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Excorporeal Normothermic Machine Perfusion Resuscitates Pig DCD Livers with Extended Warm Ischemia

Hongzhi Xu, M.D., Ph.D.^{*,†,1}, Tim Berendsen, M.D.[†], Karen Kim, M.D.^{*}, Alejandro Soto-Gutiérrez, M.D., Ph.D.[†], Francios Bertheium, Ph.D.[†], Martin L. Yarmush, M.D., Ph.D.[†], and Martin Hertl, M.D.^{*}

^{*}Transplantation Unit, Surgery Department, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

[†]Center for Engineering in Medicine, Shriners Burn Hospital for Children at Boston, Boston, Massachusetts

Abstract

Background—The shortage in donor livers has led to increased use of allografts derived from donation after cardiac death (DCD). The compromised viability in these livers leads to inferior post-transplantation allograft function and survival compared with donation after brain death (DBD) donor grafts. In this study, we reconditioned DCD livers using an optimized normothermic machine perfusion system.

Methods—Livers from 12 Yorkshire pigs (20–30 kg) were subjected to either 0 min (WI-0 group, $n = 6$) or 60 min (WI-60 group, $n = 6$) of warm ischemia and 2 h of cold storage in UW solution, followed by 4 h of oxygenated sanguineous normothermic machine perfusion. Liver viability and metabolic function were analyzed hourly.

Results—Warm ischemic livers showed elevated transaminase levels and reduced ATP concentration. After the start of machine perfusion, transaminase levels stabilized and there was recovery of tissue ATP, coinciding with an increase in bile production. These parameters reached comparable levels to the control group after 1 h of machine perfusion. Histology and gross morphology confirmed recovery of the ischemic allografts.

Conclusion—Our data demonstrate that metabolic and functional parameters of livers with extended warm ischemic time (60 min) can be significantly improved using normothermic machine perfusion. We hereby compound the existing body of evidence that machine perfusion is a viable solution for reconditioning marginal organs.

Keywords

donor after cardiac death (DCD); normothermic machine perfusion (NMP); warm ischemia; transplantation; ATP

INTRODUCTION

The only curative therapy for end-stage liver failure remains transplantation. In the search to reduce the growing shortage of liver allografts, donation after cardiac death (DCD) livers are increasingly used as an alternative source to the standard donation after brain death (DBD) livers [1–4]. This resource has remained partly untapped, due to compromised organ quality

¹To whom correspondence and reprint requests should be addressed at Center for Engineering in Medicine, Shriners Burn Hospital for Children at Boston 51 Blossom Street, Boston, MA 02114. hongzhi2008@gmail.com.

caused by a variable period of warm ischemia during the withdraw process, which has been linked to increased incidences of primary non-function, ischemic cholangiopathy, and graft survival [5–7]. In order to safely use DCD livers, novel preservation modalities that minimize insults (e.g., hypothermia and hypoxia) and provide real-time metabolic evaluation and adjustment as well as recovery of ischemic injury are needed.

Normothermic machine perfusion (NMP) mimics the physiologic state of the organ through continued perfusion after procurement, creating an optimal environment for the organ in which it has been shown to stay viable, metabolize, and even reverse sustained damage [1, 8]. Beyond its use in healthy organs, NMP has shown regenerative potential for organs discarded due to various pathologies, especially ischemia. Brockmann *et al.* showed 5-d survival rates up to 83% for DCD livers (40 min of warm ischemia), when preserved by 20 h of NMP, *versus* a survival rate of 27% in SCS-stored controls [9]. In a similar study by Schön *et al.*, 7-d survival rates of 100% for DCD livers with 1 h of warm ischemia were reported [10]. During normothermic machine perfusion, the continuous vascular flow provides several advantages over static storage, such as real-time in- and outflow analysis with feedback potential, oxygenation, drug- and nutrient delivery, and avoidance of hypothermia vascular resistance evaluation. In this study, we establish a porcine normothermic machine perfusion model, and show that it is effective in preserving healthy liver grafts as well as reconditioning warm ischemic allografts to comparable outcome parameters. Using this model, we aim to provide insight into the cellular mechanisms underlying machine perfusion, which will prove instrumental in optimizing the technique and integrating it further into the clinical field.

