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Abstract

A persistent problem in orthotopic liver transplantation is primary nonfunction (PNF) of the hepatic allograft. In an attempt to reduce the incidence of graft failure, the feasibility of pretransplant assessment of graft viability was investigated by ³¹P nuclear magnetic resonance (NMR) spectroscopy. The level of adenosine triphosphate (ATP) was measured as an indicator of liver function by ³¹P NMR spectroscopy after a 30 min normothermic reperfusion following cold-storage in University of Wisconsin (UW) solution. The mean \pm SD beta-ATP/Pi ratio after preservation for 0, 12, 24 or 48 h was 1.40 \pm 0.34, 0.85 \pm 0.27, 0.64 \pm 0.14 and 0.38 \pm 0.09, respectively. Significance was observed between 12h and 24h and between 12h and 48h of preservation. These results correlated well with the morphological changes in endothelial cells and sinusoidal lining cells examined by transmission electron microscopy. It is suggested strongly that microcirculatory disturbances due to endothelial cell injury impairs the recovery of ATP levels after reperfusion, and that ATP determination by ³¹P NMR spectroscopy, as a non-invasive modality, may help in the prediction of PNF after liver transplantation.

KEYWORDS: 31P-NMR, liver preservation, UW solution

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Brief Note

^{31}P Nuclear Magnetic Resonance Evaluation of Rat Liver Preserved in UW Solution

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A persistent problem in orthotopic liver transplantation is primary nonfunction (PNF) of the hepatic allograft. In an attempt to reduce the incidence of graft failure, the feasibility of pretransplant assessment of graft viability was investigated by ^{31}P nuclear magnetic resonance (NMR) spectroscopy. The level of adenosine triphosphate (ATP) was measured as an indicator of liver function by ^{31}P NMR spectroscopy after a 30 min normothermic reperfusion following cold-storage in University of Wisconsin (UW) solution. The mean \pm SD β -ATP/Pi ratio after preservation for 0, 12, 24 or 48 h was 1.40 ± 0.34 , 0.85 ± 0.27 , 0.64 ± 0.14 and 0.38 ± 0.09 , respectively. Significance was observed between 12 h and 24 h and between 12 h and 48 h of preservation. These results correlated well with the morphological changes in endothelial cells and sinusoidal lining cells examined by transmission electron microscopy. It is suggested strongly that microcirculatory disturbances due to endothelial cell injury impairs the recovery of ATP levels after reperfusion, and that ATP determination by ^{31}P NMR spectroscopy, as a non-invasive modality, may help in the prediction of PNF after liver transplantation.

Key words: ^{31}P -NMR, liver preservation, UW solution

Liver transplantation has become the accepted therapy for treatment of some liver diseases. Although a satisfactory prognosis after liver transplantation was reported, several problems still remain in patients undergoing liver transplantation. Among them, primary non-

function (PNF) of the graft is reported to occur in 5 to 10 % of the cases and has greatly worsened the outcome after transplantation (1, 2). The use of an inappropriate graft is considered to be one of the major causes of PNF. Accordingly, if hepatic charge could be evaluated before transplantation, the occurrence of PNF may be markedly reduced.

^{31}P nuclear magnetic resonance (NMR) spectroscopy has the advantage of being noninvasive and it continuously measures the high-energy phosphate compounds such as adenosine triphosphate (ATP) (3). Using ^{31}P NMR spectroscopy, we measured the ATP levels in the rat liver after reperfusion following cold preservation with University of Wisconsin (UW) solution. These results were compared with the morphological findings of preserved liver by transmission electron microscopy to determine whether or not there is a correlation.

Male Wistar rats, weighing 350-400 g, were used. After intraperitoneal anesthesia with pentobarbital (35 mg/kg), 400 units of heparin was injected through the penile vein. The liver was flushed out via the portal vein with 20 ml of UW solution at 4°C. The liver was removed and examined immediately or after storage for 12, 24 or 48 h at 4°C in UW solution. After cold storage, the liver was placed in a 35-mm NMR tube and measured.

