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Liver conditioning after cardiac arrest: the use of normothermic recirculation in an experimental animal model

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Abstract The aim of this study was to compare the possible role of normothermic recirculation with the role of liver transplants from non-heart-beating donor pigs after 20 min of cardiac arrest. Three groups were studied, of which two were control groups: group 1, in which the liver was harvested from a heart-beating donor; group 2, in which the liver was harvested after a period of cardiac arrest followed by total body cooling; and group 3, in which the liver was procured as in group 2, but including a period of 30 min of cardiopulmonary bypass and tissue oxygenation at 37 °C before total body cooling. Survival at 5 days; endothelial (hyaluronic acid) and hepatocellular damage (AST, ALT, and α-GST); adenine nucleotides (energy charge), and histological changes were evaluated. Normothermic recirculation during 30 min showed a significant effect on survival ($p = .03$), endothelial damage ($p < .05$), and histological changes after reperfusion ($p = .04$). Cardiopulmonary bypass significantly increased the energy charge during the normothermic recirculation period ($p = .001$). Moreover, this study shows that a significant survival (100 %) can be achieved with a liver allograft after 20 min of cardiac arrest. Although the liver suffers a major insult in terms of endothelial damage and hepatocellular damage, lesions caused by the ischemic injury are reversible. Histological changes also indicate lesion reversibility, since they almost disappear after 5 days.

Key words Liver conditioning · Cardiac arrest, liver donation · Normothermic recirculation · Liver transplantation, experimental

Introduction

The shortage of organ donors is a universal problem. The non-heart-beating donor (NHBD) is gaining importance as a potential source of transplantable organs for clinical use. Successful kidney transplantation with organs obtained from donors after cardiac arrest has been widely reported [12, 40]. All authors agree that the viability is similar to that obtained with other organs from heart-beating donors, despite an increased incidence of acute tubular necrosis, which is responsible for delayed function.

Procurement of kidneys from NHBDs is based on a shortened warm ischemia time to minimize ischemic damage. "Total body cooling", which is achieved by extracorporal cardiopulmonary bypass [13, 39], seems to be the best option. With this method, first described by Koyama et al. [27], the body temperature is progressively and quickly reduced, which provides enough time to obtain harvesting permission. It has been shown that organs obtained by this method are of better quality and exhibit a reduced incidence of acute tubular necrosis.

Due to the fact that the demand for liver donors continues to exceed the supply, the interest in the use of NHBDs has increased. However, experience in liver transplantation with this type of donors has been limited and has only been considered for donors in whom cardiac arrest occurred at a known time (a few minutes previously [4, 5]). This is mainly due to the lack of knowledge about the reversibility of histological damage and the fact that immediate liver function remains unpredictable after transplantation [37]. Despite such limited and sometimes poor experience, clinical and experimental reports suggest that the liver can tolerate a substantial period of warm ischemia, even periods up to 60 min [16, 23, 32, 33, 35].

Total body cooling may ameliorate the damage caused by cardiac arrest by decreasing the warm ischemia time; however, cardiac arrest decreases the cellular energy status. This is in turn associated with poor survival after transplantation of the liver. It has recently been reported that "extracorporal circulation with cardiopulmonary bypass (CPB) and tissue oxygenation at 37 °C" during a period of time prior to "total body cooling" may improve the compromised cellular energy status that appears after a period of cardiac arrest. This improves graft viability [22].

This procedure, called "normothermic recirculation", starting at the time of cardiac arrest and the period of "total body cooling", reduces ischemic injury and even reverses it. Furthermore, given that the variable quality of livers from cardiac-arrested donors are likely to be of variable quality, the time required to resume circulation in the donor at 37 °C may provide a means of measuring quality of the organ for transplantation.

The aim of this study was to evaluate the feasibility of liver transplantation from NHBD pigs after 20 min of warm ischemia and the role of normothermic recirculation and tissue oxygenation at 37 °C degrees during 30 min prior to total body cooling, in terms of survival, liver function, and histology.

Materials and methods

Experimental design

To determine the effect of normothermic recirculation three groups were designed:

Group 1 ($n = 5$): there was no warm ischemia time. Livers were procured from a heart-beating donor in a standard manner.

Group 2 ($n = 5$): there was a 20-min period of cardiac arrest, followed by in situ cooling until a body temperature of 15 °C was achieved (20 min). The liver was then harvested.

Group 3 ($n = 10$): is similar to group 2, but includes a period of normothermic recirculation (30 min) prior to in situ cooling.