Previous studies showed that tissue ATP level prior to transplantation is one of the most important determinants for allograft survival, and that during warm ischemia, the concentration of ATP in liver tissue decreases rapidly [11]. While cold storage of DCD livers exacerbates this ATP depletion, NMP provides a physiologically relevant environment capable of energetic replenishment. In the present study, our focus was the recovery process during normothermic perfusion, of DCD livers that had undergone 60 min of warm ischemia. We hypothesized that NMP resuscitates DCD livers by improving the energy status of the tissue.

MATERIALS AND METHODS

Animals

Male Yorkshire pigs (Tufts University Cummings School of Veterinary Medicine, North Grafton, MA) weighing 25 ± 5 kg were randomly allocated to two groups: a 60 min warm ischemia group (WI-60), and a control group without warm ischemia (WI-0) ($n = 6$ for each group). All animals were fasted but had free access to water for 12 h prior to the scheduled operation. The animals were maintained in accordance with National Research Council guidelines and the experimental protocols were approved by the Subcommittee on Research Animal Care, Committee on Research, Massachusetts General Hospital.

Surgical Procedures

After being premedicated with Telazol (1.4 mg/kg), xylazine (1 mg/kg), and glycopyrrolate (0.01 mg/kg), animals were intubated and general anesthesia was induced and maintained with 1% isoflurane, 50% nitrous oxide, and oxygen during the procedure. Fluid support consisted of intravenous administration of saline, maintained at 120 mL/h. EKG, blood pressure, oxygen saturation, and end-tidal CO_2 were continuously monitored.

All surgical procedures were performed under aseptic conditions. After opening the abdomen with a midline incision, the hilum of the liver was mobilized and the bile duct was

divided close to the pancreas. After isolation of the hepatic artery and portal vein, the infra-renal aorta was dissected free for the purpose of exsanguination. A bolus of heparinized saline (500 units/kg, i.v.) was administered. Five minutes thereafter, the aorta was cannulated with a 20-gauge cannula (Bard, Covington, GA) and blood was collected in blood bags containing citrate-phosphate-dextrose, which was facilitated using a pulse pump at 75 mL/min. After 1.5 L of blood was collected, the animal was sacrificed using sodium pentobarbital (100 mg/kg, i.v.). For the WI-60 warm ischemic study group ($n = 6$), the liver was left *in situ* for 60 min, followed by a flush through the portal vein with 2 L of ice-cold SPS-1 (UW solution) (Organ Recovery System, Inc. Chicago, IL). For the WI-0 control group, this flush was performed immediately after the sacrifice, minimizing the warm ischemic time. Under continuing portal perfusion of ice-cold UW solution, the liver was excised and placed on the back-table, where the hepatic artery and common bile duct were cannulated. Finally, the liver was stored in an organ bag with ice-cold SPS-1 solution and kept on ice until the start of machine perfusion.

Ex Vivo Normothermic Pig Liver Machine Perfusion

After 2 h of cold storage, the liver was flushed with 2 L of ice-cold Ringer's lactate through both the portal vein and hepatic artery. The liver was then placed into the perfusion chamber and connected to the perfusion system, which was primed beforehand. The perfusion setup (Fig. 1) is a dual re-circulating circuit that consists of a centrifugal pump (BP50; Medtronic Inc., Minneapolis, MN), heat exchanger, membrane oxygenator (Affinity NT; Medtronic Inc., Minneapolis, MN), bubble trap, venous reservoir, and organ chamber. To maximize sterility, the procedures were performed in a laminar flowhood. Inflow perfusate was oxygenated, warmed to 39°C and divided into two lines: The arterial branch was perfused actively using the centrifugal pump, while the portal branch was fed passively by the gravity of the venous reservoir. Both lines are perfused in a constant flow fashion. After placement into the organ chamber, the liver was immersed in perfusate, which flowed freely from the supra- and infra-hepatic vena cava. Perfusion was carried out for 4 h for each group. Flow rates and hydrostatic pressures in the arterial and venous branches were continuously monitored *via* flow- and pressure transducers (TX50 Flow Bio-Probe; Medtronic Inc., Minneapolis, MN; TSD104A Pressure; Biopac System Inc., Goleta, CA) connected to a data acquisition system (MP100; Biopac System Inc.). The total volume of perfusate was 2000mL and comprised 1.5 L whole blood, 0.5 L sterile porcine plasma (Sigma-Aldrich, St. Louis, MO), hydrocortisone (10 mg/L), insulin (2 u/L), penicillin (40,000 U/L), streptomycin (40 mg/L), and heparin (5000 U/L).

