatocytes. Evaluation was done blindly without knowing the timing of tissue collection and experimental groups.

All results were expressed as mean ± standard error of the mean. Difference between fasted and fed groups in the same preservation procedure was determined bby Mann-Whitney U test. A $p<0.05$ is considered to be significant.

Results

At the end of hypothermic preservation, livers preserved in UW solution lost, while livers perfused at 5°C or 20°C gained their weights as summarized in Table 1. But there was no difference between fasted and fed livers in each preservation procedure. LDH in fasted 5°C perfusion group and AST in fasted 20°C perfusion group were significantly higher than those in fed group (Table 1). Glucose in 20°C fed group was signficantly lower than that in fasted group. Purine nucleoside phosphorylase (PNP) is located primarily in the cytoplasm of the endothelial and Kupffer cells and used for an indicator of damage to the microvascular endothelial cells of the liver[10]. PNP and potassium in the perfusate showed no significant differences between fasted and fed groups in the same preservation procedure (data not shown).

At the end of normothermic reperfusion, fasted livers in each preservation procedure liberated much higher enzymes (AST and LDH) into the perfusate (Table 1). PNP in the perfusion procedure showed nno difference between fasted and fed livers. Slightly higher value of PNP was obtained in fasted simple storage group. No significant difference was obtained in bile production and potassium levels between fasted and fed groups in each preservation procedure (data not shown). Glucose levels in fed groups were significantly higher than those in fasted group.

Slight sinusoidal lining cells changes and vacuolization in hepatocytes were preferential in all groups. Patchy areas of hepatocytic discoloration were often seen in fasted group in each preservation procedure (Table 2).

Discussion

Among the roles of liver, co-operating with pancreas, constant supply of energy source would be one of the msot important things, since food intake is intermittent in many animals. The liver and pancreas control the distribution of energy sources, such as glycogen in liver and muscle, lipid in adipose tissue[11]. As liver is one of the center organs to maintain the energic homeostasis, the nutritional status of liver itself might affect its viability in transplantation. Previous workers demonstrated that post warm ischemic liver function was affected by its nutritional status[6]. In simple cold storage, two different reports were obtained. Palombo et al. described that effective support of adenine nucleotides by glycolysis in flush-preserved liver was dependent upon the nutritional status of the donor prior to liver procurement[8], while Caldwell-Kenkel et al. reported nutritional status (fed vs. fasted) did not affect the extent of cell damage[2]. Recently Boudjema et al. demonstrated a remarkable nutritional effect on livers preserved by simple cold storage manner in a deliberate experimental procedure[1]. In hypothermic ocntinuous perfusion models, no report has been available about the effect of nutritional status as far as our knowledge. Preliminary study on temperature effect on livers showed that there is no significant hepatic injury in livers preserved at up to 20°C[3]. Perfusion of livers at 20°C might be better at the point of improving the nutritional status of liver, as liver is working well at this temperature. We adopted temperature 20°C in addition to 5°C in present study for this reason.

In present study, the importance of nutritional status of livers both in simple cold storage and in perfusion model was clearly demonstrated especially for parenchymal cells based on enzyme release (AST, LDH) and histological findings. Simusoidal endothelial cells appeared to be less sensitive to nutritional status assessed by PN and histological findings.

Table 1 Effect of nutritional status on liver profile after simple storage or hypothermic continuous perfusion

Group	Nutritional Status	End preservation					End reperfusion			
		Weight gain (%)	AST (IU/L)	LDH (IU/L)	PNP (mU/mL)	GLU (mg/dl)	AST (IU/L)	LDH (IU/L)	PNP (mU/L)	GLU (mg/dl)
Simple storage	Fasted	-6.35±0.74					182±41***	1044±174***	17.8±1.6*	5.33±0.76***
	Fed	-6.32±0.55					24.8±2.2	97.2±12	12.8±0.75	113±5.1
5°C perfusion	Fasted	7.56±3.3	9.67±1.23	64.8±6.61*	4.67±1.63	99.3±1.28	338±39***	2103±302***	16.0±2.6	3.83±0.31***
	Fed	5.78±2.2	7.50±1.23	43.2±3.89	2.67±0.49	102±1.20	19.8±2.6	79.5±8.1	9.50±1.7	67.3±8.4
20°C perfusion	Fasted	1.64±0.58	18.4±1.21*	91.0±4.98	23.3±3.33	104±0.68**	159±57**	917±295**	20.5±8.8	6.0±0.7**
	Fed	1.36±0.17	14.8±0.52	79.2±3.70	17.5±2.42	81.0±2.07	18.0±2.0	77.8±1.1	9.83±2.6	81.7±7.7

*, $p<0.05$ versus Fed group **, $p<0.01$ versus Fed group ***, $p<0.005$ versus Fed group

Table 2 Effect of nutritional status on histological findings after one hour normothermic reperfusion

		Endothelial cell damage				Discoloration of hepatocyte				Vacuolization of hepatocyte			
		none	slight	moderate	severe	none	slight	moderate	severe	none	slight	moderate	severe
Simple storage	Fasted		5	1		1	2	3		1	4	1	
	Fed		5	1		6				1	5		
5°C perfusion	Fasted		3	3			2	3	1		5	1	
	Fed		5	1		5	1			2	4		
20°C perfusion	Fasted		5	1		1	1	3	1	1	3	2	
	Fed		6			6				1	3	2	

Results are expressed by event of histological findings graded as none, slight, moderate and severe changes.

It has been reported that renal cortex can not ustilize glucose a low temperature, and fatty acid is preferable as a substrate at hypothermic condition[5,9]. In present study, glucose level at the end of hypothermic continuous perfusion showed solely decreased value in fed 20°C perfusion group. Livers from fasted rat could not utilize glucose even at 20°C. Preminary trial to improve hepatic nutritional status using continuous perfusion with 20 mM glucose at 20°C resulted in a futile outcome (unpublished data). Glucose is consdered to be a poor substate for glycolysis in livers from fasted rats because of the high Km of hapatic glucokinase for glucose[12].

In conclusion, the nutritional status of hepatic graft is important in both simple storage and continuous perfusion preservation method. Glucose is not utilized even if the liver is perfused at as high as 20°C. Further study on substrate utilization in reference to nutritional status and different temperature should be performed.

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和文抄録

低温肝灌流における栄養状態の影響

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ラット肝灌流モデルをもちいて，栄養状態が Simple cold storage 法および，低温肝灌流保存により保存された肝臓にあたえる影響を調べた．術前36時間絶食を負荷したラットおよび，絶食負荷をかけていないラットから採取した肝臓を UW 液をもちいて18時間保存もしくは，Krebs-Henseleit 液をもちいて5度および20度で12時間持続灌流し，その後37度1時間再灌流し評価した．絶食を負荷されたラットから採取された肝臓はすべての保存法において，灌流中への AST, ALT 及び LDH により非絶食群よりも肝細胞障害が強いことが示された．灌流液中の PNP 値には絶食群，非絶食群間で差を認めなかった．組織学的所見においても絶食群は非絶食群にくらべて肝細胞障害が強い傾向が認められた．これらの結果より術前の栄養状態が肝臓保存において強い影響をあたえることが示唆された．