Donor operation for group 2

Pigs weighing 25–35 kg were fasted for 36 h prior to surgery. They were sedated with an intramuscular injection of azaperone 10 ml/kg and atropine sulfate 0.025 mg/kg. A small venous catheter was inserted into the ear vein; anesthesia was then induced with 15 mg/kg of sodium pentobarbital. The lungs were mechanically ventilated (Themel, VT/3) at a tidal volume of 15 ml/kg with oxygen (FiO_2 1) after orotracheal intubation. The respiratory rate was initially adjusted to maintain an end-tidal carbon dioxide level between 30 and 35 mmHg (Critical Care Systems, Poette Model 602-11). Anesthesia was maintained with isofluorane (0.8–1.2 MAC), fentanyl 3 μg/kg, and muscle paralysis was obtained with atracurium besilate 0.3 mg/kg. Continuous EKG monitoring was carried out.

The right external jugular vein and carotid artery were exposed by a longitudinal incision on the right side of the neck; a catheter (16-G, Arrow) was placed into the carotid artery for blood sampling and arterial pressure recording (Hewlett-Packard, 78342-A).

After the abdomen had been opened, the vena cava and aorta were exposed. At that time, heparin was given intravenously (3 mg/kg). The jugular vein, aorta, and cava were then cannulated (22 Fr, 16 Fr, and 28 Fr, respectively) and connected to a blood oxygenator (William-Harvey Blood Oxygenator, H-1700, CR Bard), a heat exchanger (Marcusor, Sorin), and a non-pulsatile roller pump (Stöckert-Shiley). The circuit was primed with saline solution 500 ml, mannitol 0.5 g/kg, and Hemoce (Hoechst Farma) 500 ml. Liver and esophageal temperature were also monitored (Mallinckrodt). Cardiac arrest was then produced by an intravenous injection of KCl (10–15 ml 2M).

After 20 min of warm ischemia, extracorporeal circulation, and tissue oxygenation at 37 °C was initiated and maintained during 30 min at the maximum flow rate to achieve 2.2 l/m^2 body surface. Sodium bicarbonate was added to the circuit to correct metabolic acidosis. After this period, total body cooling was started until the animal progressively reached a liver temperature of 15 °C (20 min). At this moment, the liver was harvested in a standard manner by perfusion with UW solution through the aorta and portal vein and then cooled and preserved at 4 °C for 6 h.

The mean arterial pressure, liver and esophageal temperature, pump flow rate, and temperature of the heat exchanger were recorded at least every 5 min. Blood samples were taken for blood gas analysis, electrolyte and hemoglobin determinations (Analyser 288, Blood Gas System, Ciba-Corning Diagnostics) 5 min after the beginning of recirculation and every 15 min thereafter.

The group 2 donor operation was similar to that of group 1, but with omission of the 30-min period of normothermic recirculation. The remainder of the procedure was identical to that of group 1.

The group 1 donor operation, anesthesia, and liver harvesting were also similar, but in this group no cardiac arrest was produced.

Table 1 Histologic changes evaluated in liver biopsies by a semiquantitative scoring system at the time of reperfusion (SII sinusoidal inflamatory infiltrate, SD sinusoidal dilatation, SC sinusoidal congestion, MHC microvacuolar hepatocyte changes, HS & D hepatocyte shrinking and disruption, FIN focal inflamatory necrosis, IN ischemic necrosis, – absent, + mild or focal, ++ moderate, +++ severe)

Group	SII	SD	SC	MHC	HS & D	FIN	IN
1	+	+	+	++	+	–	–
2	+/++	+/++	++/+++	++/+++	+++	–	–
3	+	+	+	++	++	–	–

Recipient operation

The anesthetic management was similar to that of the donor group. The right external jugular vein and carotid artery were exposed in a similar way. Single lumen sampling catheters (16-G, Arrow) were placed in both vessels and connected to a pressure transducer (Baxter-Edwards). An electrocardiogram, the arterial pressure, and the central venous pressure were recorded continuously. Blood gas was analyzed, and electrolytes and hemoglobin levels were determined during the procedure so that any metabolic imbalance could be corrected. A standard hepatectomy was performed as previously described [3]. The allograft was placed in the recipient and anastomoses were performed in the following order: the suprahepatic vena cava and portal vein first (the liver was immediately revascularized) and then the infrahepatic vena cava, the hepatic artery, and finally the biliary tract. The gall bladder was left in place. A veno-venous bypass was not used since the anhepatic stage lasted no longer than 20 min in any case. The hepatic arterial blood flow was restored between the donor celiac axis and the hepatic artery of the recipient by magnification lens. The biliary tract was reconstructed with an intraluminal stent externally secured with two silk stitches. Cold ischemia time from the beginning of the total body cooling to reperfusion in the recipient was 6 h and 30 min. The abdominal wall was closed in two layers, and the skin was closed with a running silk suture.

Postoperative care

The animals were tracheally extubated from 20 to 35 min after the operation. They were placed into metabolic cages with warm lamps. Blood gases were monitored postoperatively for several hours. Analgesia was given by intramuscular injection of meperidin 100 mg, 1 h after tracheal extubation.

The immunosuppressive regimen included methylprednisolone (500 mg) and azathioprine (1.5 mg/kg) before liver reperfusion, and oral cyclosporin (25 mg/kg daily) after the first postoperative day.

Oral fluid was permitted on the 1st postoperative day, and the animals were fed with commercial pig food and yoghourt, after the 2nd day. The animals were sacrificed on the 5th postoperative day by an i.v. overdose of sodium pentobarbital. The "Principles of laboratory animal care" (NIH publication No.86-23, 1985) were followed for the animal experiments.

Samples and measurements

Tissue samples from the donor liver and blood samples were taken in the following stages: at the beginning of the procedure in the donor, after 20 min of cardiac arrest, after 30 min of recirculation at 37 °C, before reperfusion in the recipient (biopsy only), 1 h after reperfusion and postoperatively at 3 and 5 days after transplantation (only plasma was taken).

The blood was immediately centrifuged and the supernatant plasma divided into aliquots, frozen, and stored at –20 °C for later determinations. Hepatocellular damage had been determined by SGOT, SGPT and α-GST, and endothelial damage had been determined by hyaluoronic acid (HA) at the previously established stages.

HA is an unbranched, high-molecular-weight polysaccharide. It is synthesized by mesenchymal cells and is widely distributed throughout the body. However, only a small amount enters the bloodstream [34]. The HA that reaches the circulation is eliminated mainly by way of receptor-mediated endocytosis by the liver endothelial cells (LECs). Therefore, in the absence of increased production, increased serum HA levels reflect LEC damage [7, 9, 10, 29]. In contrast, glutathione S-transferases (GSTs) comprise a family of dimeric detoxifying enzymes that catalyze the reaction of glutathione with a wide range of toxic substances. The human basic GSTs (α class) are cytosolic enzymes found in high concentrations in the liver and kidneys. Given its wide hepatic distribution and short in vivo plasma half-life, this enzyme is useful to detect hepatocellular injury [17, 19, 30, 33, 38] sensitively. The enzyme were analyzed by the standard clinical chemistry laboratory methods (Bayer Technichon, Barcelona, Spain) and the results were expressed as IU/l. HA was measured by radiometric assay with the Pharmacia HA Test (Pharmacia Diagnostics, Uppsala, Sweden) and expressed as μg/l.

Liver biopsies for histological evaluation were taken from the left lobe of the liver for with light microscopy. The specimens were fixed with 4% formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following histological changes were evaluated: sinusoidal inflammation, sinusoidal dilatation, congestion, hepatocellular vacuolization, dissociation of liver cell plates and hepatocytes, focal necrosis with inflammatory cells, vascular thrombosis, and ischemic necrosis. The intensity of these changes was evaluated by a semiquantitative scoring system as absent (–), mild or focal (+), moderate (++) and severe (+++) (Table 1). The pathologist was unaware of the period or the animal studied.

Sequential biopsies were also obtained at the same stages and quickly frozen in liquid nitrogen for determination of the energy cellular status (ATP, ADP, and AMP) [15]. This allowed subsequent calculation of tissue energy charge which was obtained by Atkinson's equation:

$$EC = ATP + 0.5\ ADP/ATP + ADP + AMP \quad (1)$$

These specimens were stored in liquid nitrogen until they were assayed with a high-performance liquid chromatography (HPLC) technique [31]. The quantities are given in nM/mg of proteins.

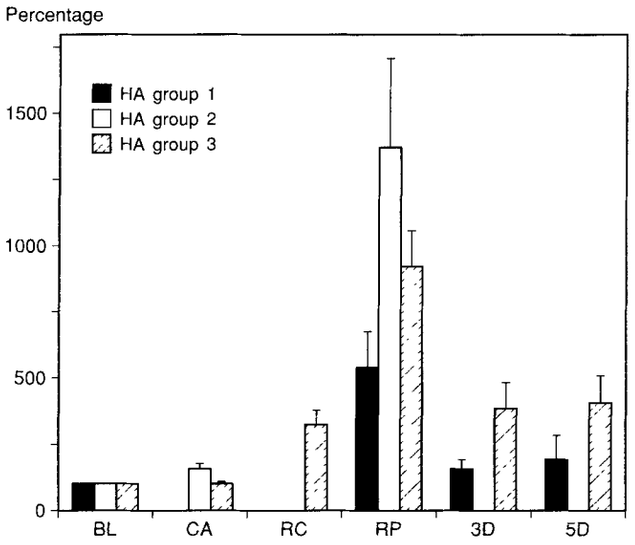

Fig. 1 Hyaluronic acid levels during the procedure in the three groups. Values at the time of reperfusion were significantly higher for group 2 than for group 1 ($P = .02$) and group 3 ($P = < .05$). However, there was no significant difference between groups 1 and 3. Normothermic recirculation showed an significant overall beneficial effect ($p < .05$) on endothelial damage. Group 1 Heart-beating donor; group 2 non-heart-beating donor without normothermic recirculation; group 3 non-heart-beating donor with normothermic recirculation; BL baseline; CA cardiac arrest; RC recirculation period; RP reperfusion; 3D 3 days postoperatively; 5D 5 days postoperatively; p = with respect to group 1. Values are expressed in percentages of the baseline value

Statistical analysis

Statistical analysis was carried out according to MANOVA repeated measures. Values of p of 0.05 or less were considered significant. The results are expressed as means ± standard error (SE) unless stated otherwise. In order to compare the three groups, values were considered relative to the baseline value (relative value, %). For comparisons with the MANOVA test, values for the period of cardiac arrest and recirculation in control group 1 were considered baseline values. At the same time, values for group 2 after recirculation, which does not exist in this group were treated as the values obtained during the previous stage.

Results

Survival analysis

As expected, all the group 1 livers transplanted from a heart-beating donor survived for 5 days. However, none of the animals with a transplanted liver survived after 20 min of cardiac arrest without normothermic recirculation (group 2). All the animals died within 24 h; two immediately after liver transplantation due to uncorrectable metabolic acidosis. At autopsy, all animals had massive serohemorrhagic ascites. The patency of all anastomoses was assessed and confirmed. However, with recirculation at 37 °C and tissue oxygenation during 30 min ten out of ten pigs lived for 5 days (100 %) ($X^2 = 6.6$ $p = .03$).

Endothelial damage

The mean baseline values of HA for all animals was of 146.1 ± 15 (range from 33 to 538). In the study group (group 3) there was a progressive, significant increase in HA levels throughout the procedure (Fig. 1), reaching the peak value 1 h after revascularization (1230 ± 104 μg/l). Thereafter, these levels decreased to normal or near normal values on the 5th day (400 ± 90 μg/l).

On comparing the three groups, we found that the effect of normothermic recirculation on HA was significant (Fig. 1). During the reperfusion stage, the relative values increased more importantly in group 2, reaching statistical significance when compared to group 1 (1371 ± 100 vs 537 ± 123, $p = 0.03$). Values in group 3 were between those of groups 1 and 2 (Fig. 1), although the difference did not reach statistical significance ($p < 0.1$). During the follow-up period, at 3 and 5 days after liver transplantation, the values remained higher bud did not differ significantly ($p = 0.09$) in the surviving pigs in group 3. Both groups showed a trend to baseline values.

Hepatocellular damage (AST, ALT, and GST)

The mean level of AST at the beginning of the procedure was 53 ± 20 UI/l. The levels increased significantly throughout the procedure, and differences were significant at all stages with respect to baseline values. The greatest values, however, were observed 3 days after transplantation (1170 ± 212 UI/l). Finally, the mean AST level reverted significantly after 5 days of survival (264.5 ± 126 UI/l, $p < 0.02$) (Fig. 2).

When the groups were compared, we found that there was no overall significant effect of normothermic recirculation on AST release. The relative AST levels were significantly higher in group 3 than in group 1 ($p < 0.03$) 3 and 5 days after liver transplantation.

The mean level of ALT at the beginning of the procedure was 41 ± 2.5 UI/l (range: 10 to 90). The levels increased in group 3 throughout the procedure, but the differences were only significant after reperfusion with respect to baseline values. Similarly to the AST levels, the greatest amount was released by the liver 3 days after transplantation (137 ± 25 UI/l). Finally, at 5 days, ALT remained increased, although it was not significantly greater than the baseline values (Fig. 2).

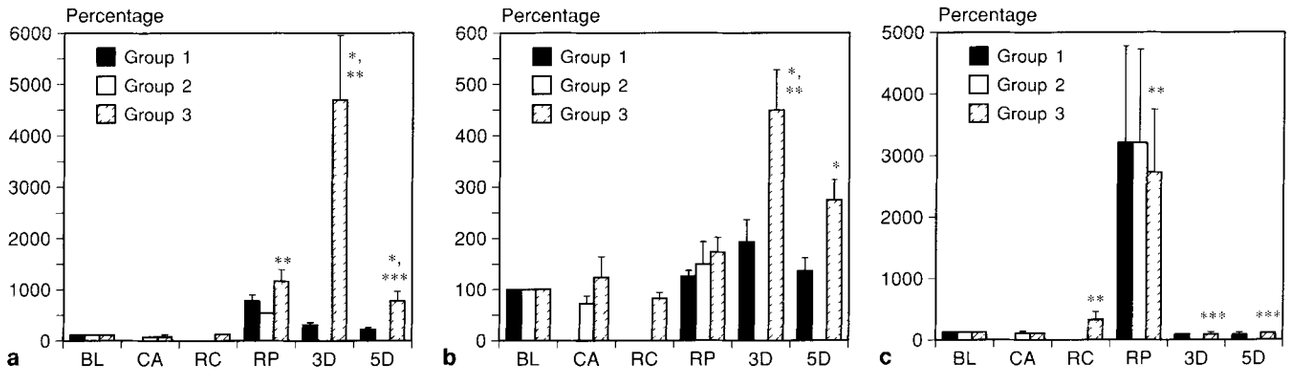

Fig. 2a–c Hepatocellular damage during the procedure. While AST and ALT peaked on the 3rd day postoperatively, the peak GST value appeared 1 h after reperfusion, the values at the at time being significantly higher than in the previous period. In group 3, GST significantly increased after normothermic recirculation. No overall effect of normothermic recirculation could be found in any of the three variables. (Group 1 heart-beating donor; G1 group 2 non-heart-beating donor without normothermic recirculation; G2 group 3 non-heart-beating donor with normothermic recirculation; BL baseline; CA cardiac arrest; RC recirculation period; RP reperfusion; 3D 3 days postoperatively; 5D 5 days postoperatively; $*P < 0.05$ with respect to group 1, $**P < 0.05$ with respect to baseline, $***P < 0.05$ with respect to the previous period. Values are expressed in percentages of the baseline value)

On comparison of the groups, there was no overall significant effect of normothermic recirculation on ALT release. As observed with AST, at days 3 and 5 after liver transplantation, the values were significantly greater in group 3 than in group 1 ($p = 0.01$).

α-*Glutathione-S-transferase (α-GST) levels*

Baseline values for GST (all animals) were 5.1 ± 1.4 ng/ml (range: 1.8 to 13). In group 3 the mean values increased gradually throughout the procedure. As with HA, the peak value appeared 1 h after reperfusion. However, a significant increase was present at the time of normothermic recirculation (21.6 ± 14 vs 5.6 ± 1 ng/ml, $p = 0.0001$). Moreover, a rapid decrease towards normality was observed, with figure at 3 days similar to baseline values, but significantly different with respect to values of the reperfusion stage (5.9 ± 1.5 vs 218 ± 130, $p = 0.0001$).

Comparing the three groups, there was no overall effect of normothermic recirculation on the GST levels. Although the values were lower in group 3 (2716 ± 1046 vs 3215 ± 1600 for group 1 or 3210 ± 1553 for group 2) during reperfusion, the difference was not significant. During the follow-up period, the GST levels decreased similarly in the two surviving groups.

Energy status

The mean ATP value in baseline conditions was 1.27 ± 0.92 nM/mg protein, decreasing significantly to 0.44 ± 0.51 nM/mg protein ($p < 0.0007$) after 20 min of cardiac arrest in the donor. This mean value did not change after 30 min of recirculation at 37 °C and was maintained at this level for 1 h after reperfusion in the recipient. After 5 days of survival, the ATP level reached the baseline value (1.58 ± 0.26 nM/mg protein).

The mean value for ADP at the baseline was 2.17 ± 1.25 nM/mg protein which decreased significantly to 1.02 ± 0.43 nM/mg protein ($p < 0.002$) after 20 min of cardiac arrest. However, this value increased to 1.09 ± 0.62 nM/mg of protein after 30 min of recirculation at 37 °C. After 5 days of survival, ADP values achieved those observed at baseline (Fig. 3).

AMP decreased progressively during the operation from a mean value of 9.65 ± 4.49 nM/mg of protein to 3.84 ± 1.97 nM/mg of protein with the lowest values being observed 1 h after reperfusion in the recipient.

In summary, adenine nucleotides decreased significantly after cardiac arrest, and only the quantity of ADP seemed to improve after 30 min of extracorporal circulation and tissue oxygenation.

Accordingly, the following changes were observed with regard to the energy charge; a significant decrease after 20 min of cardiac arrest (from 0.164 ± 0.05 to 0.113 ± 0.06, $p < 0.01$), followed by a significant ($p = 0.001$) return to normal values after 30 min of recirculation at 37 °C (0.191 ± 0.08) loss of the significant difference with respect to baseline levels (Fig. 3). The energy charge was maintained within these values during the remainder of the procedure, reaching a mean value of 0.170 ± 0.08 after 5 days.

When the three groups were compared for the three nucleotides, only the ADP values (Fig. 3) tended to be higher in group 3 (with respect to the other groups), before and after reperfusion in the recipient, but no overall effect of normothermic recirculation was observed over the mean ADP levels ($p < 0.1$).

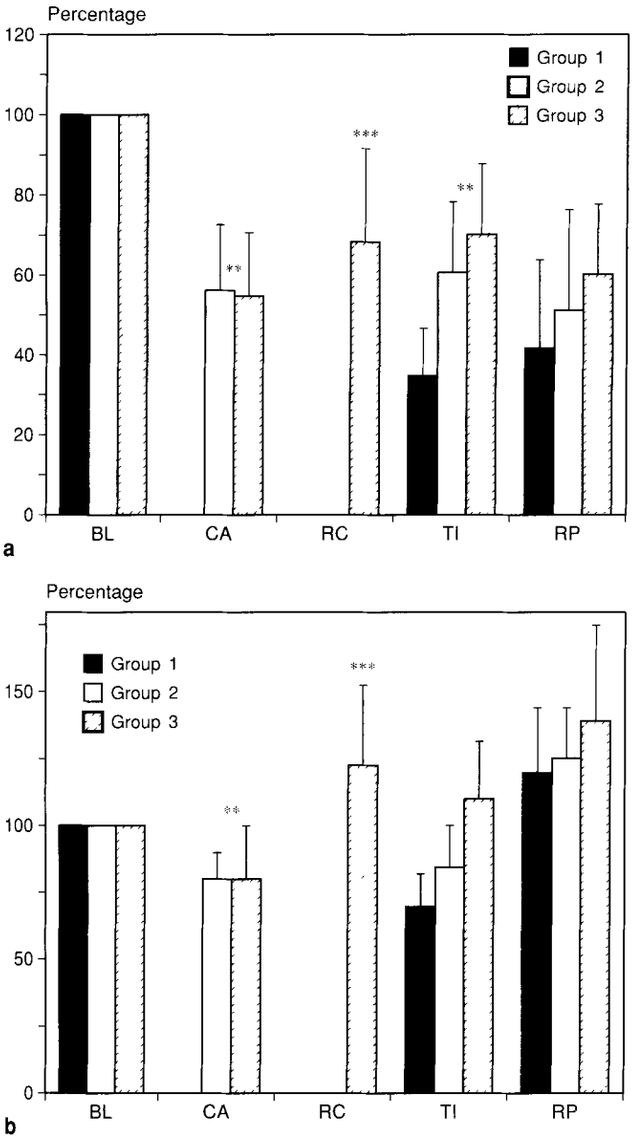

Fig. 3a,b Adenine nucleotides and energy charge. In study group 3, there was a significant reduction in energy charge after 20 min of cardiac arrest, which returned to normal values after normothermic recirculation. However, no effect of normothermic recirculation was found in any of the variables studied. (Group 1 heart-beating donor; group 2 non-heart-beating donor without normothermic recirculation; group 3 non-heart-beating donor with normothermic recirculation; BL baseline; CA cardiac arrest; RC recirculation period; RP reperfusion; 3D 3 days postoperatively, 5D 5 days postoperatively, $^{**}P < 0.05$ with respect to baseline, $^{***}P < 0.05$ with respect to the previous time period. Values are expressed in % of the baseline value)

Thus, on comparing the energy charge among the three groups (Fig. 3), the mean values were always greater at any period in group 3; however, the overall effect or normothermic recirculation did not reach statistical significance ($p = 0.1$).

Histological damage

Intraoperative changes

In group 1, liver biopsies taken during transplantation showed a mild degree of sinusoidal inflammation, sinusoidal dilatation, and congestion just before and after reperfusion. Only two cases showed hepatocellular vacuolization.

On including a period of cardiac arrest, changes before reperfusion were almost similar to those found in the control group, but there was a moderate degree of sinusoidal inflammation, sinusoidal dilatation, congestion, and hepatocellular vacuolization. One hour after reperfusion (Table 1), the severity of sinusoidal congestion, hepatocellular vacuolization, and disruption were markedly increased and significantly different from that of group 1 ($p = 0.01$).

When a period of normothermic recirculation was added to the procedure (group 3) before reperfusion, changes were again similar, but the severity of sinusoidal dilatation was more important in group 2 ($p = 0.019$). However, histological changes after reperfusion (Table 1) were less severe in group 3 than in group 2 ($p = 0.04$).

On postoperative day 5

Histology was normal in two animals in group 1. In those with lesions, a mild degree of cellular congestion, portal inflammation, and scarce areas of hepatocellular necrosis were found. The mean necrotic area was about 6.5%.

In group 3, three animals had normal or near normal histology. Three other biopsies demonstrated minor foci of ischemic necrosis in perivenular hepatocytes. More extensive ischemic necrosis of perivenular hepatocytes was observed in two pigs, but there were large periportal areas of well-preserved hepatocytes. Finally, extensive ischemic necrosis with absence or minor areas of non-necrotic hepatocytes was seen in two other biopsies, coinciding in the two pigs with higher AST levels. The mean necrotic area was about 31%. Eight of the ten animals had a normal biliary tract.

None of the histological changes evaluated after cardiac arrest and after normothermic recirculation had any predictive value.

Discussion

This study was mainly directed at determining the feasibility of liver transplantation from NHBDs with a CPB and a period of recirculation at 37 °C with tissue oxygenation prior to total body cooling. Post-transplant liver viability was determined by recipient survival during the first 5 days. After 6 h of cold preservation, all the pigs in the control group (group 1) and the study group (group 2) retained immediate life-sustaining graft function following transplantation and survived for 5 days. These results suggest that, although graft viability may be compromised, liver transplantation is feasible after 20 min of warm ischemia and that this type of organ is capable of sustaining life immediately after transplantation. Moreover, the use of cardiopulmonary bypass and tissue oxygenation at 37 °C may have a beneficial effect on the liver so procured by ameliorating the ischemic injury after reperfusion. A fact that deserves further comment is related to the "all-or-nothing" effect of normothermic recirculation on survival after 20 min of cardiac arrest. It must be taken into account that no further treatment (except for correction of metabolic acidosis) was given thereafter. Probably, if the animals were initially managed in an intensive care manner, better survival would have been achieved in group 2. However, *survival* was always considered within the concept of *"immediate life-sustaining organ function"*. Recently, Takada et al. [35] shows that survival can be achieved with liver allografts submitted to a warm ischemia time of as much as 60 min. However, the number of animals in each group was low, and liver histology was not investigated at the end of the study. Survival, however, is not the only end point requiring study, since lesion reversibility is also of paramount importance.

This experimental model, first described by Hoshino [20, 21] can easily be transferred to a clinical setting. CPB and total body cooling have already been successfully used for kidney retrieval [26], and the addition of a period of extracorporeal circulation and oxygenation can be used to evaluate the quality of the potential donor organ. Not all donors can be maintained with this type of extracorporal circulation, specially those with massive hemorrhage. Moreover, this model cannot be translated to a clinical situation with this type of donors in whom resucitating maneuvers and medication have been used. However, one of the main aims of experimental research with NHBDs is long-term survival. Any experimental model in which this aim is not achieved will never have an important impact on the clinical setting. Contrary to other experiences [8], our survival rate was very high, and the results suggest lesion reversibility. Furthermore, the uniformity of the ischemic time (20 min) will allow the maximum time for lesion reversibility and provide the opportunity of ameliorating these pathological changes, so that they can be established in future studies with longer periods of cardiac arrest.

HA is a high-molecular-weight polysaccharide produced by fibroblasts ubiquitously distributed in the extracellular space. Its specific site of uptake and degradation is in the LECs [9]. In the absence of increased production, serum levels reflect LEC function [6, 7]. Therefore, HA appears to be a useful marker of sinusoidal endothelial cell damage. Recently, it has become accepted that LEC damage is the predominant form of injury following cold preserveration of the liver [24] and some studies have shown that HA is related to graft viability in pigs [25] and humans [2]. In the present study, we assessed HA changes during the whole procedure of liver procurement and transplantation, as well as postoperatively. A progressive increase during the procedure, similar to that described by Itasaka et al. [25], was observed. Thie peak values also appeared 1 h after reperfusion. These levels tended to decrease during the postoperative period, becoming almost normal on day 5. This fact suggests the reversibility of endothelial damage. Furthermore, an overall beneficial effect of normothermic recirculation on endothelial cell damage was shown by significantly improved values 1 h after reperfusion compared to animals directly submitted to total body cooling ($p < .05$).

Hepatocyte injury of the allograft was evaluated by serum transaminase levels, including the recently described α-GSTs. These measurements indicate the degree of hepatocellular membrane breakdown, which indirectly reflects hepatocellular functional integrity [11, 33]. In our study, AST and ALT levels increased progressively, showing a peak value on the 3rd postoperative day, and significantly decreasing thereafter. Again, this trend to normality in liver hepatocellular function suggests ischemic injury reversibility. Nonetheless, GST changes during the procedure were totally different from those of the other enzymes. It has already been shown that GST is a very sensitive indicator of acute hepatocellular injury because its intracellular concentration is high and it is expressed in both centrilobular and periportal [17, 19, 33]. Its half-life is very short [36]. This enzyme is widely distributed in the liver and the kidney, but while renal injury gives rise to a significant release of the enzyme in the urine, liver injury causes a significant release of the enzyme to the blood stream [19]. Consequently, the levels we obtained are directly related to the degree of liver injury. We have confirmed previously published data showing that GST is a very sensitive, early marker of ischemia reperfusion injury. Its peak value appears immediately after reperfusion, as a HA peak value similarly does, but, in contrast, as a transaminase value does not (peak value on the 3rd day). No significant effect of normothermic recirculation was shown on hepatocellular damage (AST, ALT, or GST). This fact could be related to the difference in

the onset of warm ischemia and reperfusion injury. Nakagami et al. [28] have recently suggested that a time lag occurs in the onset of injury between parenchymal and endothelial cells, and that endothelial cells are temporarily earlier in failing than parenchymal cells when the liver is exposed to short-term warm ischemia and subsequent reperfusion. The timing of our determinations are probably too short to obtain any difference with respect to hepatocellular damage (although it appears that it does not get to its greatest level). This possibly explains why there is a lack of a relationship between hepatocellular damage and histological features. Further studies with longer periods of warm ischemia are needed to prove this hypothesis in which the sequence of events would be as follows; first, endothelial cell damage; second, microvascular injury; and third, hepatocellular hypoxia.

Various studies have shown that the energy charge is significantly correlated to graft viability either in the experimental or the clinical setting [14, 18]. As others have [21, 35], we also have shown, that the energy charge decreases significantly after a period of cardiac arrest. Moreover, we show that extracorporal circulation at 37 °C and tissue oxygenation may not only arrest the process of ischemic injury, but may also ameliorate the compromised cellular status by increasing the energy charge significantly, becoming similar to that before cardiac arrest. Furthermore, as have Hoshino et al. [22], we have shown that normothermic recirculation and tissue oxygenation were determinant factors for organ viability, since after 20 min of warm ischemia, all pigs subjected directly to total body cooling failed to sustain life. They concluded, as we have been able to confirm, that normothermic recirculation has an overall beneficial effect in this type of potential organ donor.

All these results correlate well with the histological studies. After cardiac arrest, various types of acute cellular injury significantly different from those of the control group were observed immediately before reperfusion in the recipient. After the addition of a period of normothermic recirculation, no beneficial effect could be found immediately. However, histological changes were significantly ameliorated 1 h after reperfusion in the recipient. After 5 days, lesion reversibility became evident. Although the mean necrotic area was greater (31 %), all the livers were considered viable. Only two grafts showed severe lesions of the biliary tract.

In summary, liver transplantation is feasible after 20 min of cardiac arrest. The use of a period normothermic recirculation prior *"in situ cooling and liver procurement"* may have a beneficial effect on liver viability in terms of a significant amelioration of endothelial cell damage, energy charge, and histological changes. Moreover, survival after 5 days (100 %) suggests the reversibility of the histological lesions in the majority of the grafts. Further studies with longer periods of warm ischemia are needed to confirm these data.

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