Liver Function, Viability, and Metabolic Assessment

Bile was collected every hour and gravimetrically measured. Aliquots of perfusate from the liver outflow were taken hourly for analysis of liver enzymes (AST, ALT, ALP) using a Piccolo blood chemistry analyzer (Abaxis, Union City, CA). For analysis of the hepatic oxygen consumption, samples were taken from the inflow and outflow of the liver and analyzed immediately using a handheld blood gas analyzer (Abaxis). OURs were determined as follows:

$$\text{OUR} = \frac{V \times [\text{CO}_2, \text{inflow} - \text{CO}_2, \text{outflow}]}{\text{liver weight}}$$

where V is the perfusion flow rate (mL/min), C_{O_2} is the oxygen concentration (nM) calculated as $\text{C}_{\text{O}_2} = 0.0031 \cdot \text{PO}_2$, where PO_2 is the oxygen partial pressure (mmHg), and 0.0031 (mL O_2 /(mmHg· dL)) is the solubility of oxygen in aqueous solutions.

Liver tissue samples were harvested from the left lateral lobe before the warm ischemic period, before cold flush with SPS-1, and 0, 1, 2, and 4 h after the start of machine perfusion. Tissues were immersed immediately in liquid N₂ and store at -80°C. For ATP analysis, liver tissues were homogenized in cold 4% HClO₄, after centrifugation and pH adjustment (pH 8.5), the ATP concentration was analyzed using a ATP Colorimetric Assay Kit (K354-100; Biovision Inc., Mountain View, CA).

Histologic Evaluation

After perfusion, liver tissue samples were harvested from the left lateral lobe for histologic analysis. The samples were immersed in formalin and evaluated for hepatocyte morphology and overall structure using light microscopy.

Statistical Analysis

Analysis of variance by *post hoc* Sheffe *F*-test was used in order to assess significant differences in data obtained from the WI-60 warm ischemia group and the WI-0 control group. Significance was defined as $P < 0.05$. The data were expressed as means $\pm$ standard error of the mean.

RESULTS

Hemodynamics

The livers were hemodynamically stable throughout the 4 h of perfusion. The flow rate, which was maintained at 1.2–1.5 L/min for the portal branch and 0.3–0.4 L/min for the arterial branch, corresponded to a portal pressure of 5–8 mmHg and an arterial pressure of 70–80 mmHg.

Parenchymal Liver Enzymes

Liver enzyme levels were measured before, during, and after perfusion. Notably, as the perfusion system recirculates the perfusate, parenchymal injury to the liver is represented by the net increase in enzyme levels rather than the absolute value. At the start of perfusion, perfusate ALT levels in the WI-60 group were markedly higher compared with the base level (i.e., the enzyme level before the liver was connected to the system or $t = -5$ min) and the level in WI-0 group [66.0 ± 22.60 (WI-60) *versus* 23.38 ± 2.88 (WI-0) at 0 min *versus* 18 ± 3.46 at -5 min, $P < 0.05$], indicating significant damage to the liver as a result of the 60-min warm ischemia (Fig. 2). The ALT levels in the WI-60 group were elevated slightly after 1 h of perfusion and then remained stable during the rest of 3 h [ALT: 81.67 ± 28.24 (WI-60, 1 h) *versus* 83.83 ± 25.02 (WI-60, 2 h) *versus* 83.5 ± 23.42 (WI-60, 3 h) *versus* 85 ± 22.80 (WI-60, 4 h), $P > 0.05$]. There was no significant change in ALP levels observed during the perfusion.

