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ORIGINAL PAPER

Protection of mitochondria during cold storage of liver and following transplantation: comparison of the two solutions, University of Wisconsin and Eurocollins

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Abstract Injury to allografts during ischaemia/reperfusion contribute to the development of graft failure following transplantation with significant morbidity and mortality to patients. The development of University of Wisconsin solution has significantly improved the quality of graft preservation and transplant outcome relative to formerly used solutions such as Eurocollins. The aim of this study was to further characterize mitochondrial structural and functional alterations occurring in rat livers following cold storage and transplantation. Mitochondrial impairment after prolonged storage in Eurocollins included decreased cyt. $c+c_1$, cyt. b and cyt. $a+a_3$ concentration and dramatic falls in the activities of the respiratory chain enzymes ubiquinol-cyt. c oxidoreductase and cytochrome oxidase. Under the same conditions the highest hydroperoxide but lowest vitamin E concentrations were also found. Although both the Eurocollins and University of Wisconsin preservation solutions have limitations in preventing oxidative injuries following cold storage and reperfusion, our data indicate that mitochondrial impairment was higher in Eurocollins- than in University of Wisconsin-stored livers. Further improvements are necessary in maintaining the stability of mitochondria in order to optimize preservations solutions used in transplantations.

Keywords Vitamin E · Coenzyme Q · Hydroperoxides · Conjugated dienes · Cytochromes · Respiratory chain · Ischaemia/reperfusion

Abbreviations

AAPH	2,2′-azobis(amidinopropane hydrochloride)
CD	conjugated dienes; CoQ_9, coenzyme Q_9
CoQ_9H_2	reduced coenzyme Q_9
DGF	delayed graft function
EC	Euro-Collins preservation solution
FOX2	ferrous-oxide xylenol orange
HP	hydroperoxides
HPLC	high performance liquid chromatography
IRI	ischaemia/reperfusion injury
PNF	primary non-function
ROS	reactive oxygen species
TN	turnover numbers
TPP	tryphenylphosphine
UW	University of Wisconsin preservation solution
Vit. E	vitamin E

Introduction

In liver transplantation cold preservation followed by reperfusion of the allograft at the time of transplantation, known as

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ischemia/reperfusion (IR), is an unavoidable step and results in graft damage. Such injury to the allografts might contribute to the development of graft failure following transplantation, with significant morbidity and mortality to patients (Deschenes *et al*., 1998; Jassem and Roake, 1998; Strasberg *et al*., 1994).

The underlying mechanisms of IR are poorly defined; however, there is evidence that mitochondrial alterations during this process may be critical. Several studies have demonstrated that IR leads to impairment of adenosine triphosphate (ATP) production in animal models (Bor *et al*., 1999; Jeon *et al*., 2001; Kobayashi *et al*., 1991; Oishi *et al*., 2001) and clinical studies (Kamiike *et al*., 1988), which were correlated to the extents of cold preservation. Mitochondria have been seen to be an important source of free radicals during cold preservation and reactive oxygen species (ROS) following reperfusion (Jassem *et al*., 2002). Cold preservation also leads to the depletion of mitochondrial antioxidants substrate and enzymes (Ahlenstiel *et al*., 2003; Gondolesi *et al*., 2002; Oishi *et al*., 2001) and alteration of the NADH/NAD^+ ratio (Thorniley *et al*., 1995). Finally mitochondria become over loaded with calcium during IR and there is opening of megachannels that contribute to the release of pro-apoptotic proteins and cell death (Kroemer and Reed, 2000).

Organ preservation is based on cooling organs by perfusion in situ with preservation solutions (Muhlbacher *et al*., 1999), cold storage and pharmaceutical intervention. Preservation solutions were developed along with research efforts to find appropriate cardioplegic solutions. The first solution was developed by Collins for kidney transplantation and modified to Eurocollins solution in the 1980s (Collins, 1976). Collins or Eurocollins solution mimics the composition and concentration of intracellular electrolytes. Eurocollins solution contains phosphate as a buffer and glucose to maintain hypertonic osmolarity (420 mOsmol). However, while kidney can be safely preserved up to 24 h in EC solution, livers tolerate no more than 6–8 h. In the late 1980s, Belzer described the University of Wisconsin solution (UW) for organ storage (Belzer and Southsard, 1988). This was based on maintaining osmotic concentration by inert substrates such as raffinose and colloid carrier hydroxyethyl starch, instead of glucose. Furthermore, UW contained antioxidants such as allopurinol, adenosine and glutathione. In animal models UW solutions are efficient in preserving livers for up to 48 h. However, in clinical practice UW solution was shown to preserve livers safely for up to 18–24 h and was superior to Eurocollins to prevent the development of graft failure following transplantation (Ploeg *et al*., 1993).