The liver was then reperfused at 30 ml/min for 30 min with 37°C oxygenated Krebs-Henseleit (KH) buffer in a nonrecirculating mode and NMR spectra were recorded at 10-min intervals for a total of 30 min. All NMR spectra were acquired using a 4.7-tesla General Electric CSI spectrometer (General Electric Medical System, Milwaukee, WI, USA). The spectral conditions were as fol-

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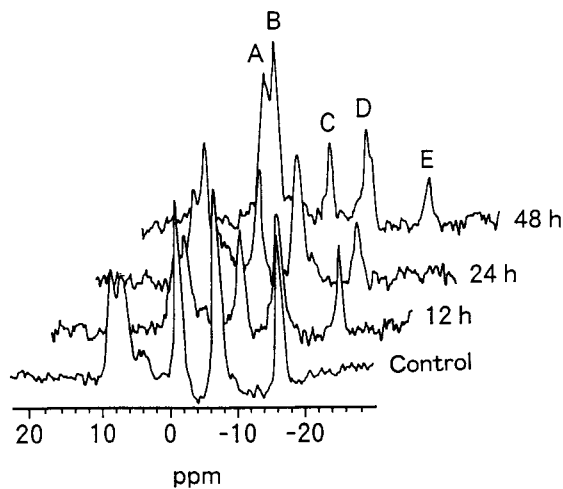


Fig. 1 ^{31}P -NMR spectra of the reperfused rat liver in the control and after preservation for 12h, 24h and 48h. A: Phosphomonoesters (PME), B: Inorganic phosphate (Pi), C: γ -ATP and β -ADP, D: α -ATP and α -ADP, E: β -ATP.

lows: pulse width = $20\ \mu\text{s}$, signal acquisition = 1000, repetition rate = 0.2 s.

Data are presented as the mean \pm SD. Statistical analysis was carried out by Student's *t*-test for unpaired data.

Fig. 1 shows the ^{31}P -NMR spectra acquired from the reperfused rat liver in the control (non-stored), and after preservation for 12h, 24h and 48h. The peaks of interest are those of phosphates which were attributed to adenosine nucleotides and inorganic phosphate. Only the β -ATP peak was used for measurement of adenosine triphosphate (ATP) in this study since it contains only signals from ATP. The value of β -ATP/Pi was used since the ATP level is a good indicator of mitochondrial function (4).

The values of β -ATP/Pi after a 30-min reperfusion in controls ($n = 5$), and after preservation for 12h ($n = 4$), 24h ($n = 5$) and 48h ($n = 5$) were 1.40 ± 0.34 , 0.85 ± 0.27 , 0.64 ± 0.14 and 0.38 ± 0.09 , respectively (Fig. 2). A significant difference was observed between preservation for 12h and 24h and between 12h and 48h.

For transmission electron microscopy, the preserved rat liver was fixed first by perfusion via the portal vein with 2% glutaraldehyde in phosphate-buffered saline (PBS), and then liver was cut into small cubes ($1 \times 1 \times 1\ \text{mm}^3$). Then the tissue was postfixed in 1% osmium

tetroxide. The samples were dehydrated by dipping into ethanol and were embedded in Epon 812. Ultrathin sections of the samples were stained with 2% uranyl acetate followed by lead citrate and examined under a JEM-100s transmission electron microscope (JEOL Ltd., Tokyo, Japan).

In the liver preserved for 12h, sinusoidal endothelial cells (SEC) were not swollen and the sinusoidal endothelial lining was almost intact virtually. No blebs were found (Fig. 3a). In the liver preserved for 24h, SEC were moderately swollen and sinusoidal endothelial lining was partially disrupted. No alterations were found in the hepatocytes (Fig. 3b). In the liver preserved for 48h, Kupffer cells were activated and protruded into the lumen, the sinusoidal endothelial lining was disrupted completely and detached from the underlying hepatocytes, and blebs were seen. Although slight swelling of mitochondria, an obvious decrease in microvilli on hepatocytes, and

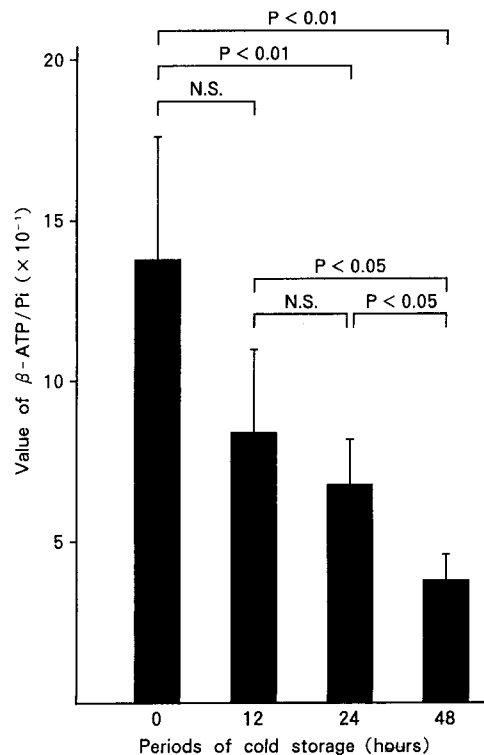


Fig. 2 β -ATP/Pi ratio measured by ^{31}P -NMR after 30 min of normothermic reperfusion for 0, 12h, 24h, 48h in the rat liver. NS., not significant: Control versus 12h and 12h versus 24h. $P < 0.05$: 12h versus 24h and 12h versus 48h. $P < 0.01$: Control versus 24h and control versus 48h.

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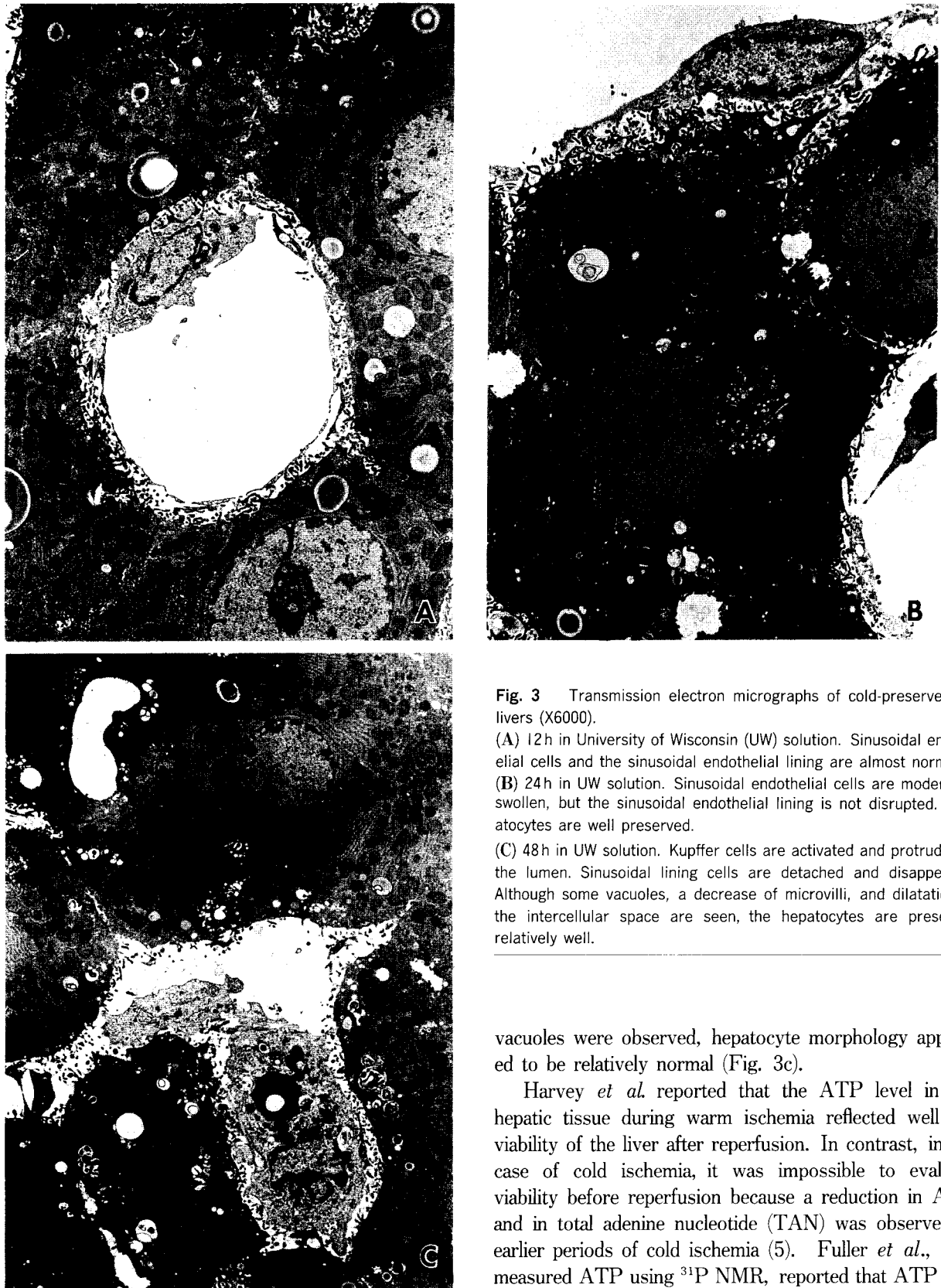


Fig. 3 Transmission electron micrographs of cold-preserved rat livers (X6000).

(A) 12h in University of Wisconsin (UW) solution. Sinusoidal endothelial cells and the sinusoidal endothelial lining are almost normal.

(B) 24h in UW solution. Sinusoidal endothelial cells are moderately swollen, but the sinusoidal endothelial lining is not disrupted. Hepatocytes are well preserved.

(C) 48h in UW solution. Kupffer cells are activated and protruded to the lumen. Sinusoidal lining cells are detached and disappeared. Although some vacuoles, a decrease of microvilli, and dilatation of the intercellular space are seen, the hepatocytes are preserved relatively well.

vacuoles were observed, hepatocyte morphology appeared to be relatively normal (Fig. 3c).

Harvey *et al.* reported that the ATP level in the hepatic tissue during warm ischemia reflected well the viability of the liver after reperfusion. In contrast, in the case of cold ischemia, it was impossible to evaluate viability before reperfusion because a reduction in ATP and in total adenine nucleotide (TAN) was observed in earlier periods of cold ischemia (5). Fuller *et al.*, who measured ATP using ^{31}P NMR, reported that ATP was

not detected 60 min after initiation of cold storage, and therefore, prediction of viability after liver transplantation was difficult (6). On the other hand, Kamiike *et al.* (7) reported a significant correlation between the levels of ATP, adenosine diphosphate (ADP) and TAN, which were measured after liver transplantation in humans in which PNF developed. These reports suggested that ATP measurement in the reperfused liver before transplantation may allow determination of the viability of the graft. Then, we analyzed the correlation between the ATP measurements at reperfusion and the morphological findings of hepatocytes on transmission electron microscopy. The electron microscopic findings can be characterized as follows: apparent swelling of endothelial cells was observed in the liver preserved for 24h, disruption of sinusoidal lining cells became more evident in the liver preserved for 48h, but no clear morphological changes were observed even after preservation for 48h.

In the study by Guyomard *et al.* (8), ATP levels in isolated rat hepatocytes were measured after preservation in UW solution for 48h at 4°C. They found that ATP levels after storage decreased to 73% of that before storage, and cell viability was well maintained. In our study, ATP levels decreased to 46% and 27% after reperfusion following 24h and 48h storage in UW solution as compared with non-preserved graft.

Furthermore, Manner *et al.* (9) measured posttransplantation liver microcirculation in a porcine model by hydrogen gas clearance and reported a good correlation with graft survival.

Taken together, these results strongly suggest that microcirculation injury due to endothelial cell damage is related to the recovery of ATP after reperfusion, and that it is involved in the occurrence of PNF after transplantation. ATP measurements before transplantation using

³¹P NMR spectroscopy may be useful for predicting the occurrence of PNF. However, further studies are planned to confirm these early results of ³¹P NMR measurements.

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