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Prediction of hepatic graft viability before reperfusion: an analysis of effluent from porcine allografts

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Abstract. Rapid and reliable assessment of hepatic graft viability is important for successful orthotopic liver transplantation (OLTx). OLTx was performed in 11 pairs of pigs via a venovenous bypass. Six of these grafts were transplanted immediately (group A), while the other five were preserved in University of Wisconsin (UW) solution for 24 h and then transplanted (group B). All grafts were flushed with 300 ml of chilled (4 °C) Ringer's lactate solution before reperfusion of the graft, when 20 ml of effluent from the graft was collected and the concentrations of ammonia, lactic acid, GOT, and LDH were measured. Four of the six pigs in group A survived longer than 3 days, while the other two pigs died of causes other than graft dysfunction. All five pigs in group B died either of hemoperitoneum or hemodynamic instability due to liver failure. The histology of postperfusion biopsies in group A showed minimal pathological changes, while the grafts in group B revealed moderate to severe ischemic injuries. Ammonia and lactic acid in the effluent of group B were significantly higher than those of group A (1511 ± 216 vs 417 ± 333 μg/dl and 114.1 ± 12.2 vs 91.4 ± 12.2 mg/dl, respectively; $P < 0.05$ in both cases). Before reperfusion, the rate of total adenine nucleotides in all of the substances in the graft, which were measured using high performance liquid chromatography (HPLC), inversely correlated with the ammonia levels in the effluent. We conclude that an analysis of the effluent, (i.e. the levels of ammonia and lactic acid), flushed from a hepatic graft before reperfusion could serve as a predictor of hepatic graft viability.

Key words: Liver transplantation, viability test – Viability, liver transplantation, pig – Venous effluent, liver perfusion

In spite of recent advances in orthotopic liver transplantation (OLTx) [2, 10, 17, 20], primary nonfunction (PNF) of hepatic allografts remains one of the most serious problems. The incidence of PNF is approximately 10%, and it is a devastating complication that is associated with an 80% mortality rate without retransplantation [1, 14]. The condition of PNF is presumably multifactorial, and possible etiologies include donor factors, such as preexisting liver disease, allograft ischemia, quality of preservation, and preservation time, as well as hyperacute rejection [3, 5].

On post-transplant evaluation of hepatic grafts, several parameters have recently been reported in clinical OLTx [4, 8, 11, 15, 19]. The focus of interest of liver transplant surgeons has been on the early assessment of the graft, either before reperfusion of the graft or before the actual transplant procedure, rather than on the post-transplant assessment. Little, however, has been elucidated on the discriminating methods of graft viability before reperfusion [6, 12, 13].

In clinical OLTx, the hepatic graft is usually flushed in situ with chilled Ringer's lactate solution to wash out the preservation solution and air in the graft before reperfusion, where the effluent could serve as an indicator of allograft viability. The purpose of the present study is to evaluate the significance of effluent components, which are flushed out from a hepatic graft prior to reperfusion, as a predictor of graft viability in pigs.

Materials and methods

Eleven pairs of pigs weighing 24–34 kg were used for the experiment. Body weights were closely matched between donor and recipient. The animals were anesthetized with nitrous oxide, oxygen, and halothane under controlled respiration using an intermittent administration of i.v. pancronium bromide and ketamine.

The techniques for allograft hepatectomy have been described elsewhere [21]. Briefly, the hepatic graft was procured during in situ flushing with 2000 ml of Ringer's lactate solution (4 °C) via the portal vein and then stored cold (4 °C) after being flushed with a preservation solution. The grafts were divided into two groups, according to the preservation period. In group A ($n = 6$), the grafts were implanted immediately; in group B ($n = 5$), the grafts were implanted after 24 h of preservation in University of Wisconsin (UW) solution.

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Table 1. Survival following orthotopic liver transplantation

Group	Survival (days)
A ($n = 6$)	$> 200, 8, 6, 4, 1^a, < 1^a$
B ($n = 5$)	$< 1^b, < 1^b, < 1^b, < 1^c, < 1^c$

[a] Death due to disconnection of the arterial line
[b] Death due to hemoperitoneum
[c] Death due to hemodynamic instability

During the OLTx, the native liver was removed via a veno-venous bypass from the portal vein and the left iliac vein to the left external jugular vein using a centrifugal pump (Centrimed System 1, Centrimed, Minn, USA) and a hepatic graft was implanted orthotopically. The blood pressure of the animal was continuously monitored during the operation by a catheter placed in the left internal carotid artery, which was used as a blood sampling route postoperatively. The order of vascular anastomoses was as follows: the suprahepatic vena cava, the infrahepatic vena cava, the portal vein, and the hepatic artery. After anastomosis of the posterior wall of the infrahepatic vena cava, the operative procedure was interrupted while the graft was flushed with 300 cc of chilled (4 °C) Ringer's lactate solution through a catheter placed in the portal vein to remove both the preservation solution and air. Immediately after the initiation of the in situ flush-out, the initial 20 ml of the effluent was aspirated in a syringe and the concentrations of ammonia, lactic acid, glutamic oxaloacetic transaminase (GOT), and lactate dehydrogenase (LDH) were measured. After the anastomosis of the portal vein, the graft was reperfused. The anastomosis of the hepatic artery was then performed between the donor's common hepatic artery or celiac axis and the recipient's common hepatic artery at the bifurcation of the gastroduodenal artery in an end-to-end fashion. Billiary reconstruction was by choledochocholedochostomy. No immunosuppressant was administered during this experiment.

At 120 min after reperfusion of the graft, blood samples were drawn to determine blood cell counts (white blood cells, red blood cells, platelets), the coagulation profile [prothrombin time (PT), activated partial thromboplastin time (APTT), and hepaplastin test (HPT)], and chemistry (GOT, LDH, and β-glucuronidase). A biopsy of the graft was obtained before reperfusion, at 30, 60, and 120 min after reperfusion, and then before the abdominal closure. The biopsy specimens were immediately immersed in liquid nitrogen and were then assayed for adenine nucleotides and purine compounds, using high performance liquid chromatography.

High performance liquid chromatography (HPLC)

For the preparation of hepatic extracts, liver samples were placed in 1 ml of 6% perchloric acid and ultrasonically homogenized. The homogenate was centrifuged at 4 °C for 10 min at 3000 rpm, and the precipitate was homogenized and centrifuged in the same manner. The supernatant was neutralized with 6N potassium carbon trioxide and was centrifuged at 4 °C for 10 min at 3000 rpm. The supernatant was used for the assay of adenine nucleotides and purine compounds.

HPLC was performed using a system LC-6A (Shimadzu, Kyoto, Japan) equipped with a data processor for the measurement of the following substances. Adenine nucleotides [adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP)] and purine compounds (xanthine, hypoxanthine) were measured by reverse-phase HPLC with a column, CLC-ODS (0.15 m × 6.0 ∅; Shimadzu, Kyoto, Japan), which was equilibrated by a buffer containing 48 m*M* diethylaminoethanol and 14.6 m*M* of citrate with a pH of 3.5 adjusted by H_3PO_4. The effluent rate was 1.5 ml/min and the eluate was continuously monitored at 260 nm. Each peak was analyzed by a comparison with the retention times of authentic compounds. The concentrations were estimated by measuring the peak areas. The levels of total adenine nucleotides (TAN) were then calculated as a summation of ATP, ADP, and AMP. The energy charge (EC) was defined as $(ATP + {}^1/_2ADP)/(ATP + ADP + AMP)$.

For the measurement of adenine nucleotides and purine compounds, three samples in each group were available. TAN was expressed as the rate of TAN in all the measured substances.

For the histological examination, biopsies were taken before and after reperfusion and immersed for hematoxylin and eosin staining.

Statistical analysis

Data were expressed as the mean ± SD and were analyzed using the Wilcoxon rank sum test.

Results

Table 1 shows the survival of both groups. All allografts in group A produced a fair amount of bile soon after revascularization, and the animals quickly awoke from anesthesia. Four of six pigs survived longer than 3 days after OLTx. The deaths of the other pigs in group A were due to an accidental disconnection of an arterial line. All animals in group B died within 1 day after OLTx. Three of the five pigs died of hemoperitoneum, which was judged to be secondary to primary liver failure, while the other two pigs died of hemodynamic instability after reperfusion, which was presumably due to myocardial depression from ischemic products produced by nonviable hepatic allografts.

Figure 1 shows the changes in energy charge (EC) during OLTx. The EC of group A quickly recovered after reperfusion, while that of group B decreased without returning again to normal levels.

As for the comparison of post-transplant data, PT and APTT were significantly prolonged in group B (16.6 ± 0.9 vs 13.2 ± 0.6 s and 59.8 ± 24.6 vs 34.3 ± 4.9 s, respectively; $P < 0.05$ in both cases). HPT in group A (52.4% ± 11.1%) was significantly better than that in group B (25.8% ± 12.9%, $P < 0.01$). With regard to liver chemistry, GOT, LDH, and β-glucuronidase in group B were significantly higher than those in group A (1174 ± 401 vs

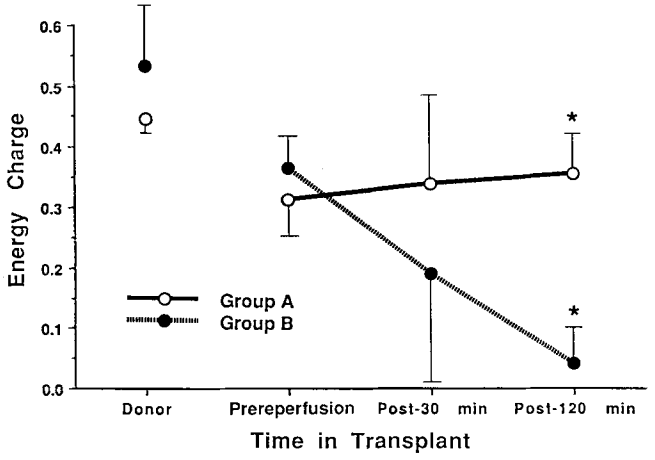

Fig. 1. Changes in energy charge during orthotopic liver transplantation. The energy charge of group A was quickly restored, while that of group B did not return to normal levels. * $P < 0.05$

Table 2. Comparison of contents in the perfusate flushed from hepatic grafts. GOT, glutamic oxaloacetic transaminase; LDH, lactate dehydrogenase

Variable	Group A ($n = 6$)	Group B ($n = 5$)
Ammonia (μg/dl)	417 ± 333[a, b]	1511 ± 216[b]
Lactic acid (mg/dl)	91.4 ± 12.2[c]	114.1 ± 12.2[c]
GOT (IU/l)	440 ± 217	750 ± 711
LDH (IU/l)	1134 ± 557	1863 ± 1770

[a] Mean ± SD
[b, c] $P < 0.05$

307 ± 87 IU/l, 3163 ± 1097 vs 1355 ± 390 IU/l, 2610 ± 444 vs 443 ± 200 μg/dl, respectively; $P < 0.05$ in all cases).

As to the histological evaluation of the liver biopsies taken before reperfusion, no significant difference was detected except for damage to the sinusoidal endothelial cells, which was more prominent in group B. On the other hand, the histology of postperfusion biopsies in group A showed nearly normal liver appearances, while that in group B revealed moderate to severe preservation damage, such as midzonal necrosis, diffuse hepatocellular swelling, and microvesicular steatosis.

Table 2 shows the concentrations of parameters measured in the effluent. The levels of ammonia and lactic acid in the effluent in group B were significantly higher than those in group A (1511 ± 216 vs 417 ± 333 μg/dl, 114 ± 12.2 vs 91.4 ± 12.2 mg/dl, respectively; $P < 0.05$ in both cases). Only one of the six grafts in group A had an ammonia level over 1000 μg/dl while, on the other hand, all grafts in group B had a level over 1000 μ/dl (Fig. 2). The values of GOT and LDH in group B tended to be higher than those in group A (750 ± 711 vs 440 ± 217 IU/l, 1863 ± 1770 vs 1134 ± 557 IU/l, respectively).

The rate of TAN in the measured substances immediately before reperfusion of group A (69.8% ± 1.0%) tended to be higher than that of group B (48.9 ± 13.7, $P = 0.081$). There was also an inverse correlation between the level of ammonia in the effluent and the rate of TAN of the graft ($r = 0.748$, $P = 0.087$), while there was no significant difference in the EC of the graft immediately before reperfusion between the two groups.