We previously found that the concentration of rat liver mitochondrial glutathione and the activity of related enzymes such as glyoxalase fell during cold storage, in relation to the preservation solution used (Jassem *et al*., 1996, 2000). The aim of this study was to further characterize the structural and functional alterations occurring in rat liver mitochondria following cold storage and transplantation.

Materials and methods

Experimental procedure and surgical technique

The entire experimental protocol was approved by the local ethical committee. Male inbred Wistar rats, obtained from INRCA colonies (Instituto Nazionale di Ricerca e Cura Anziani, Ancona) weighing 250–300 g were used as donors and recipients. Orthotopic liver transplantation was performed using the method of Kamada and Calne (1983), without reconstruction of the hepatic artery. Briefly, donors and recipients were anesthetized with isofluran. After ligation of the left phrenic vein, the liver was freed from its attachments, flushed with 5 mL of heparinized cold UW or EC solutions through the portal vein, and then it was removed and placed in cold storage solution for cuff preparation. Livers were immediately transplanted or were stored at 4°C for 7 h or 24 h before transplantation. In the recipient, after removal of the native liver, the suprahepatic vena cava of the graft was anastomosed by a running suture with 7–0 Prolene, portal and the infrahepatic vena cava using the cuff technique. The average warm ischemia time (anastomotic time) was 12–15 min; no attempt was made to perform the biliary anastomosis. All livers were removed 30 min after the reperfusion of the grafts for analysi.

There were six experimental groups: (1) control (*C*) three animals, (2) transplanted ($C_{transpl}$) three animals, (3) transplanted after 7 h storage in UW solution (UW7) four animals, (4) transplanted after 24 h of cold storage in UW solution (UW24) six animals, (5) transplanted after 7 h of cold storage in EC solution (EC7) four animals, and (6) transplanted after 24 h of cold storage in EC solution (EC24) four animals.

All animals were randomly allocated in groups of four per cage and maintained on a 12 h light/12 h darkness cycle with free access to food and drinking water and were fed a standard semi-synthetic and isoenergetic diet according to the AIN93 criteria (Reeves, 1997). Surgical procedures were carried out at the same time of the day to avoid any circadian fluctuation.

Chemicals

All chemicals used were purchased from Boehringer Mannheim (GmSH, Germany), Fluka Chemie GmbH (Buchs, Switzerland), and Sigma Chemical Co. (St. Louis, MO) and were of the highest purity available.

Mitochondria isolation

Portions of liver (10–12 g) were homogenized 1:10 (w/v) in ice-cold buffer, pH 7.5 containing 75 mM sucrose, 225 mM mannitol, 1 mM EDTA, 5 mM HEPES and 0.5 mg/mL fatty-acid-free bovine serum albumin. The homogenate was centrifuged for 10 min at 600 × *g* at 4°C. The nuclear pellet was discarded and the supernatant was centrifuged for 15 min at 12,000 × *g* at 4°C, obtaining a mitochondrial pellet and a supernatant. The mitochondrial pellet was washed two times and purified mitochondria were carefully resuspended in 1 mL of ice-cold homogenisation buffer (Armeni *et al*., 2003).