Bile Production

Livers from the WI-0 control group displayed a constant rate of bile production during the 4-h perfusion, whereas the WI-60 group initially showed low bile production rates (4.43 ± 1.51 ml (WI-60, 1 h) *versus* 10.5 ± 2.84 mL (WI-0, 1 h), $P < 0.05$) within the first hour of perfusion. Concomitant with the profile of the ALT levels in the WI-60 group, after 1 h of machine perfusion the bile production rates started to increase to similar levels as those in the WI0 group, indicated by the similar slope of the bile production curve (Fig. 3).

Oxygen Consumption

Oxygen consumption rates for both groups are shown in Figure 4. Interestingly, we observed higher oxygen consumption rates in the WI-60 group at 2 and 3 h after perfusion [2 h: 19.41

± 3.79 (WI-60) *versus* 16.38 ± 1.24 (WI-0); 3 h: 18.86 ± 3.82 (WI-60) *versus* 13.85 ± 2.58 (WI-0); $P > 0.05$], though the difference between these two groups did not reach the statistical significance (Fig. 4). This occurrence of high oxygen demands indicates highly active metabolic processes in DCD liver during the perfusion.

Energetic State

To further explore the mechanisms by which normothermic machine perfusion improves organ function, we measured tissue ATP levels at different stages throughout the experiments (Fig. 5). Sixty minutes of warm ischemia caused the total liver tissue ATP levels to decrease to 30% of the original level (WI-60 min: 0.37 ± 0.08 $\mu\text{mol/g}$ *versus* WI-0 min: 1.26 ± 0.14 $\mu\text{mol/g}$). During cold storage, the ATP levels continued to decline to nearly undetectable at the end of the 2 h period (0 min: 0.01 ± 0.03 $\mu\text{mol/g}$). Four hours of normothermic perfusion restored the tissue ATP levels to approximately 80% of the initial value (240 min: 1.07 ± 0.12 $\mu\text{mol/g}$).

Gross Morphology and Histology

At the start of normothermic perfusion, dark patchy areas were visible on the surface of the liver, generally understood to be a sign of ischemic damage. During perfusion, these areas gradually started to disappear. At the end of the 4 h perfusion period, the livers were homogeneous in color, and resembled the physiologic appearance. Microscopically, after 60 min warm ischemia and 2 h cold storage, there was prominent steatotic degeneration of the hepatocytes, as evidenced by numerous lipid vacuoles accumulating in the cytoplasm. In addition, necrosis and apoptosis could be observed in the hepatocytes (Fig. 6A and B). These morphologic changes were almost completely reversed by 4 h continuous NMP, with only few instances of hepatocyte necrosis observed (Fig. 6C and D).

DISCUSSION

Maintaining organ viability during preservation is essential for successful liver transplantation. With the current practice to accept extended criteria donor organs, it is imperative to improve organ preservation techniques so that the viability of these marginal organs can be enhanced. Previously, this group and others have shown that NMP is an effective method to resuscitate DCD livers [1, 9, 12–14]. In the present study, we provide further evidence that the recovery of DCD livers with extended warm ischemia time by NMP occurs after as early as 1 h of NMP perfusion. In addition, we demonstrated that the improvement of viability and functionality is associated with restored liver tissue ATP content, providing a possible approach towards elucidating the molecular mechanisms of NMP.