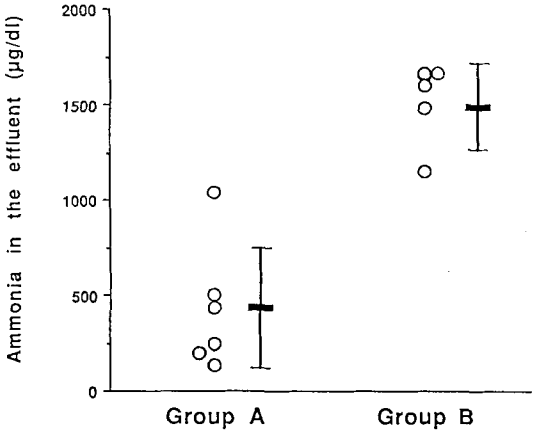

Fig. 2. Comparison of ammonia levels in the effluent before reperfusion. A significant difference ($P < 0.05$) of ammonia levels in the effluent between the groups is observed

Discussion

In the present study, we used two groups: grafts preserved for a short period of time and ones stored cold for 24 h in UW solution. The biochemical data, histology, intraoperative bile production, and survival indicate that the hepatic grafts in these two groups are extremely different: the viability of the grafts in group A was judged to be good, whereas grafts in group B were nonviable. In spite of the fact that the same technique was used, the hemoperitoneum occurred only in group B, but not at all in group A. We believe that the hemoperitoneum in group B was caused by the failed graft and not due to technical difficulty, since all animals in group B exhibited generalized oozing from multiple raw surfaces at the end of the operation and failed to produce any significant amount of bile after reperfusion. Furthermore, post-transplant liver histology of group B showed moderate to severe preservation damage, unlike the near-normal findings in group A.

It was interesting to observe that a porcine graft preserved in UW solution for 24 h was not viable since human livers can be preserved successfully for up to 24 h [20]. Isai et al. [7] suggested in 1990 that the 24-h preservation of a porcine graft in UW solution would fail due to the severe damage of sinusoidal endothelial cells. We also feel that the difficulty with 24-h preservation of a porcine hepatic graft using UW solution is due to the difference between species in susceptibility to preservation damage of sinusoidal endothelial cells.

With regard to the effluent, we believe that the initial flush-out solution is important because the initial effluent should contain preservation fluid that represents the sinusoidal status and graft viability.

For the assessment of hepatic graft viability following OLTx, some progress has recently been made in clinical OLTx, such as the measurement of plasma lecithin: cholesterol acyltransferase (LCAT) activity [15], restoration of ATP of the graft [11], arterial ketone body ratio [19], clearance of free amino acids [4, 8], and total body oxygen consumption [18]. All of these parameters could be useful in monitoring graft function after OLTx. However, the evaluation of graft viability prior to reperfusion or actual transplantation is more valuable and practical than post-transplant assessment. To date, only a few studies have reported on the pretransplant assessment of hepatic graft viability. In 1990, Ollerich et al. [13] showed that the liver's capacity to metabolize lidocaine in the donor correlated with graft outcome following OLTx. Lanir et al. [12], in 1988, reported that there was a positive relationship between hepatic allografts with a high ATP content and energy charge and a successful outcome of OLTx.

Exogenous ammonia is derived from the intestine by bacterial action, while endogenous formation of ammonia takes place in the liver and kidneys, as well as in the peripheral tissues and brain, though to a lesser extent. Moreover, ammonia is known to be produced in the process of adenine nucleotide degradation during ischemia [9]. Theoretically, the degradation of one molecule of ATP can produce one molecule of ammonia. Therefore, any increased level of ammonia in the effluent of an ischemic hepatic graft can be reflected in the reduction of the energy

status of the graft, which was reported to closely correlate with the graft outcome following OLTx [12]. In the present study, we demonstrated a significant correlation between the ammonia and lactic acid levels in the effluent and hepatic graft viability. Our preliminary report, in which the level of the ammonia in the effluent flushed out from the graft correlated well with the graft quality and ATP content of the graft before OLTx using an extracorporeal OLTx model in the dog [16], reinforces the above-mentioned fact.

During anaerobic glycolysis, glucose is metabolized into the pyruvate, and then the pyruvate, which goes to the TCA cycle in aerobic glycolysis, changes into lactic acid. Lactic acid finally causes tissue acidosis, and the level of lactic acid in the effluent can therefore indicate the degree of acidosis and anaerobic metabolism of the graft.

In summary, the measurement of ammonia and lactic acid levels in the effluent from the grafts prior to reperfusion appears to offer potential as a reliable and simple predictor of graft viability in OLTx.

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