Respiratory chain activities

Mitochondria were assayed for respiratory chain activities at 25°C in a 25 mM K-phosphate buffer, pH 7.5, under quasi-saturating substrate concentrations in a Beckman DU 640 spectrophotometer, as previously described in detail by Battino *et al*. (1991). Briefly, complex II (succinate-ubiquinone oxidoreductase; EC 1.3.5.1) activity was measured as a rate of reduction of ubiquinone-2 by succinate followed by the secondary reduction of 2,6-dichlorophenolindophenol (DCPIP) by the ubiquinol formed. The decrease in absorbance was measured at 600 nm after the addition of DCPIP, ubiquinone, and the mitochondrial fraction. Complex III (ubiquinol-cytochrome c oxidoreductase; EC 1.10.2.2) activity was assayed by monitoring the rate of reduction of cytochrome c by ubiquinol-2. The reaction was started by adding ubiquinol–2 to cytochrome c in a Tris–HCl buffer containing potassium cyanide. Reduced cytochrome c was measured as an increase in absorbance at 550 nm. Complex IV (cytochrome c- oxidase; EC 1.9.3.1) activity was measured as a rate of oxidation of reduced cytochrome c. The decrease in reduced cytochrome c was monitored at 550 nm.

Cytochrome contents

Cytochrome contents were evaluated by a dithionite-reduced minus ferricyanide-oxidized difference spectrum, in the presence of 1% deoxycholate in a Beckman (DU-640) spectrophotometer as previously described (Battino *et al*., 1991; Quiles *et al*., 2001).

Coenzyme Q_9, CoQ_9H_2 and vitamin E contents

CoQ_9, CoQ_9H_2 and vitamin E were assayed by HPLC as recently reported (Battino *et al*., 2001, 2004). The HPLC system consisted of a Beckman Model 126 pump, a Rheodyne model 7125 valve fitted with a 20 μl loop, a stainless steel column 15 cm long 4,6 mm i.d. packed with 3 μm ODS Supelcosil from Supelchem, an ESA Coulochem model 5100 A electrochemical detector and a model 5011 Analytical cell. Chromatograms were integrated with Model 4290 Varian integrator.

The mobile phase consisted of lithium 20 mM perchlorate, 10 mM perchloric acid, 20% ethanol, 80% methanol; electrode 1 was set at −0.5 V, electrode 2 was set at + 0.35 V. Time of analysis was 18 min. Briefly, 50 μl of the sample were precipitated with 150 μl of isopropanol and vortexed for 60 s. After centrifugation at 1,1200 × *g* for 10 min in a bench top centrifuge for eppendorf vials, 20 μl of supernatant was injected into the HPLC.

Hydroperoxide contents

The ferrous-oxide xylenol orange (FOX2) method was used for determining hydroperoxides (HP). HP concentration were assayed according to the principle of the rapid peroxide-mediated oxidation of Fe^{2+} to Fe^{3+} under acidic conditions (Jiang *et al*., 1992) slightly modified (Nourouz-Zadeh *et al*., 1994) using tryphenylphosphine (TPP), an agent that avoids artifactual color generation in samples that might contain substantial quantities of loosely available iron. Authentic mitochondrial HP can be determined by this strategy in which the HP reductant, TPP, is used to discriminate between the background signal generated by ferric ions present in the sample and the one generated by HP in the sample. Briefly, mitochondria (0.1 mg) were incubated at 37°C for 30 min with and without 1 mM TPP. Then FOX2 reagent was added to each sample and incubated again at 37°C for 30 min in a water shaking bath. After centrifugation (2000 × *g* for 5 min) the supernatants were monitored at 560 nm. The samples were also challenged, in vitro, by further peroxidative attack in order to elucidate the peroxidation rate constant for each group of mitochondria. This aim was reached by incubating mitochondria at 37°C for 30, 60, 120, 180 and 240 min in the presence of 5 mM 2,2′-azobis(amidinopropane hydrochloride) (AAPH), a chemical free radical initiator.

Mitochondrial fatty acid oxidation

Oxidation of mitochondrial fatty acids was determined as conjugated dienes (CD) production by continuously monitoring the increase in absorbance at 234 nm according to a slightly modified method of Esterbauer (Ziouzenkova *et al*., 1998). Briefly, mitochondria suspensions (150 μg mitochondrial protein/mL) were incubated with 5 μM $CuSO_4$ at 37°C in a Beckman DU64 Spectrophotometer. The increase in the absorbance at 234 nm from basal values were automatically recorded at each 5 min interval during 120 min. The rate of lipid peroxidation (i.e., maximal propagation rate (CD rate) and the maximum diene concentration (CD max)) after 2 h were evaluated.

Table 1 Comparison of rat liver mitochondrial cytochrome contents (expressed in nmol/mg protein) and respiratory chain activities (expressed in μmol/min/mg protein) in control and transplanted organs after cold storage in different preservation solutions (see text for details).

