Tolerance of the Cirrhotic Liver to Cold Ischemia : Ex vivo Analysis in Rats

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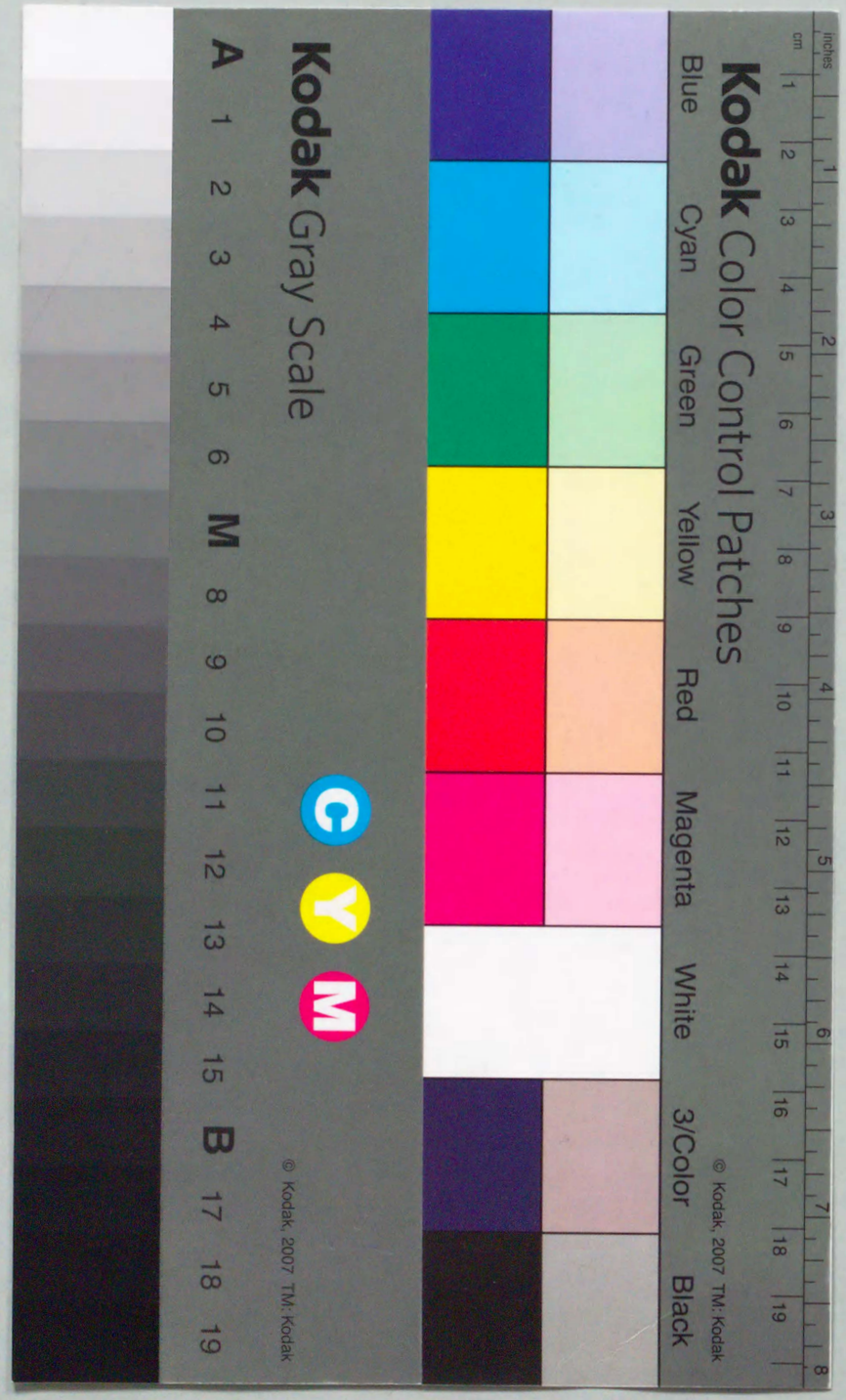
https://doi.org/10.11501/3128602

出版情報：九州大学, 1997, 博士（医学）, 論文博士
バージョン：
権利関係：



Original Paper

European Surgical Research

Eur Surg Res 1996;28:111–118

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Key Words

Cold ischemia, cirrhotic liver
Hyaluronic acid
Isolated perfusion model, rat liver
Liver cirrhosis, cold ischemia tolerance

Abstract

We investigated the viability of rat cirrhotic livers preserved cold using an isolated rat liver perfusion model. Cirrhosis was induced by intravenous thioacetamide injection. Normal and cirrhotic livers were reperfused immediately or following 6 h of preservation through the portal vein with Krebs-Henseleit buffer and hyaluronic acid added. Cirrhotic livers with short cold ischemia showed higher portal venous resistance than their normal counterparts ($p < 0.05$), while cirrhotic livers preserved for 6 h exhibited marked elevation of the portal venous resistance as compared with their fresh counterparts or normal liver preserved cold for 6 h ($p < 0.05$). Similarly, the oxygen consumption of cirrhotic livers with short cold preservation was lower than that of normal livers ($p < 0.05$), which was further reduced by 6 h of cold preservation ($p < 0.05$). The bile output was not different between normal and cirrhotic livers. The sinusoidal endothelial cell function of cirrhotic livers as assessed by the clearance of hyaluronic acid was impaired even after a short period of cold ischemia. Histologically, cirrhotic livers showed severe sinusoidal endothelial cell damage, hepatocellular swelling, and marked septal edema which became more prominent by cold preservation. We conclude that the viability of the cirrhotic liver is impaired even by a short cold exposure, and that the prolongation of cold ischemia of the cirrhotic liver leads to further deterioration of the viability.

Introduction

Pichlmayr et al. [1] advocated new surgical techniques for previously unresectable hepatic tumors, extracorporeal hepatic resection or in situ perfusion, which are extensions of liver transplantation with the use of veno-venous bypass. The only well-established contraindication for extracorporeal hepatic resection is cholestasis due to obstructive jaundice [1] for which we tentatively added liver cirrhosis and obvious fatty liver [2] that are known factors for postoperative liver failure after hepatic resection [3, 4]. To date, little is known about the tolerance of cirrhotic liver to cold ischemia, and, therefore, it is unclear if patients

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0014–312X/96/0282–0111$10.00/0

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Received:
January 26, 1995
Accepted:
June 26, 1995

with concomitant liver cirrhosis can be candidates for such new operations. The purpose of this study was to clarify the viability of the cirrhotic liver after cold ischemia with the use of an ex vivo perfusion of rat livers.

Materials and Methods

Animals

Inbred male Lewis rats aged 6 weeks (Sciwa, Fukuoka, Japan) were used for the experiments. The animals were housed in a temperature-controlled room with 12-hour light-dark cycles and maintained on normal rat diet and water ad libitum.

Induction of Liver Cirrhosis

The rats were injected with thioacetamide 200 mg/kg i.v. every other day for 3 months to induce liver cirrhosis [5] which was confirmed grossly and histologically in each animal.

Experimental Groups

The rats were divided into four groups as follows: group LC-Fr (n = 8): cirrhotic livers perfused immediately after hepatectomy; group LC-Pr (n = 8): cirrhotic livers perfused after 6 h of cold preservation in lactated Ringer solution (4 °C); group N-Fr (n = 8): normal livers perfused immediately after hepatectomy, and group N-Pr (n = 8): normal livers perfused after 6 h of cold preservation in lactated Ringer solution (4 °C).

For groups N-Fr and N-Pr, the animals were fed without intravenous injection for 3 months.

Hepatectomy

Under general anesthesia by intraperitoneal injection of pentobarbital sodium 70 mg/kg, the abdomen was entered via a midline incision, and the common bile duct was cannulated with a fine Silastic tube (Dow Corning, Midland, Mich., USA; Cat. No. 602-105, 0.3 mm inner, 0.64 mm outer diameter) [6]. With in situ perfusion of the liver via the terminal aorta with 40 ml of cold lactated Ringer solution (4 °C), the right gastric, splenic, and adrenal veins were tied and divided, while portal vein and suprahepatic vena cava were cannulated with 14-gauge catheters. The infrahepatic inferior vena cava was ligated and divided. The liver was then resected and placed in cold lactated Ringer solution (4 °C). We used lactated Ringer solution, since we have documented using orthotopic liver transplantation in rats that 6 h of static preservation in lactated Ringer solution is not consistent with survival, while such a period lasting 4 h is [7].

Perfusion System

The liver was reperfused via the portal vein for 2 h with a pressure of 12 cm H_2O. The perfusate was recirculated through a filter with 7-μm pores and oxygenated through the artificial lung [8] by 95% O_2 and 5% CO_2. The perfusate consisted of 300 ml of Krebs-Henseleit buffer with additional 2.5 m*M* $CaCl_2$, to which 300 μg hyaluronic acid (HA; Wako Chemical, Osaka, Japan) was added just before reperfusion. The portal flow was measured continuously by an electromagnetic flowmeter (model MF-27; Nihon Kohden, Tokyo, Japan) with a flow transducer (model FF-050T, inner diameter 5 mm; Nihon Kohden). pH and oxygen concentrations of inflow and outflow perfusates were measured every 30 min using an ABL-30 acid-base analyzer (Radiometer, Copenhagen, Denmark) for pH correction and calculation of the oxygen consumption.

Biochemical Analyses

The glutamic-pyruvic transaminase (GPT) levels of the perfusate were measured by an ultraviolet or enzymatic method 15 min after starting reperfusion and every 30 min thereafter. For the measurement of adenine nucleotides, tissue samples were taken after 15 and 30 min and then every hour. Tissue samples were immediately soaked and stored in liquid nitrogen for later analysis. In this study freeze-sampling was not employed to avoid the influence of frozen tissue on perfusate GPT. Adenine nucleotides were measured by reverse-phase liquid chromatography [9] (LC6A system; Shimazu, Kyoto, Japan) using a CLC-ODS 6 × 160-mm column with 24 m*M* dimethylaminoethanol and 16 m*M* citrate as a mobile phase. For analytical standards of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP), commercially available kits (Sigma, St. Louis, Mo., USA) were used. For the protein assay, a DC Protein Assay Kit II (Bio-Rad Laboratories, Richmond, Calif., USA) was used [10]. The HA level was measured 15, 30, 60, and 120 min after reperfusion using a specific radioactivity-binding assay [11].

GPT release, bile production, portal venous resistance, oxygen consumption, and energy charge were calculated according to the following formulae:

GPT release (IU/g liver) =
GPT (IU/l) × 0.3 (l)/ liver weight (g)

Bile production (l/g liver/15 min) =
BO_{15} (l/15 min)/liver weight (g)

Table 1. Liver weight and preservation time (mean ± SD)

Group	Liver weight, g	Preservation time, min
LC-Fr (n = 8)	14.9 ± 3.20	26.6 ± 12.63
LC-Pr (n = 8)	16.2 ± 4.81	366.0 ± 11.54
N-Fr (n = 8)	12.4 ± 1.33	15.7 ± 4.5
N-Pr (n = 8)	12.3 ± 0.67	366.0 ± 5.45

Portal venous resistance (cm H_2O min/ml/g liver) = (P_{PV} – P_{IVC}) (cm H_2O)/portal flow (ml/min)/liver weight (g)

Oxygen consumption (mol/g liver/min) = (A_{PO_2} – E_{PO_2}) (mm Hg) × 0.00136 (mol/ml/mm Hg) × F_a (ml/min)/liver weight (g)

Energy charge = (ATP + $^1/_2$ × ADP)/(AMP + ADP + ATP)

BO_{15} = Bile output during each 15-min period (l/15 min); P_{PV} = portal vein pressure (cm H_2O); P_{IVC} = inferior vena cava pressure (cm H_2O); A_{PO_2} = oxygen pressure of the affluent (mm Hg); E_{PO_2} = oxygen pressure of the effluent (mm Hg); F_a = affluent perfusate flow (ml/min).

Liver Weight

The liver weight was measured immediately after donor hepatectomy and at the end of reperfusion.

Histological Examination

Tissue samples were taken before preservation and at the end of the reperfusion, fixed in 10% formalin, and stained with hematoxylin and eosin.

Statistical Analyses

The data are expressed as mean values ± SD. Two-tailed Student t or Wilcoxon tests were used. $p < 0.05$ was considered significant.

Results

Table 1 lists liver weight and preservation time for the four groups. For the liver weight, no statistically significant difference was present among the four groups.

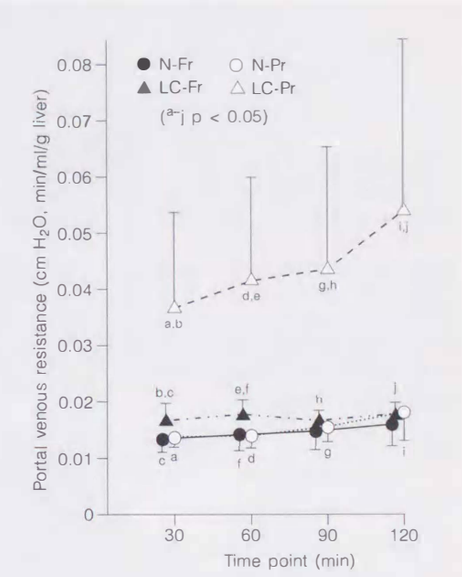

Fig. 1. Changes in portal venous resistance. The group LC-Pr exhibited a markedly higher portal vein resistance than groups N-Pr and LC-Fr at any time point ($^{a, b, d, e, g, h, i, j}$ $p < 0.05$), and group LC-Fr showed a higher portal venous resistance than group N-Fr at 30 and 60 min after reperfusion ($^{c, f}$ $p < 0.05$). The portal vein resistance of the cirrhotic liver showed a rather steady increase during perfusion. There was no difference between groups N-Fr and N-Pr.

Figure 1 demonstrates the portal venous resistance. Group LC-Pr exhibited a markedly higher portal vein resistance than group LC-Fr at all time points ($p < 0.05$). Group LC-Fr showed a higher portal vein resistance than group N-Fr 30 and 60 min after reperfusion ($p < 0.05$). The portal venous resistance of the preserved cirrhotic liver showed a steady increase during reperfusion. There was no difference between groups N-Fr and N-Pr.

Figure 2 exhibits the bile production during each 15-min period of reperfusion. During the first 15 min, groups N-Pr and LC-Pr pro-

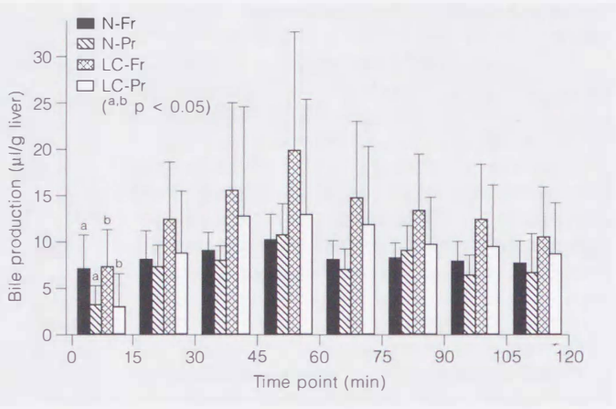

Fig. 2. Changes in bile production. During the first 15 min, group LC-Pr produced less bile than group LC-Fr (^{a} p < 0.05). A similar difference was observed between groups N-Pr and N-Fr (^{b} p < 0.05). The bile in groups LC-Fr and LC-Pr was thin, yellowish, and with a lower viscosity as compared with their normal counterparts.

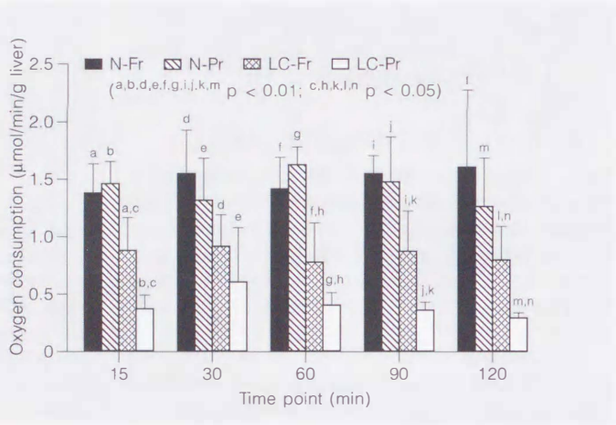

Fig. 3. Oxygen consumption. Group LC-Fr consumed less oxygen than group N-Fr at any time point (^{a,d,f,i} p < 0.01; ^{l} p < 0.05). Group LC-Pr consumed less oxygen than either group N-Pr (^{b,e,g,j,m} p < 0.01) or group LC-Fr (^{c,h,k,n} p < 0.05).

duced less bile than their fresh counterparts (p < 0.05). The bile of groups LC-Fr and LC-Pr was thin and less viscous as compared with their normal counterparts.

Figure 3 demonstrates the oxygen consumption. Group LC-Fr consumed less oxygen than group N-Fr at all time points (p < 0.05). Group LC-Pr consumed even less oxygen than either group LC-Fr or group N-Pr at any time point (p < 0.05).

Figure 4 shows the GPT release into the perfusate. In each group, the GPT release increased along with the reperfusion time. In group N-Pr, the GPT release was significantly higher than in groups N-Fr and LC-Pr (p < 0.05) at early time points.

Figure 5 demonstrates the changes in the energy charge which tended to increase after reperfusion in groups N-Fr and N-Pr, while that of group LC-Fr showed no significant change.

Figure 6 exhibits the HA clearance. While the HA concentration declined linearly in group N-Fr, the other three groups failed to show a reduction (p < 0.01 120 min after reperfusion).

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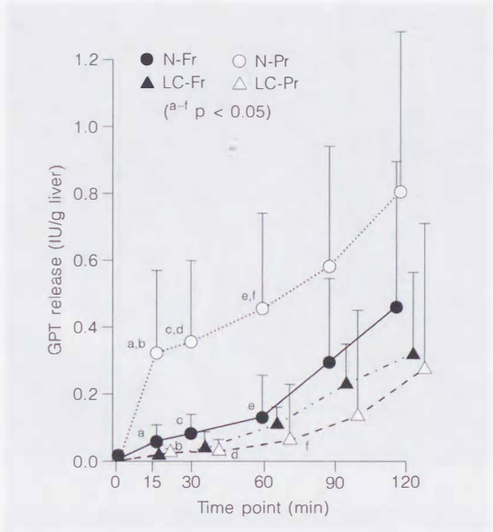

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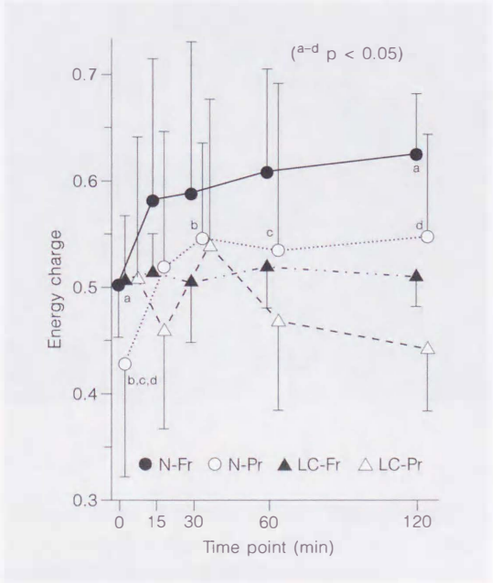

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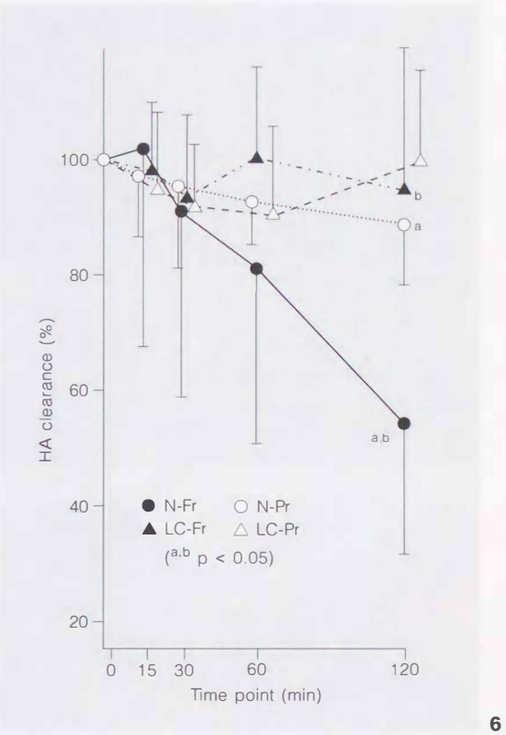

Fig. 4. Changes in GPT release. In each group, the GPT levels releasing into the perfusate increased along with the reperfusion time. In group N-Pr, the GPT releases were significantly higher than in groups N-Fr and LC-Pr at early time points ($^{a-f}$ p < 0.05).

Fig. 5. Changes in energy charge. The energy charge of the groups N-Fr and N-Pr increased significantly after reperfusion ($^{a-d}$ p < 0.05), while that of the groups LC-Fr and LC-Pr failed to show an increase.

Fig. 6. HA clearance. Only group N-Fr exhibited a linear reduction of HA, while the other groups failed to show any significant reduction. At 120 min after reperfusion, the HA levels of group N-Fr were significantly lower than those in groups LC-Fr and N-Pr (^{a,b} p < 0.05).

Histologically, thioacetamide-treated livers revealed liver cirrhosis (fig. 7). A biopsy specimen obtained before reperfusion from groups LC-Fr and LC-Pr showed occasional spotty acidophilic necrosis and edema of the interstitium, while the sinusoidal endothelial cells were well maintained (fig. 8). After reperfusion, the livers of group LC-Fr demonstrated hepatocyte swelling and sinusoidal stenosis (fig. 9a), while those of group LC-Pr

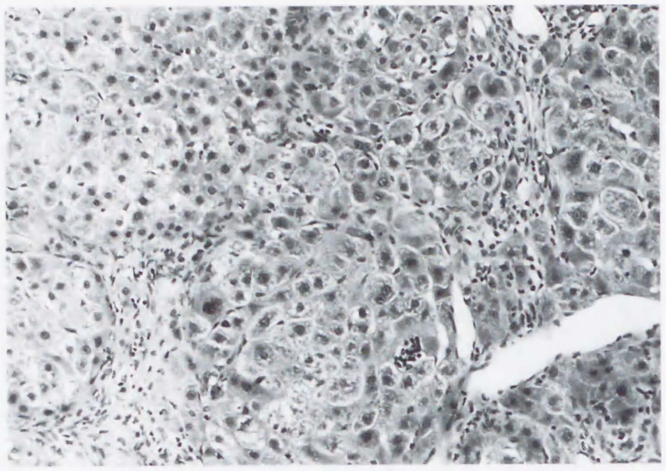

Fig. 7. Photomicrograph of a thioacetamide-treated liver. HE. ×77. Regenerative nodules surrounded by fibrosis are observed.

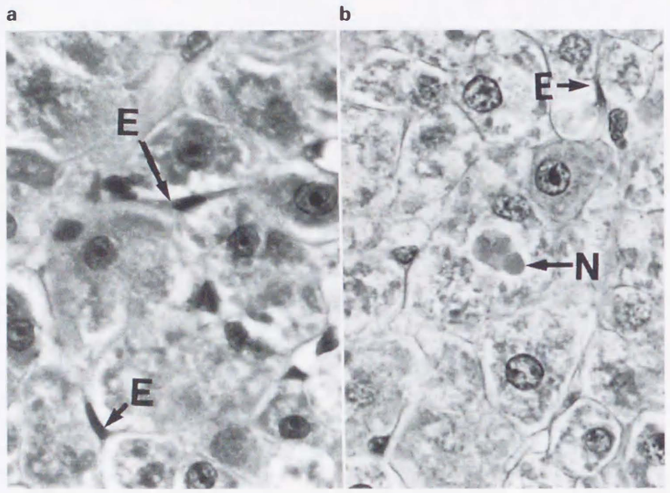

Fig. 8. Photomicrographs of the liver before reperfusion. HE. ×302. **a** Section of group LC-Fr. Sinusoidal endothelial cells (E) are well maintained. **b** Section of group LC-Pr. Hepatic sinusoidal endothelial cells (E) are well maintained. Some areas of spotty acidophilic necrosis (N) are occasionally seen.

exhibited prominent septal edema and hepatocyte swelling with sinusoidal stenosis (fig. 9b). Focal hepatocyte necrosis with sinusoidal dilatation was also present. Furthermore, various degrees of endothelial cell damage were seen in the regenerating nodules. Group LC-Fr (fig. 10a) demonstrated less endothelial cell damage than group LC-Pr (fig. 10b).

Discussion

As to the absence of literature on the effect of cold ischemia on the cirrhotic liver, this is because there had been no necessity to investigate the tolerance of cirrhotic livers to cold ischemia. However, recently introduced extracorporeal or in situ perfusion techniques for extensive liver resection allow excision of a liver tumor which was deemed inoperable

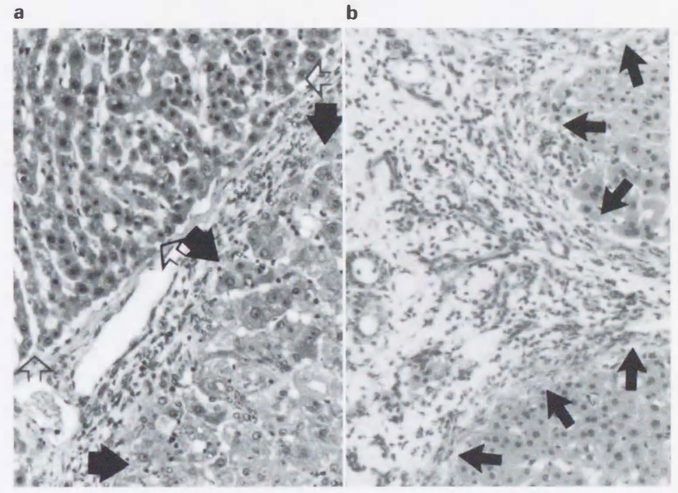

Fig. 9. Photomicrographs of the liver after reperfusion. HE. ×75. **a** Section of group LC-Fr. Interstitial edema is present, and various degrees of hepatocyte swelling with sinusoidal stenosis (black arrows) and dilation (open arrows) in regenerative nodules are noted. **b** Section of the group LC-Pr. Interstitial edema (arrows) is prominent.

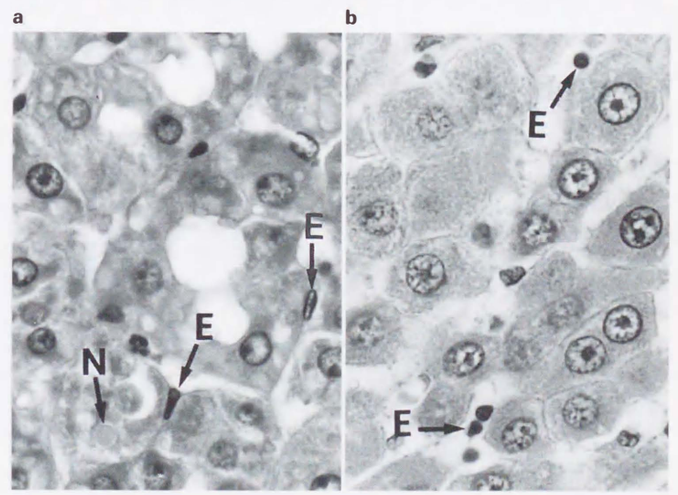

Fig. 10. Photomicrographs of the liver in group LC-Pr after reperfusion. HE. ×302. **a** Section of group LC-Fr. Hepatic sinusoidal endothelial cells (E) are maintained, and spotty acidophilic necroses (N) are present. **b** Section of group LC-Pr. The majority of hepatic sinusoidal endothelial cells (E) are denuded.

by conventional means [1, 2]. The application of these techniques to cirrhotic livers could increase the resectability of liver tumors with underlying liver disease.

In this study, cirrhotic livers exposed to cold ischemia demonstrated a high portal venous resistance and a low oxygen consumption which were amplified when the livers were preserved cold for 6 h. The absence of energy recovery and the production of thin bile of the cirrhotic liver were also obvious even after a short period of cold exposure.

We used HA clearance as a parameter of hepatic sinusoidal endothelial cell function [7, 12]. HA is a glycosaminoglycan synthesized in the plasma membrane and is rapidly and selectively absorbed into the hepatic sinusoidal endothelial cells were degradation follows [13]. Elevation of serum HA has been reported in rheumatic disease and primary bil-

iary cirrhosis [14]. The elevation has been attributed to an increased production or a decreased absorbance due to hepatic endothelial cell injury. Since hepatic endothelial cells are easily damaged by cold preservation [15], we have reported specific elevation of the serum HA level after transplantation of a liver with cold ischemic damage [7]. In this perfusion study, we loaded the livers with 1,000 μg/l of HA before reperfusion and measured the clearance of HA. The failure of the liver graft to clear HA in the perfusate indicates disturbance of sinusoidal endothelial cell function or reduced perfusate flow through the liver. Histological findings and the elevation of portal venous resistance are consistent with the results of HA clearance in our study.

It is reasonable for the cirrhotic liver to have high vascular resistance, but the elevation of vascular resistance was much more prominent when the liver was stored cold. However, the marked difference in the degree of endothelial cell damage between the normal and cirrhotic liver is unclear. The characteristics of the cirrhotic liver exposed to cold ischemia may be explained by septal edema.

The poor viability of the cirrhotic livers after cold preservation with the use of lactated Ringer solution in the present study seems to indicate that extreme care should be taken when extracorporeal hepatic resection or the in situ cold perfusion method is used in cirrhotic patients with hepatic neoplasms.

References

1 Pichlmayr R, Grosse H, Gubernatis G, Lameshch P, Bretshneider HJ: Technique and preliminary results of extracorporeal liver surgery (bench procedure) and of surgery on the in situ perfused liver. Br J Surg 1990;77:21–26.

2 Yanaga K, Kishikawa K, Shimada M, Kakizoe S, Higashi H, Nishizaki T, Matsumutu T, Kanematsu T, Sugimuchi K: Extracorporeal hepatic resection for previous unresectable neoplasms. Surgery 1993;113:637–643.

3 Huguet C, Nordlinger B, Galopin JJ, Bloch P, Gallot D: Normothermic hepatic vascular exclusion for extensive hepatectomy. Surg Gynecol Obstet 1978;147:689–693.

4 Bismuth H, Castaing D, Garden OJ: Major hepatic resection under total vascular exclusion. Ann Surg 1989; 210:13–19.

5 Dashti H, Jeppson B, Hägerstrand I, Hultberg B, Srinvas U, Abdulla M, Bengmark S: Thioacetamide and carbon tetrachloride induced liver cirrhosis. Eur Surg Res 1989;21:83–91.

6 Bower BA, Branum GD, Rotolo FS, Watters CR, Meyers WC: Bile flow: An index of ischemic injury. J Surg Res 1987;42:565–569.

7 Ikeda T, Yanaga K, Kishikawa K, Kakizoe S, Shimada M, Sugimachi K: Ischemic injury in liver transplantation: Difference in injury sites between warm and cold ischemia in rats. Hepatology 1992;16:454–461.

8 Hamilton RL, Berry MN, Williams MC, Severinghaus EM: A simple and inexpensive membrane 'lung' for small organ perfusion. J Lipid Res 1974;15:182–186.

9 Jungling E, Kammermeier H: Rapid assay of adenine nucleotides or creatine compounds in extracts of cardiac tissue by paired-ion reverse-phase high-performance liquid chromatography. Anal Biochem 1980; 102:358–361.

10 Bradford MM: A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72: 248–254.

11 Tengblad A: Quantitative analysis of hyaluronate in nanogram amounts. Biochem J 1980;185:101–105.

12 Deaciuc I, Bagby G, Lange C, Spitzer J: Hyaluronic acid uptake by the isolated, perfused rat liver: An index of hepatic sinusoidal endothelial cell function. Hepatology 1993;17:266–272.

13 Laurent TC, Fraser JR: The properties and turnover of the hyaluronan. Ciba Found Symp 1986;124:9–29.

14 Lindqvist U, Engström-Laurent A, Laurent U, Nyberg A, Björklund U, Eriksson H, Pettersson R, Tengblad A: The diurnal variation of serum hyaluronan in health and disease. Scand J Clin Lab Invest 1988;48: 765–770.

15 McKeown CMB, Edward V, Phillips MJ, Harvey PRC, Petrunka CN, Strasberg SM: Sinusoidal lining cell damage: The critical injury in cold preservation of liver allografts on the rat. Transplantation 1988;46: 178–191.