Levels of Purine Nucleoside Phosphorylase (PNP) as a Viability Marker of Nonparenchymal Cells in Cold Preserved Livers

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COLD preservation of the liver with currently used solutions is limited to a few hours and is often accompanied by increased rates of postoperative graft complications.1,2 Although the University of Wisconsin (UW) solution has significantly increased the optimal preservation period, primary nonfunction of the graft remains a serious problem in liver transplantation.3-5 While the exact mechanism underlying storage and reperfusion injury is still unknown, injury to the microvasculature by toxic oxygen radicals is the most favored hypothesis.6,7 Presently, no method is available to predict the viability of the preserved liver, and biochemical assessments of graft viability after preservation are mainly focused on enzyme release, bile production, and energy status.8-12 We had recently determined that the extent of reperfusion injury to the microvascular endothelial cell could be accurately predicted by levels of PNP, an enzyme localized mainly in the cytoplasm of endothelial and Kupffer cells.13,14 Therefore, this study was carried out to assess if PNP could be used to predict the extent of preservation injury to the endothelial cell and hence the viability of the preserved grafts.

MATERIALS AND METHODS

Male Lewis (LEW) rats (Charles River Laboratories, Wilmington, Mass), weighing 250 to 300 g were used as liver donors. All animals were induced and maintained with inhalational methoxyflurane. Three hundred units of heparin were administered via the penile vein prior to harvesting the liver. For bile collection, the common bile duct was intubated with polyethylene tubing (Clay Adams PE-10, ID 0.2 mm). Immediately after cannulating the abdominal aorta, a thoracotomy was performed and after clamping the thoracic aorta the right atrium was opened. The liver was gently flushed with 20 cc ice cold UW solution via the aorta, and the portal vein intubated with a 16-gauge Teflon angiocatheter. Finally the suprahepatic IVC was ligated, infrahepatic IVC cannulated with a 14-gauge catheter, and the liver immediately harvested.

Isolated Perfusion Technique (IPRL)

The IPRL apparatus was designed to perform 2 liver perfusions simultaneously. The perfusate was circulated by a Masterflex pump controller (Cole Parmer Instruments, Chicago, Ill), in each of the perfusions circuits. The oxygenator, constructed from a single container and separated into 2 chambers, served for both perfusion systems. The oxygenation was maintained using a 95% O₂ and 5% CO₂ mixture at a flow rate of 3 ml/min, resulting in a P0₂ of 450 to 550 mm Hg. After the oxygenator, the perfusion medium (Krebs buffer containing 2% bovine serum albumin and 0.5% glucose) passed through a microfilter, reservoir, debubbler, and effluent collecting basins which were placed in line within each circuit. The temperature was controlled by a digital ther-...
Fig 1. Changes in hepatocellular enzyme concentrations during 120 minutes of reperfusion after 6, 12, 24 and 48 hours of cold storage as compared to controls. Values were averaged and shown as natural logarithm of enzyme concentrations (± SD) at each time point. Twenty-four and 48 h caused more enzyme release ($P < .05$) indicating nonviable livers. Similar to hepatocellular enzymes, levels of PNP increased dramatically and became significantly different after 24 and 48 hours storage from those preserved for 6 and 12 hours. After 120 minutes reperfusion, PNP levels up to 50 U/L seemed to be a marker of reversible microvascular damage whereas higher levels indicated irreversible damage.

Statistical Analysis

Data is presented as mean ± SD. In order to investigate differences between groups, nonparametric analysis of variance was performed. Correlations were expressed by a Spearman regression coefficient. Maximum likelihood discriminant analysis was performed with a normal distribution model. Variance was pooled if Bartlett’s test on homogeneity of variances was significant at the 5% level. Error rates were estimated by a posterior probability estimator in order to reduce their variance (SAS/STAT PC-Program).

RESULTS

Transaminase Release

Enzyme values are presented in Fig 1. AST and LDH were the most sensitive hepatocellular enzymes in this model. No statistically relevant differences in AST and LDH release could be observed throughout the 120-minute reperfusion between livers stored for 6 (A1) or 12 (A2) hours, and controls, that did not undergo a preservation period. Livers stored for 24 (A3) and 48 (A4) hours leaked significantly more AST, ALT, and LDH into the perfusate indicating nonviability and reflecting continuing damage during reperfusion. The discriminant analysis between the viable (A2) and nonviable (A3) groups was calculated from measurements during 0 to 60 minutes and 60 to 120 minutes, respectively. The error estimation for hepatocellular enzymes was clearly lower during the second reperfusion period compared to the first 60 minutes (Fig 4).