	C	$C_{transpl}$	UW7	UW24	EC7	EC24
cyt. c + c_1	0.24 ± 0.03 a	0.22 ± 0.03 a	0.21 ± 0.02 a	0.19 ± 0.04 a	0.17 ± 0.02 ab	0.15 ± 0.02 b
cyt. b	0.11 ± 0.01 a	0.12 ± 0.01 a	0.10 ± 0.02 a	0.09 ± 0.01 a	0.09 ± 0.01 ab	0.07 ± 0.01 b
cyt. a + a_3	0.18 ± 0.01 a	0.18 ± 0.02 a	0.16 ± 0.01 a	0.15 ± 0.01 ab	0.14 ± 0.00 b	0.11 ± 0.00 c
Succinate-DCPIP	0.22 ± 0.04 a	0.21 ± 0.02 a	0.20 ± 0.03 a	0.19 ± 0.02 a	0.18 ± 0.04 a	0.16 ± 0.02 a
Ubiquinol$_2$-cyt c	2.31 ± 0.09 a	2.00 ± 0.12 a	1.88 ± 0.11 ab	1.73 ± 0.10 bc	1.51 ± 0.14 cd	1.22 ± 0.13 d
Cytochrome Oxidase	2.57 ± 0.24 a	2.23 ± 0.19 a	2.06 ± 0.18 ab	2.01 ± 0.22 bc	1.77 ± 0.17 c	1.36 ± 0.15 d

Note. Data are means ± SD. For each variable, values in a row not sharing same letters are significantly different ($p < 0.05$). C, control; $C_{transpl}$, transplanted; UW7, transplanted after 7 h storage in UW solution; UW24, transplanted after 24 h storage in UW solution; EC7, transplanted after 7 h storage in EC solution; EC24, transplanted after 24 h storage in EC solution.

Protein determinations

Protein content was determined by the Lowry method (Lowry *et al*., 1951) using bovine serum albumin as standard.

Statistical analysis

Results are reported as mean ± standard deviation (SD). One-way ANOVA was used to compare groups. Prior to any statistical analysis, all variables were checked for normal and homogeneous variance using the Kolmogorov–Smirnov test and the Levene test respectively. When significant differences were detected ($P < 0.05$), Scheffe's test was used for a *post hoc* comparison. All tests were performed using a PC Statistical Package (SPSS for Windows, SPSS Inc., Chicago, IL).

Results

In Table 1, structural and functional mitochondrial features have been reported. Thirty minutes following reperfusion of the livers, cytochrome contents were similar in mitochondria obtained from groups C, $C_{transpl}$, UW7 and UW24 livers, and limited differences were found in mitochondria from EC-stored organs. Mitochondria from EC24 livers always showed the lowest significant cytochrome contents. EC7 livers always contained mitochondria with lower cytochrome levels than mitochondria from C, $C_{transpl}$, and UW7 livers, but, only in the case of cyt. $a + a_3$ were the levels significantly different.

Succinate-DCPIP activity did not change under the experimental conditions tested. Ubiquinol$_2$-cyt. c reductase activity decreased in mitochondria of UW-stored livers (the decrease was significant after 24 h of cold storage) and dramatically dropped in EC24 samples.

Similarly, cytochrome oxidase activity was largely compromised in mitochondria isolated from EC-stored livers.

Table 2 summarizes mitochondrial contents of two key lipophilic antioxidants coenzyme Q_9 and vitamin E (CoQ_9 and vit. E). This table also shows the degree of oxidative damage that can be found in mitochondria (lipid hydroperoxides) before and after cold storage and that can be maximally elicited in vitro (maximum amount of conjugated dienes (CDmax)) in mitochondria isolated from treated livers. Vit. E decreased after liver transplantation ($C_{transpl}$ vs. C) and after cold storage, with >65% of mitochondrial vit. E lost in the EC-stored livers. In contrast total CoQ_9 concentration increased after liver transplantation ($C_{transpl}$ vs. C), remained

Table 2 Rat liver mitochondrial vitamin E, CoQ_9, basal hydroperoxide contents (expressed in nmol/mg protein), $\%CoQ_9H_2$ and maximal diene concentration induced by $CuSO_4$ (expressed in $A_{234\,nm}$/mg protein) in control and transplanted organs after cold storage in different preservation solutions (see text for details).