As a major “energy currency,” ATP is essential to maintain biological processes in cells. There is abundant evidence based on both animal and human studies suggesting that the level of ATP in the liver prior to transplantation is an important determinant for allograft and recipient survival [15–17]. In warm ischemic livers, ATP levels have been shown to decline so rapidly that they were nearly undetectable after 15 min of warm ischemia [18]. In line with these findings, we observed a marked decrease in ATP in pig livers after 60 min of warm ischemia. Conceivably, in warm ischemic circumstances with ATP depletion of this magnitude, tolerance to the hypothermia that accompanies cold storage, as well as the subsequent re-warming phases, may be significantly compromised. In addition, although ATP is a universal cellular compound, its depletion can play a specific role in different cell-types. For instance, Doctor *et al.* [19] have found that 60 min of warm ischemia in cholangiocytes caused striking alterations in the membrane-cytoskeleton organization, with loss of apical microvilli coinciding with selective disassociation of the linking protein

“ezrin” from the microvillar cytoskeleton. Because these membrane structures are essential for differentiated epithelial function, their dysregulation may play a role in transplantation-related pathology, such as impaired biliary function or development of non-anastomotic biliary strictures after transplantation, which are more frequently observed in DCD allografts [20]. Possibly, while a certain threshold level of ATP is likely to be crucial in regular liver transplantation, in DCD livers this threshold is not always met. Hence, restoring the ATP content might be a prerequisite for successful resuscitation of DCD livers. In fact, several strategies of modifications on hypothermic storage/perfusion aiming to increase tissue ATP levels were reported to improve DCD liver viability. In contrast to these hypothermia-based strategies, NMP provides an ideal platform for organ recovery by mimicking the physiological environment, without hypothermia-induced discontinuity in the endothelial lining and alterations in the endothelial cell cytoskeleton, which were found to be major culprits of post-ischemic liver dysfunction [21]. We concede that in the clinical setting, a degree of cold preservation will always be incorporated. However, NMP seems able to assuage cold insults and regenerate the liver towards a state of high metabolism and energy content.

In this study, we demonstrate that during normothermic perfusion, DCD livers initially show inferior viability and functionality. Within 1 h of machine perfusion, parameters such as liver enzyme release, bile production rate, and vascular pressure improved to similar levels as the control group. These observations were accompanied by a slightly higher oxygen consumption rate and a rapidly increasing ATP content, which may indicate an active recovery process. ATP deficiency in DCD livers is linked directly to the occurrence of mitochondrial dysfunction, which has been frequently reported in ischemic livers [22]. Concordantly, we found that both overall liver architecture and mitochondrial integrity were significantly improved after NMP. Given our results as well as the critical role of ATP in allograft viability, we propose that the improvements seen in function, morphology, and viability have an origin in the restoration of ATP levels in the mitochondria of the various cell types in the allograft. Further investigation towards the processes underlying NMP and energy restoration is warranted.

The perfusion system used for these experiments incorporates dual (portal and arterial) perfusion. Similar to the system used by Jamieson and Friend *et al.* [13], we use a centrifugal pump to provide constant pressure on the arterial branch. The flow in the portal vein was passively driven by the gravity of the venous reservoir. To minimize pressure-injury, the organ was immersed in perfusate in a customized organ chamber, which is also designed to avoid “bruising” of the organ by its own weight, which would disrupt perfusion in the peripheral microcirculation of the organ. The stable hemodynamics demonstrated by our system shows that it is capable of avoiding such damage.

In summary, the present study provides evidence that DCD livers that have undergone 60 min of warm ischemia can be resuscitated by normothermic machine perfusion. NMP effectively restored the tissue ATP content and improved the mitochondrial integrity, which might contribute to the improvement of DCD liver viability and functionality. The information provided in study might help us to better understand the repair mechanism of NMP, which has great potential to successfully use DCD livers as an alternative resource and enlarge the total number of viable donor livers available for transplantation.