Bile Production

Bile production commenced 5 minutes after the livers were connected to the circuit. The amounts of bile produced per gram of liver/min during the 2-hour perfusion are summarized in Fig 2. At the end of reperfusion the volumes of excreted bile were not statistically different from each
Fig 2. Effect of different storage periods on rate of bile excretion (µL/g liver weight/min) during 120 minutes reperfusion. Between the 4 storage groups only livers stored for 48 hours showed a significant reduction in bile production.

other or from controls in livers stored in UW for 6, 12, and 24 hours, resulting in higher probability error rates (0.43) than cellular enzymes or PNP (Fig 4). The bile flow averaged 0.43 ± 0.12, 0.39 ± 0.06, and 0.37 ± 0.04 µL/g liver min, respectively. Forty eight hours of storage led to a distinctly depressed bile flow over 120 min (0.22 ± 0.011 µL/g liver/min). However, bile production was significantly lower than controls in 24-hours preserved livers after shorter periods of reperfusion up to 90 minutes.

Purine Nucleoside Phosphorylase
A1 (6-h) and A2 (12-h) preservation in UW solution resulted in an increase of PNP concentrations from 33.4 ± 3.0 and 33.8 ± 2.9 U/L at time 0 to 49.2 ± 8.5 and 49.3 ±

Fig 3. Livers stored at 4°C for 6, 12, 24 and 48 hours manifested a significant reduction in tissue ATP content when compared with nonischemic livers. All preserved organs were able to regenerate ATP. After 120 minutes reperfusion, recovery of ATP was not significantly different within the 4 storage groups.

Fig 4. PNP showed a distinctly reduced error rate (0.038) during the first 60 min as well as throughout the whole perfusion period (0 to 120 min) compared to conventional viability parameters (AST 0.29, ALT 0.35, LDH 0.16, and bile 0.43 probability error). In the second perfusion period (60 to 120 min) no difference could be shown except using bile flow as a viability parameter (0.42).

7.9 U/L after 120 min reperfusion (Fig 1). No difference could be shown between these groups throughout the whole perfusion period. Levels of PNP increased dramatically and became significantly different after 30 min of reperfusion between livers stored for 24 h (94.5 ± 25 U/L) (A3) and 48 hours (152.7 ± 31.9 U/L) (A4) following a pattern similar to that of hepatocellular enzymes. This is confirmed by a distinctly reduced error rate for the first 60 min of reperfusion (0.038).

Adenosine Triphosphate (ATP)
The amount of ATP measured in organs at the end of cold preservation period was low, ranging from 0.04 ± 0.02 and 0.06 ± 0.06 µmol/g wet weight liver. All cold preserved livers were able to regenerate ATP (Fig 3). After 120-minutes reperfusion the ATP levels were not significantly different in livers preserved for either 6, 12, 24, or 48 hours. ATP levels in livers preserved for 48 hours were significantly lower (P < .05) than control livers.

DISCUSSION
Recent results have established that the microvasculature is the site of reperfusion injury following cold preservation.18-20 PNP localized mainly in the cytoplasm of endothelial and Kupffer cells has been demonstrated to be a reliable marker to assess reperfusion damage to the microvascular endothelial cell.18 In the present study, no significant increase in PNP levels was observed in livers pre-
PNP served for either 6 or 12 h. Viability of the livers was also confirmed by normal levels of hepatocellular enzymes. Significant increases in PNP levels were, however, observed when livers were reperfused following either 24 or 48 hours of cold preservation and this increase occurred within the first 60 minutes of reperfusion. Nonviability of grafts was also confirmed by significant increases in hepatocellular enzymes.

It has been determined in other studies that only grafts preserved for up to 18 h are capable of survival. Therefore, it is apparent that PNP levels can accurately predict this, since in our studies, although 12 h of cold preservation was associated with normal levels of PNP, 24 h and more of cold preservation resulted in significant injury to the endothelial cell. Bile production is considered to be a measure of ATP generation and has traditionally been used as a marker of graft viability. In our study, however, bile production dropped significantly only after 48 hours of cold preservation, a period when the organ is no longer viable. Therefore, it is obvious that in this model, bile production has a limited usefulness as a marker of graft viability. Tissue ATP levels were similarly nonpredictive.

In conclusion, PNP levels appear to be an early indicator of preservation injury to the microvascular endothelial cell, and could prove to be an accurate diagnostic aid of graft viability following liver transplantation.

REFERENCES