	C	$C_{transpl}$	UW7	UW24	EC7	EC24
Vitamin E	0.37 ± 0.03 a	0.29 ± 0.03 b	0.18 ± 0.04 c	0.23 ± 0.03 b	0.12 ± 0.02 d	0.13 ± 0.02 d
Coenzyme Q_9 (total)	1.87 ± 0.08 a	2.45 ± 0.30 b	2.23 ± 0.27 b	2.17 ± 0.33 ab	1.75 ± 0.23 ab	1.54 ± 0.26 a
% Coenzyme Q_9H_2	37 ± 14 a	26 ± 18 a	32 ± 15 a	26 ± 12 a	4 ± 5 b	7 ± 7 b
Hydroperoxides	1.98 ± 0.09 a	3.88 ± 0.19 b	6.68 ± 0.28 c	7.67 ± 0.41 c	10.01 ± 0.67 d	12.54 ± 1.44 d
CDmax	0.53 ± 0.04 a	0.60 ± 0.03 a	0.47 ± 0.04 b	0.52 ± 0.08 ab	0.37 ± 0.03 c	0.37 ± 0.03 c

Note. Data are means ± SD. For each variable, values in a row not sharing same letters are significantly different ($p < 0.05$). C, control; $C_{transpl}$, transplanted; UW7, transplanted after 7 h storage in UW solution; UW24, transplanted after 24 h storage in UW solution; EC7, transplanted after 7 h storage in EC solution; EC24, transplanted after 24 h storage in EC solution.

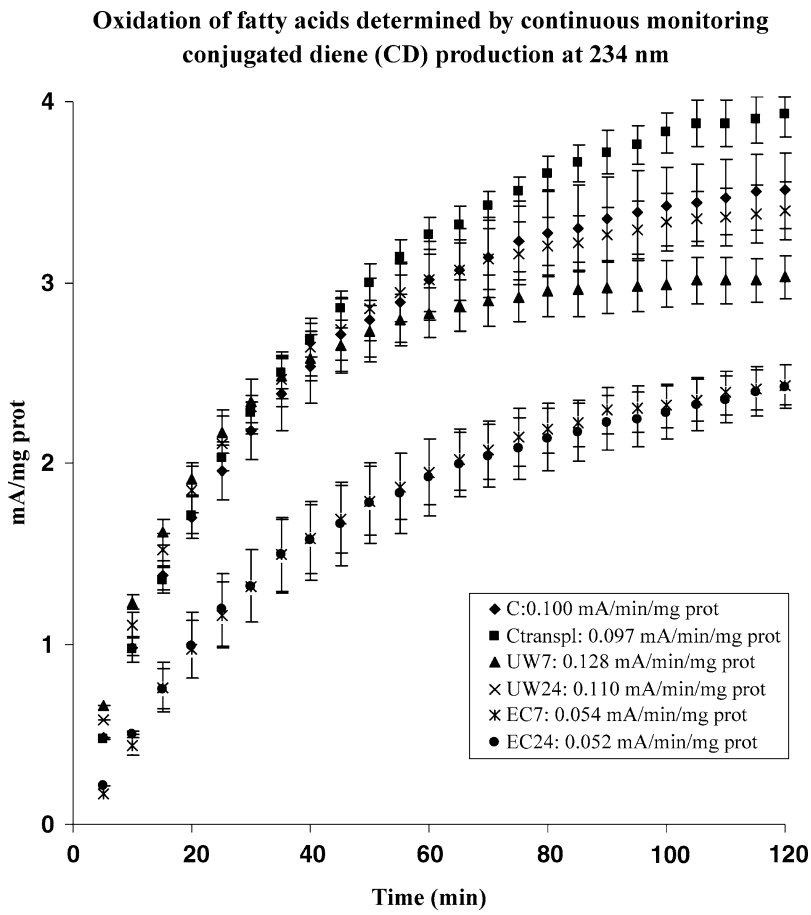

Fig. 1 Oxidation of mitochondrial fatty acids determined by continuous monitoring at 234 nm of the conjugated dienes (CD) production. Mitochondria suspensions (150 μg mitochondrial protein/mL) were incubated with 5 μM $CuSO_4$ at 37°C in a Beckman DU64 Spectrophotometer. The increase in the absorbance at 234 nm from basal values was automatically recorded at each 5 min interval during 120 min. In the inset are indicated the rate constants of CD formation for each group expressed as $mA_{234\,nm}$/min/mg protein *C*, control; $C_{transpl}$, transplanted; UW7, transplanted after 7 h storage in UW solution; UW24, transplanted after 24 h storage in UW solution; EC7, transplanted after 7 h storage in EC solution; EC24, transplanted after 24 h storage in EC solution

constant in the mitochondria of livers stored in UW solutions and partially decreased in the mitochondria of the EC-stored livers. The percentage of reduced CoQ_9 (i.e., CoQ_9H_2) remained constant in *C*, $C_{transpl}$, UW7 and UW24 and nearly disappeared in EC7 and EC24.

Native lipid hydroperoxide concentrations rose significantly along the series $C_{transpl}$, UW7, UW24, EC7 and EC24 relative to the very low concentration in control mitochondria.

CDmax values were significantly higher for mitochondria obtained from *C* and $C_{transpl}$ livers, lower for mitochondria obtained from EC7- and EC24-stored livers and at intermediate values for mitochondria isolated from UW7- and UW24-stored livers.

Finally, CDrate (Fig. 1) monitoring showed that the rate constant of CD formation was at least twofold higher in mitochondria from *C*, $C_{transpl}$, and UW7- and UW24-stored livers than in mitochondria from EC7- and EC24-stored livers.

Discussion

In liver transplantation high quality initial graft function after cold preservation and transplantation is essential for patient recovery and increases the chances of survival (Strasberg *et al*., 1994; Deschenes *et al*., 1998). It is widely recognized that IR leads to mitochondrial as well as cellular damage in hepatic parenchyma. Thus in order to achieve good graft function, one of the aims of organ preservation must be to minimize mitochondrial damage and improve bioenergetics. The present study in EC and UW preserved rat livers identifies significant reductions in activity of the mitochondrial electron transport chain complexes. This increases mitochondrial oxidative stress via adverse events that demand prevention by successful preservation.

In this study, cytochrome contents of mitochondria were maintained for 30 min following reperfusion in livers preserved in UW solution. In contrast all livers preserved in EC showed a significant depletion of cytochrome c following reperfusion (Table 1). These findings are in accordance with other observations on IR in liver (Hirakawa et al, 2003), and heart (Borutaite *et al.,* 2003). Borutaite *et al*. (2003) showed that cytochrome *c* oxidase activity decreased during ischemia and that full respiration rates are restored by external addition of cytochrome *c* (Borutaite *et al*., 2003). The authors also showed that cytochrome *c* is found in the perfusate of hearts, following IR, indicating its loss from mitochondria perhaps in relation to the opening of

permeability transition channels that is known to occur during IR (Kroemer and Reed, 2000). Cytochrome *c* mediates electron transfer between complexes III and IV, from the intermembrane space in mitochondria. Depletion of cytochrome c may decrease the activity of the electron transport chain and subsequent production of ATP. The cytochrome c release from mitochondria contributes to the development of apoptosis with the formation of apoptosome complex and activation of pro-caspase 9 (Li *et al*., 1997). It is possible that UW solution maintains the levels of cyt c because it better preserves the mitochondrial membrane and leads to less occurrence to permeability transition phenomena. This may relate to the presence of Mg in UW solution as a membrane stabilizer and to colloids that avoid cellular and mitochondrial swelling. Glucose which is present in EC solution provides osmotic pressure but permeates hepatocytes causing cellular and mitochondrial swelling (Belzer and Southsard, 1988).

Following reperfusion, complexes III and complex IV activities are maintained for up to 7 h in UW solution, but decrease significantly when preservation is extended to 24 h. In contrast livers preserved in EC solution lose complex III and IV activities. These data are in agreement with studies on failure in complex III activity reported both in cold IR rat liver model (Sammut *et al*., 1998) and warm IR dog heart model (Rouslin, 1983). Veitch et al showed that 5 min. of reperfusion could cause a significant loss of complex III activity after 60 min of ischemia (Veitch *et al*., 1992) The loss was associated with decreased content of two components of complex III cytochrome *b* and *c*1 and attributed to cardiolipin degradation by ROS. It is not clear how UW solution maintains complex III and IV activities following reperfusion of rat livers. However, this may be related to a better preservation of mitochondrial antioxidants such as glutathione by UW solution in contrast to the significant depletion early after preservation with EC solution (Jassem *et al*., 1996). Maintaining high levels of glutathione in mitochondria may help to attenuate the oxidative damage by ROS of mitochondrial components including the electron transport chain complexes. Decrease in complexes III and IV activities will lead to reduction in electron flow into the electron transport chain and lower ATP production following reperfusion, and has been associated with graft failure following transplantation (Kobayashi *et al*., 1991). Complex II activity did not change under all experimental conditions suggesting that complex II seems to be resistant to ischemia and reperfusion. These data are in accordance with other data obtained from warm IR models in hearts (Rouslin, 1983).

Specific attention is drawn to our data on the native antioxidant/peroxidation markers of the mitochondrial membranes as well as to that indicating the susceptibility of such membranes to a peroxidation insult in vitro (Table 2 and Fig. 1). Although vit. E decreased after transplantation and liver storage and HP contents increased under the same conditions, total CoQ_9 concentrations surprisingly increased after transplantation in C or in UW-stored livers and remained constant in EC preservation. The failure of vit. E in both transplantation and cold storage probably resulted from the peroxidation processes as suggested by the sharp increase of HP content several fold from baseline values following liver transplantation or storage.

The behavior of CoQ_9 deserves separate consideration because an apparently improbable increase of total CoQ_9 in $C_{transpl}$ and in both UW7 and UW24 livers was found and it is evident that a mere improved biosynthetic mechanism should be rejected. CoQ, because of its pivotal role as both an antioxidant and an essential component of the electron transfer chain, is often considered a marker of mitochondrial welfare (Ebadi *et al*., 2001). CoQ levels fluctuate during aging, in quasi-physiological conditions and in some pathologies involving oxidative stress (Armeni *et al*., 2003; Battino *et al*., 1995, 1999). These variations occurs simultaneously with a sudden decrement in plasma CoQ concentration and have been ascribed to a possible recall of the CoQ molecules by damaged tissue from blood, acting as a large CoQ "reservoir." Therefore, the increase in CoQ after transplantation might be explained through a similar mechanism that would recall the CoQ molecules from the blood once it starts reperfusing the liver tissue. Oxidative injuries that accumulated during cold ischemia or depended on IRI could stimulate the mechanism.

Another interesting finding is the percentage of reduced CoQ_9 (i.e., CoQ_9H_2) representing active CoQ_9 in the sense of actual antioxidant protection: This proportion decreased following each transplantation procedure but, disappeared if the livers were stored in EC before being transplanted, suggesting that CoQ dual roles were largely compromised.

As seen in CDmax and CDrate the maximum amount and rate of formation of conjugated dienes was lower in EC-stored livers, suggesting that these organs could be less damaged or are less prone to injury. However, an alternative explanation more consistent with outcome is that the lower values of CDmax and CDrate obtained from mitochondria of EC7- and EC24-stored liver reflect almost complete mitochondrial membrane dysfunction. Such extensive damage from the cold storage and transplantation procedure IR suggests that further stress induced in vitro would have no additional effects. It is likely that most of the peroxidizable lipid structures have already been damaged and they were found in the HP form. Thus, lower CDmax and CDrate, in this case, merely confirm the absence of oxidizable substrate rather than an increased strength against peroxidation.

Both the EC and UW preservation solutions have limitations in preventing oxidative injuries following cold storage and reperfusion; however, the data of the present study indicate that mitochondrial impairment was higher in EC- than in UW-stored livers, confirming several other reports from clinical and experimental studies suggesting superiority of the UW solution for organ preservation (Alshaibani *et al*., 1998; Eberl *et al*., 1999; Jassem *et al*., 2000; Peters *et al*., 1998) also at a mitochondrial level (Smrekova *et al*., 2000). Nevertheless, UW solution is clearly far from being considered the best possible preservation medium. Further improvements are desirable in order to optimize solutions which sustain mitochondrial structure and function and resist the effects of IR, so reducing the incidence of graft failure post-transplantation.

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