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References

1. Tolboom H, Pouw RE, Izamis ML, et al. Recovery of warm ischemic rat liver grafts by normothermic extracorporeal perfusion. *Transplantation*. 2009; 87:170. [PubMed: 19155970]
2. Wheelton DR, Potter CD, Oduro A, et al. Transforming the “unacceptable” donor: Outcomes from the adoption of a standardized donor management technique. *J Heart Lung Transplant*. 1995; 14:734. [PubMed: 7578183]
3. Strasberg SM, Howard TK, Molmenti EP, et al. Selecting the donor liver: Risk factors for poor function after orthotopic liver transplantation. *Hepatology*. 1994; 20(4 Pt 1):829. [PubMed: 7927223]
4. Kocak Z, Celik Y, Uzal MC, et al. Isolated bilateral sixth nerve palsy secondary to metastatic carcinoma: A case report with a review of the literature. *Clin Neurol Neurosurg*. 2003; 106:51. [PubMed: 14643919]
5. Chan EY, Olson LC, Kisthard JA, et al. Ischemic cholangiopathy following liver transplantation from donation after cardiac death donors. *Liver Transpl*. 2008; 14:604. [PubMed: 18433032]
6. Merion RM, Pelletier SJ, Goodrich N, et al. Donation after cardiac death as a strategy to increase deceased donor liver availability. *Ann Surg*. 2006; 244:555. [PubMed: 16998364]
7. Reich DJ, Hong JC. Current status of donation after cardiac death liver transplantation. *Curr Opin Organ Transplant*. 2010; 15:316. [PubMed: 20414112]
8. St Peter SD, Imber CJ, Lopez I, et al. Extended preservation of non-heart-beating donor livers with normothermic machine perfusion. *Br J Surg*. 2002; 89:609. [PubMed: 11972552]
9. Brockmann J, Reddy S, Coussios C, et al. Normothermic perfusion: A new paradigm for organ preservation. *Ann Surg*. 2009; 250:1. [PubMed: 19561463]
10. Schön MR, Kollmar O, Wolf S, et al. Liver transplantation after organ preservation with normothermic extracorporeal perfusion. *Ann Surg*. 2001; 233:114. [PubMed: 11141233]
11. Miyagi S, Iwano T, Akamatsu Y, et al. The significance of preserving the energy status and microcirculation in liver grafts from non-heart-beating donor. *Cell Transplant*. 2008; 17:173. [PubMed: 18468247]
12. de Rougemont O, Lehmann K, Clavien PA. Preconditioning, organ preservation, and postconditioning to prevent ischemia-reperfusion injury to the liver. *Liver Transpl*. 2009; 15:1172. [PubMed: 19790166]
13. Jamieson RW, Friend PJ. Organ reperfusion and preservation. *Front Biosci*. 2008; 13:221. [PubMed: 17981540]
14. Jain S, Lee SH, Korneszcuk K, et al. Improved preservation of warm ischemic livers by hypothermic machine perfusion with supplemented University of Wisconsin solution. *J Invest Surg*. 2008; 21:83. [PubMed: 18340625]
15. Berendsen TA. Hepatocyte viability and adenosine triphosphate content decrease linearly over time during conventional cold storage of rat liver grafts. *Transplant Proc*. 2011 (in press).
16. Sumimoto K, Inagaki K, Yamada K, et al. Reliable indices for the determination of viability of grafted liver immediately after orthotopic transplantation. Bile flow rate and cellular adenosine triphosphate level. *Transplantation*. 1988; 46:506. [PubMed: 3051557]
17. Kamiike W, Burdelski M, Steinhoff G, et al. Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation. *Transplantation*. 1988; 45:138. [PubMed: 3276042]
18. Kamiike W, Watanabe F, Hashimoto T, et al. Changes in cellular levels of ATP and its catabolites in ischemic rat liver. *J Biochem*. 1982; 91:1349. [PubMed: 7096291]
19. Doctor RB, Dahl RH, Salter KD, et al. Reorganization of cholangiocyte membrane domains represents an early event in rat liver ischemia. *Hepatology*. 1999; 29:1364. [PubMed: 10216117]
20. Pine JK, Aldouri A, Young AL, et al. Liver transplantation following donation after cardiac death: An analysis using matched pairs. *Liver Transpl*. 2009; 15:1072. [PubMed: 19718634]
21. Ghonem N, Yoshida J, Stolz DB, et al. Treprostinil, a prostacyclin analog, ameliorates ischemia-reperfusion injury in rat orthotopic liver transplantation. *Am J Transplant*. 2011; 11:2508. [PubMed: 21668631]

22. