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An underlying mechanism for improved liver preservation with a combined histidine-lactobionate-raffinose flush solution

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Abstract In previous experimental liver transplant studies, it was possible to extend cold ischaemic time (CIT) by using a flush/storage solution combining histidine, lactobionate and raffinose (HLR). In this study, energy metabolism, glycolytic substrate (glucose) and anaerobic end-product (lactate) were examined in rat liver over 24 h of cold storage to determine the mechanism of action of the HLR solution. In livers subjected to simple flush and storage with the HLR solution, levels of ATP and ADP were considerably higher than livers stored with modified UW throughout 24 h of storage; at 4 h of storage, ATP and ADP levels were 1.1 and 3.1 μmol/g for HLR solution versus 0.18 and 0.81 μmol/g for UW solution. Total adenylate contents (TA = ATP + ADP + AMP) also remained 1–2 μmol/g higher in HLR-treated livers than those preserved in UW; TA values ranged from 3.8 to 5.7 μmol/g. Glucose increased to 20–35 μmol/g by 10–24 h of storage (similar to the UW group). Lactate rose to almost twice that in livers stored in UW; total lactate accumulation was approximately 10.0 μmol/g. This study demonstrated that the combined HLR solution is able to prolong the maximum 'safe' CIT by increasing anaerobic metabolism and consequently preserving liver energetics. The second part of the experiment examined the effect of continuous perfusion (with/without O_2) over the 1st h of cold ischaemia. Under current methods of liver flushing and excision, the 1st h of cold storage may be the critical time of metabolic 'adjustment' since most of the pH and ATP changes occur during this period. Therefore, we tested the hypothesis that the combination of a simple flush with an additional brief 1-h perfusion period prior to storage would enhance the maintenance of hepatic energetics. There was no beneficial effect of 1 h of perfusion without O_2 compared to simple HLR flush and storage. However, perfusion with O_2 resulted in prolonged maintenance of high energy adenylates and total adenylates; at 10 h of storage ATP was 1.0, ADP 3.3, and TA 5.7 μmol/g. However, any improvement in ultimate viability following long-term storage of the livers in these two groups needs to be tested in an animal transplant model.

Key words Liver preservation, rat, histidine-lactobionate-raffinose · Histidine-lactobionate-raffinose solution, liver preservation, rat · Preservation, liver, histidine-lactobionate-raffinose

Introduction

There have been many attempts to improve liver preservation with respect to improving graft viability and prolonging cold ischaemic time (CIT). Perhaps the most dramatic was the advent of the University of Wisconsin (UW) solution, which enables successful graft viability after at least twice the CIT of the original citrate-based solution [12, 14]. UW solution is now widely accepted as the 'gold standard' for liver preservation.

In recent years, Bretschneider's histidine-based HTK cardioplegia solution has been tested experimentally and used clinically [6, 11, 20] as an alternative to UW solution for liver preservation with no apparent differences in the ultimate survival rates between the two solutions. Most recently, Sumimoto et al. [22] reported that a considerable improvement in CIT is possible by combining the osmotic impermeants of UW solution (lactobionate and raffinose) with the increased buffering capacity (provided by histidine) of HTK solution. In their study, a survival rate of 85 % was achieved for livers flushed and stored for 24 h in the combined histidine-lactobionate-raffinose (HLR) solution, whereas the survival rate for livers stored in UW solution was only 29 % [22]. Although the authors were able to demonstratc an improvement in prolonging CIT for several organs (heart, pancreas, liver) with the new solution, they were unable to determine by what underlying mechanism the HLR solution was able to effect the improved preservation [22–25]. Sumimoto and colleagues suggested that the improved survival of transplanted livers stored for 24 h in HLR solution might be a result of increased buffering capacity provided by histidine. The progressive acidification that typically occurs during cold storage would be prevented and, thus, preserve the status of key metabolic processes that directly influence homeostatic control of the organ. Previous studies carried out in our laboratory involving ^{31}P NMR spectroscopy of a histidine-based solution have shown an improved maintenance of ATP and ADP in parallel with the control of intrahepatic pH [9]. Therefore, we proposed that anaerobic energy metabolism via glycolysis should be further investigated during hepatic storage with the HLR solution. More specifically, we investigated the progressive effects of this solution on levels of high energy phosphates, glycolytic substrate and anaerobic end-product over a 24-h period of cold storage. Furthermore, we believe that under the current methods of liver flushing and excision, the 1st h of cold storage may be the critical time of metabolic 'adjustment' since most of the pH and ATP changes occur during this period [2, 4, 9]. Therefore, we also tested the hypothesis that the combination of a simple flush with an additional brief 1-h perfusion period prior to storage would enhance the maintenance of hepatic energetics with the improved HLR-based solution for subsequent long-term hypothermic storage.

Materials and methods

All chemicals and biochemicals were AR grade and were purchased from Sigma Chemical, Boehringer Mannheim, or BDH. Male albino Sprague-Dawley rats were used as liver donors and all experiments were conducted in accordance with regulations in the Animals (Scientific Procedures) Act (1986) and the „Principles of laboratory animal care" (NIH publication No. 86–23, revised 1985).

Surgical and cannulation procedure

Rats (200–300 g) were anaesthetised with halothane/oxygen, followed by an intramuscular injection of 4.0 ml/kg hypnorm (Janssen Pharmaceuticals) plus 5 mg/kg diazepam (Phoenix Pharmaceuticals). The procedures for surgery and portal vein cannulation have been described previously [8]. A volume of 60 ml (approximately 4 ml/g liver) of ice-chilled flush/storage solution – either modified UW solution or histidine-lactobionate-raffinose (HLR)-based solution – was allowed to flow through the portal vein (hydrostatic pressure head 20 cm) to cool the organ and clear blood from the vascular bed.

Composition of preservation solution

The modified UW solution used in this study is used clinically at the Royal Free Hospital and contained: lactobionate (108 mmol/l), raffinose (31 mmol/l), KOH (105 mmol/l), potassium acid phosphate (26 mmol/l), magnesium sulphate (22 mmol/l), NaOH (38 mmol/l), glutathione (3 mmol/l), allopurinol (1 mmol/l). The solution had a pH of 7.2–7.3.

The HLR flush/storage solution was based approximately on that used by Sumimoto et al. [22] and contained: histidine (90 mmol/l), lactobionate (90 mmol/l), raffinose (20 mmol/l), KOH (90 mmol/l), NaOH (30 mmol/l), magnesium sulphate (20 mmol/l). The solution was adjusted to a pH of 8.0 (360 mosmol/l).

Experimental groups

The UW group and the first experimental group (group *A*: 'HLR-simple flush') of livers were then stored in 50 ml of ice-chilled UW or HLR solution, respectively, on ice and sampled at the subsequent time points. The second experimental group (group B: 'HLR-perfusion without O_2') and the third experimental group of livers (group C: 'HLR-perfusion + O_2') were flushed with HLR and then transferred to a perfusion circuit where they were continuously perfused over the 1st h post-flush with the HLR solution ± oxygenation rat 4 °C (flow rate 1 ml/g per minute). For group C, oxygenation was facilitated by an inline unit in the circuit that directly bubbled 99 + % O_2 through the perfusate prior to introduction into the tissue. Both groups subjected to 1 h perfusion were then transferred to HLR solution and stored on ice. Control liver samples at $t = 0$ were taken immediately following liver flush. For groups B & C, the lobe sampled as control liver tissue was tied off in order to prevent any leakage during the 1-h period of perfusion. Following removal of the 1-h time point sample at

the conclusion of the 1-h perfusion period, the liver was transferred to 50 ml of ice-chilled HLR (either air- or O_2-equilibrated, respectively) and 1- to 2-g tissue samples were taken at the specified time points. All liver samples were 'freeze clamped' in liquid nitrogen and subsequently stored at –65 °C.

Sample preparation and metabolite assay

Samples of frozen liver were weighed and then extracted 1:5 w/v in 6 % perchloric acid (containing 1 mM EDTA). Precipitated protein was removed by centrifugation (20 min at 3700 *g*) and extracts were neutralised by the addition of 3 N KOH/0.4 M TRIS/0.3 M KCl and recentrifuged. Aliquots of the neutralised extract were immediately used for assays of ADP and AMP; the remaining extract was frozen at –65 °C for subsequent use. ATP was assayed within 24 h of being extracted. Metabolites were assayed enzymatically based on the absorbance of NADH at 340 nm, using a Pye Unicam PU8700 spectrophotometer. Assays were performed as described elsewhere [16]. For determination of tissue water contents, tissues were dried to constant weights in an 80 °C oven for 48–72 h. Wet weight/dry weight ratios were reported.

Statistical analysis

Data are reported as means ± SEM for four to six livers. Statistical significance for changes in group A (HLR-simple flush) compared to data from livers stored with modified UW solution was determined using an unpaired Student's *t*-test; a *P* value below 0.005 was reported. Additionally, changes within group A over time were determined to be significantly different from the respective control value using an ANOVA test followed by the Dunnett's test; a *P* value below 0.05 was reported. Statistical significance for differences in groups B & C compared to the corresponding time point in group A was assessed using an ANOVA test followed by Dunnett's test; a *P* value below 0.05 was reported.

Results

Tissue water content

Wet weight/dry weight ratios were assessed in order to detect any changes in tissue water as a direct result of the 1-h experimental perfusion period and simple flush/storage. There were no significant changes in water contents of livers in any of three experimental groups; wet weight/dry weight ratios ranged from 2.7 to 3.0. Thus, all metabolite values are reported in terms of 'μmol per gram wet weight'.

Livers flushed with modified UW
Energetics (Fig. 1)

These data were adapted from an earlier study [4] and were included for comparison. Levels of ATP dropped rapidly upon entry into cold storage; within 2 min post-flush, approximately 50 % of control ATP had been depleted, and by 2 h 85 % had been used. Changes in levels of ADP complemented the decrease in ATP, although at a more gradual level; values dropped from a control level of 1.77 μmol/g to 1.05 μmol/g over the first 2 h of storage and subsequently continued to decline. While AMP levels increased during storage, the difference between the decrease in ATP + ADP and increase in AMP was accounted for by a decrease in total adenylate content (TA); over the first 2 h, TA dropped by 1.9 μmol/g. Combined decreases in high energy adenylates (and TA) and the increase in AMP resulted in a rapid decrease in 'energy charge' [EC = (ATP + ADP/2)/TA] [1]; values fell to less than 50 % of control over the first 2 h (control EC = 0.70) and subsequently continued to decline. Both glucose and lactate levels rose abruptly at 2 min and by 2 h of storage, levels had reached upper levels of 38.8 and 7.1 μmol/g, respectively.

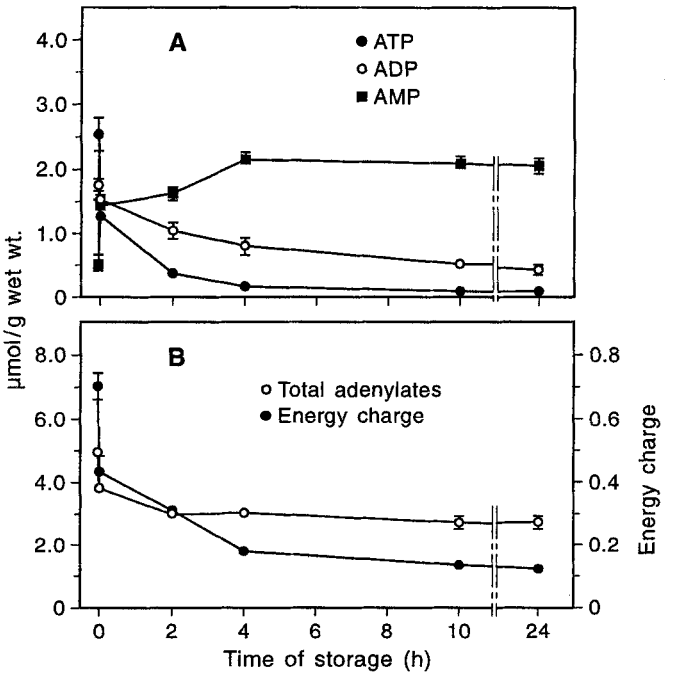

Fig. 1 Effect of cold storage with modified UW solution on levels of ATP, ADP and AMP; total adenylates (ATP + ADP + AMP) and energy charge [(ATP + ADP/2)/(ATP + ADP + AMP)] values in rat liver. Data represent means ± SEM ($n = 6$). All values (2 min–24 h) were significantly different ($P < 0.05$) from the respective control value

Group A – HLR solution (simple flush)
Energetics (Figs. 2A, 3A)

ATP levels remained at control values of 2.68 μmol/g for the 1st h of storage (Fig. 2A). Levels began to drop by 2 h ($P < 0.05$) and continued to decline over the remainder of the 24-h storage period. However, at times

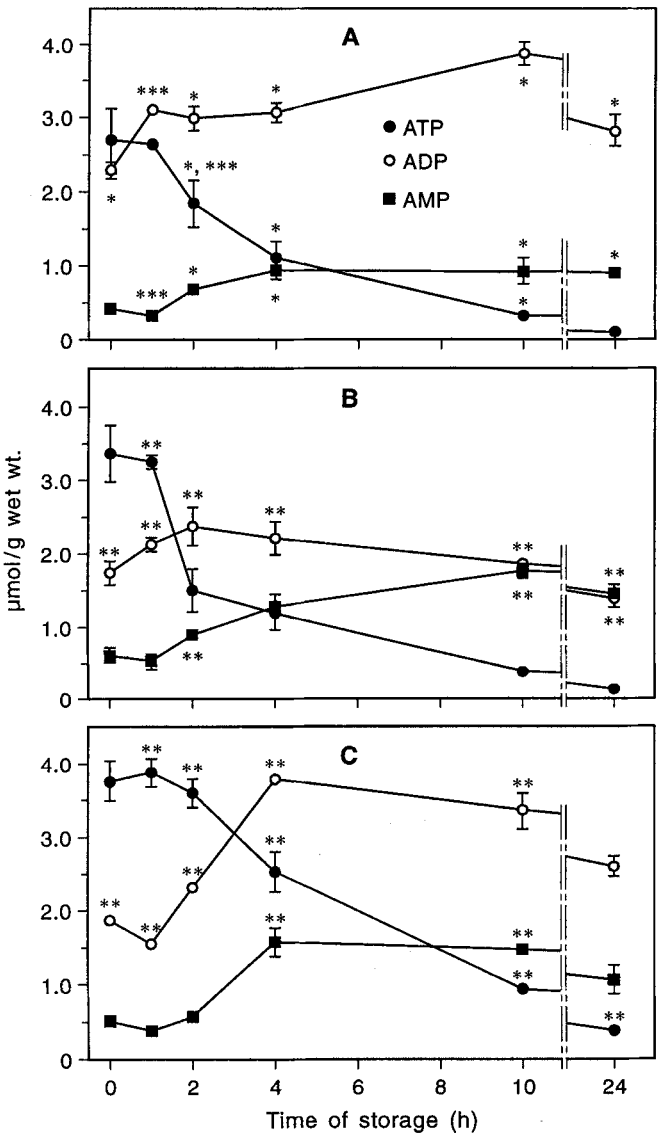

Fig. 2A–C Effect of cold storage with HLR solution on levels of ATP, ADP and AMP in rat liver after: **A** simple flush/storage (group A); **B** 1-h perfusion without O_2 (group B); **C** 1-h perfusion with O_2 (group C). Data represent means ± SEM, ($n = 4$). * $P < 0.005$ compared to corresponding value in UW-stored livers; ** $P < 0.05$ compared to corresponding value in group A; *** $P < 0.05$ compared to control ($t = 0$) values

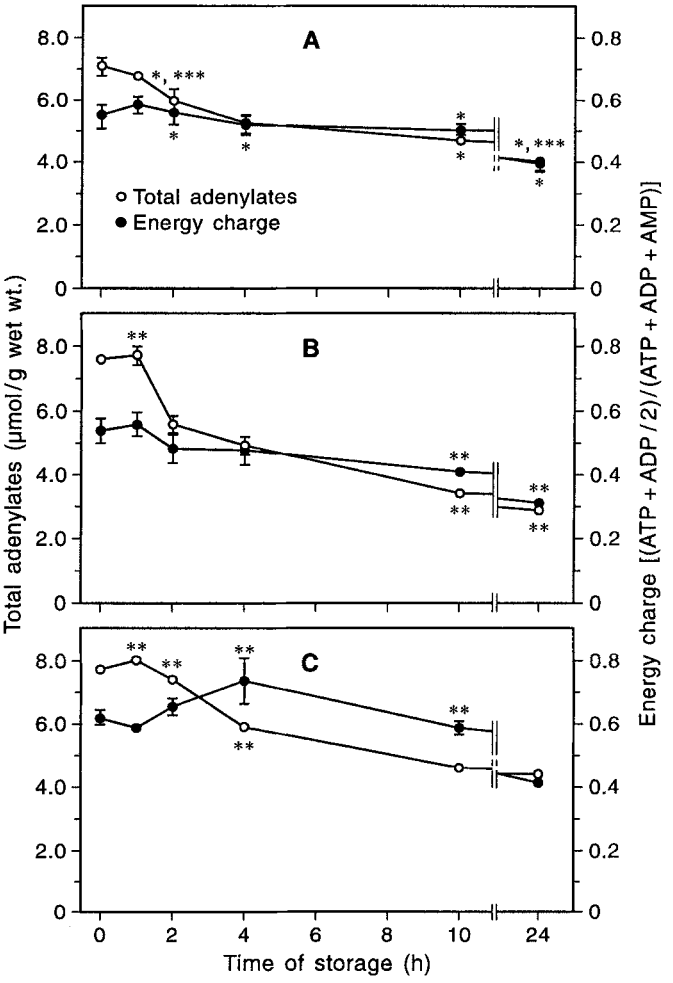

Fig. 3A–C Effect of cold storage with HLR solution on total adenylate contents and „energy charge" values in rat liver after: **A** simple flush/storage (group A); **B** 1-h perfusion without O_2 (group B); **C** 1-h perfusion with O_2 (group C). Details as in Fig. 2

between 2 and 10 h, values were significantly higher than those found in livers stored in modified UW; values were higher by 1.5, 1.0 and 0.24 µmol/g at 2 h, 4 h and 10 h, respectively ($P < 0.005$). ADP exhibited no decrease throughout the 24 h period (Fig. 2A), as was seen with the modified UW solution, but instead increased by 0.5–1.7 µmol/g compared to control values ($P < 0.05$). Increases in levels of low energy adenylate, AMP, accounted for a minor proportion of the decreases observed in ATP levels. Of particular note, final AMP levels were approximately 50 % of those in livers stored in modified UW solution [0.95 µmol/g (Fig. 2) vs 2.1 µmol/g (Fig. 1), respectively]. The net effect of the changes in individual adenylate levels was reflected in TA contents (Fig. 3A). Over the first 10 h, TA values ranged from 4.9 to 5.8 µmol/g, with no significant change from control values until 24 h of storage. EC ratios remained high and constant at control values over the 1st h of storage (EC = 0.67–0.70; Fig. 3A) and then exhibited a progressive drop throughout the remainder of the storage period; the final value at 24 h was EC = 0.39. Compared to livers stored in modified UW solution (Fig. 1), TA and EC values were significantly higher in livers stored in HLR solution at times between 2 and 24 h ($P < 0.005$).

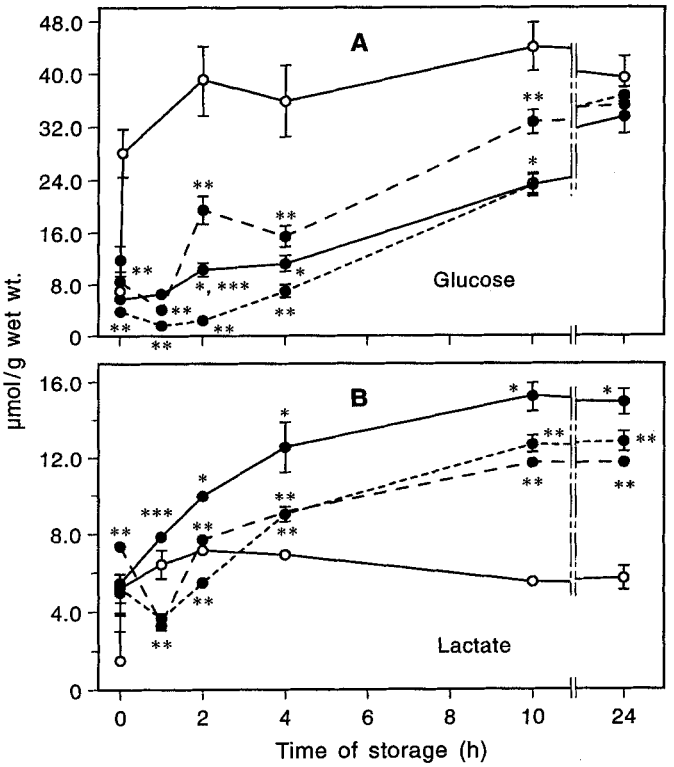

Fig. 4A,B Effect of cold storage with HLR solution on: **A** glucose and **B** lactate levels in rat liver after: simple flush/storage (group A, -); 1-h perfusion without O_2 (group B, —); 1-h perfusion with O_2 (group C, . . .), (● HLR solutions, ○ modified UW solution). Details as in Fig. 2 for groups A–C and as in Fig. 1 for the modified UW group

Glycolytic substrate and anaerobic end-product (Fig. 4)

Glucose levels remained at control values over the 1st h, but increased at a gradual rate between 2 h of storage ($P < 0.05$) and final 24-h values of approximately 33 µmol/g. This was in contrast to livers stored with modified UW solution, where 100 % of glucose (and lactate) accumulation occurred over the first 2 h of storage. Lactate also exhibited a more gradual increase than the UW stored counterparts; however, not only was the rate of accumulation less but the magnitude of total lactate accumulated was almost twice as high with HLR treatment.

Group B – HLR solution (perfusion without O_2)
Energetics (Figs. 2B, 3B)

The additional treatment of 1 h perfusion (without O_2) prior to storage had no apparent positive effect on energy levels. ATP and EC levels were similar to those in group A with the exception of a higher value ($P < 0.05$) at 1 h (immediately post-perfusion). ADP levels were consistently lower than those of group *A* ($P < 0.05$), which may have accounted for the greater increase in AMP (approximately twice as much as group *A*). TA contents remained at control values (not significantly different from group A) until 10 h, at which point contents dropped to 0.8–1.0 µmol/g less than those of group A; this was presumably a result of the much lower ADP levels. Additionally, the combined effect of lower ADP and TA resulted in a lower EC value at 10–24 h ($P < 0.05$).

Glycolytic substrate and anaerobic end-product (Fig. 4)

Glucose levels were significantly different from those of group *A* livers between control and 10 h ($P < 0.05$), however, no distinct pattern emerged until after 2 h of storage. Between 2 h and 10 h of storage, levels were 4.3–9.2 µmol/g higher than those in group A ($P < 0.05$); by 24 h, values were not significantly different from livers stored without the 1-h perfusion period. Although lactate levels exhibited a gradual yet progressive increase, similar to those in group A, between 1 and 24 h, values remained 2.4–4.3 µmol/g less than group A levels ($P < 0.05$). Even though 24 h levels were lower than group A values, total lactate accumulation was comparable [8.1 µmol/g (group B) vs 10.0 µmol/g (group A)].

Group C – HLR solution (perfusion plus O_2)
Energetics (Figs. 2C, 3C)

The most effective group in maintaining liver energetics was group C; elevated levels of ATP were seen throughout the entire 24-h storage period ($P < 0.05$). Between 1 and 4 h, ATP levels sustained values that were 1.2–1.7 µmol/g higher than those in group A. Although levels of the high energy adenylate dropped at 10–24 h, values were 0.3–0.6 µmol/g higher than those of livers subjected to the HLR solution (simple flush). Levels of ADP were considerably lower than those of group A (similar to group B); however, there was a large increase at 4 h, presumably as a direct result of ATP hydrolysis. Patterns of AMP increase were similar to those of group B and did not exhibit any patterns inconsistent with group A and B results.TA contents never dropped below those of group A, and exhibited an increase at 4–10 h of storage that was consistent with the increase in ADP.Interestingly, EC values remained high and constant (EC = 0.73–0.79) throughout the first 2 h of storage and then began to decrease at subsequent time points. Nevertheless, EC at 4 h was significantly greater than that of group A. By 10–24 h, EC values were not significantly different from livers treated with HLR (simple flush; group A).

Glycolytic substrate and anaerobic end-product (Fig. 4)

Glucose levels were 2–8 μmol/g less than group *A* values ($P < 0.05$), but by the latter part of the storage time course showed no differences from the other groups. Lactate levels were similar to group B values. However, total lactate accumulation was comparable to group A taking into consideration that hypoxia did not begin until immediately post-perfusion.

Discussion

The maintenance of energy levels within the liver is one of the key factors in prolonging tissue/organ viability during cold ischaemic storage. There have been many attempts to retard the rapid degradation of high-energy adenylates during the storage period. However, many of the attempts have been unsuccessful due to a lack of understanding of the factors governing survival of mammalian tissue during hypothermic ischaemia. Not only is the supply of oxygen cut off during harvesting and storage but there are added complications of hypothermia, including perturbation of key functional aspects of metabolism, ion regulation, and membrane fluidity/processes [7, 10, 26]. Nevertheless, it is necessary to expose the target organ to hypothermic storage in order to reduce the degeneration in cellular homeostasis. Advances made in organ preservation have been empirically based and the underlying mechanisms not fully elucidated. As well, the potential benefits of any improvement in preservation solution can only be realised when the basic mechanisms involved are fully understood. For these reasons, we designed the present study to investigate the protective effects of a combined HLR solution on hepatic energetics and flux through the sole anaerobic energy-producing pathway, glycolysis.

The increase in CIT sustaining graft viability using HLR solution was clearly shown for liver (as well as heart and pancreas) in the series of experimental transplantation studies carried out by Sumimoto and colleagues [22–25]. The results of the present study demonstrate that one major protective effect of the HLR solution is the maintenance of liver energetics during cold storage. ATP and EC values remained at control levels throughout the 1st h of storage. A degeneration of ATP and EC levels (typically observed with citrate- and lactobionate-based solutions [3, 4]) was clearly indicated; however the rate of ATP disappearance was much more gradual and levels were appreciably higher than the UW-treated livers stored for up to 10 h.

Interestingly, levels of ADP were also higher in livers stored with HLR (simple flush) than with UW-treated livers; this however was particularly evident at the latter time points (10–24 h of storage). The high EC values at 24 h of storage were directly attributable to high ADP, since most ATP had been depleted by this time. It is unusual to find a preferential accumulation of ADP since pools of the three phosphorylated adenylates are interconverted via the equilibrium enzyme, adenylate kinase, ($2ADP < — > ATP + AMP$) and, theoretically, should not increase without proportional increases in ATP and AMP. The maintenance of high ADP, TA, and EC levels is likely to be indicative of the improvement in viability after 24 h of storage that was clearly demonstrated in the transplant studies using HLR [22–24]; however, this may not be the sole aspect of cellular homeostasis that is positively affected by the HLR formulation. One additional point is that the total adenylate pool was also maintained for a considerably longer time than that of the UW-treated livers. Elevated ADP levels would partially explain this, however there is another possibility that must be considered. Accompanying the decreases in ATP levels and EC values during a typical bout of cold storage is a delayed degradation of the total adenine nucleotide content. Approximately 40 % of the total adenylate pool is diminished over 24 h of cold storage [3, 4]. Any reduction in flux through the purine catabolic pathway that converts AMP into adenosine, inosine, hypoxanthine, etc. may influence graft viability to a similar degree as actual quantities of ATP and ADP. Thus, even though TA content was 2.7 μmol/g at 10 h and 24 h of storage in UW-treated livers, the total purine catabolite pool may be determinant in organ viability; thus explaining differences in survival of transplanted rats with 10-h and 24-h stored livers [17, 19]. The decrease in flux through the purine catabolic pathway demonstrated in this study is presumably a result of direct or indirect (by mass action) activation of the pathway. Indeed, examining the factors governing regulation (specifically pH) of specific steps of this pathway may provide additional clues to the efficacy of the HLR solution in prolonging adenylate (high- or low-energy) degradation.

Several experimental and clinical transplantation studies have investigated the effects of histidine-based solutions (without lactobionate) on the preservation of various organs and found comparable survival rates of transplanted organs compared to the UW solution [6, 21]. Specifically in the case of the liver, preliminary studies carried out in our laboratory have shown that ATP is higher over short-term storage (0–2 h) in histidine-based solutions than in UW solution, although levels were not as high as those found with the HLR solution in the present study. As well, pH measurements via non-invasive ^{31}P NMR techniques have shown that the pH drop over storage time parallels the decrease found with low buffering capacity preservation solutions, but pH values shift to a constant higher value at all times [2]. Clearly, the added buffering capacity of the combined HLR solution would partially explain the

improvement in liver energetics. The decrease in pH that occurs in citrate- and UW-stored livers appears to be one of the predominant factors in the inhibition of anaerobic energy production [5, 18]. Thus, the inhibition should be alleviated with the high pH, histidine-based buffer solution; flux through glycolysis would not be impeded and ATP generation would be supported. Our results indicate that the maintenance of liver energetics is such a pH-sensitive process. More specifically, the regulation of glycolysis is kept under stringent control and this control exists at several key enzyme steps along the pathway [15, 21]. The drop in intracellular pH during cold storage is large enough to result in the inhibition of the most pH-sensitive enzyme, phosphofructokinase (PFK), as well as other glycolytic enzymes [5, 18]; pH values have been found to fall from 7.6 to 6.8 within 10 h of storage [9]. However, we have found that PFK exhibits a regulatory role by an activation at 8–10 h of storage [3, 4] and, thus, even though the inhibitory effect of pH may not be predominant in the regulation of PFK, other enzymes may be negatively influenced by the drop in pH and, thus, undergo inactivation as the period of cold storage progresses. Presumably, the presence of histidine in the HLR solution used in this study alleviated any potential inhibition that would otherwise occur in the pathway. Increased flux through the glycolytic pathway was apparent in the present study by the increased levels of lactate compared to comparably stored UW-treated livers. Flux through glycolysis was increased twofold with the HLR solution (lactate increased at least twice as much as that of UW livers). This would enable the continued production of glycolysis-derived ATP, thereby maintaining the ratio of high-energy adenine nucleotides and total nucleotide pool.

The second part of this investigation was based on the premise that the period of hypoxia during the initial flush and 1st h of storage is a critical time since most of the pH and ATP changes occur during this time period [2, 4, 9]. Our hypothesis was that if the sudden drop in ATP and pH (over the 1st h of storage) could be avoided and still be able to reduce the temperature of the liver to hypothermia, then the subsequent decline of these two factors influencing glycolytic flux would occur at a more gradual rate. Thus, flux through glycolysis would be maintained for reasons already discussed and liver energetics would be better preserved. Both groups B and C of this study were designed to examine the effects of a simple flush followed by a brief 1-h perfusion period (without/with deliberate O_2) with the HLR solution. Even though group B (without O_2) livers maintained energetics at control values throughout the 1st h, there was a subsequent depletion in ATP and TA that was equivalent to, or greater than livers treated with a simple flush. The data suggest that there is no additional benefit from the 1-h perfusion period (without O_2). However, the group supplemented with oxygen exhibited a prolonged maintenance of high energy adenylates (ATP and ADP) over at least the first 4 h of storage. Consequently, EC values were also elevated throughout short-term storage. This increased support of liver energetics was clearly due to aerobic metabolism stimulated by oxygen present in the HLR solution since there was no accompanying increase in lactate and presumably once the oxygen was depleted, metabolism reverted to the anaerobic pathway. The effect of HLR plus oxygen was evident for as long as 10 h of cold ischaemia, but after 24 h there was no indication that energy levels (with the exception of slightly higher ATP) were improved over those in livers subjected to the simple flush alone. The higher ATP at 24 h was comparable to levels at 10 h in livers without perfusion (HLR simple flush) and may influence maximal safe cold ischaemic times. However, this point is somewhat tenuous since it has been difficult in the past to correlate absolute levels of ATP with ultimate graft viability, and any potential improvement in viability with the HLR plus oxygen treatment in this study ultimately requires testing in an animal transplant system. Additionally, one must also consider that the high concentration of oxygen used during perfusion may have induced oxidative damage to membrane structures as well as perturbation of important functional proteins [13], although exposure times were relatively short.

In conclusion, this study clearly demonstrates a direct correlation between the maintenance of high-energy adenylates and total adenine nucleotide content with the improved graft viability after prolonged storage previously shown with HLR solution [22–25]. This increased support of hepatic energetics is partially a result of increased glycolytic flux and represents the underlying mechanism of action for preservation solutions combining histidine, lactobionate and raffinose.

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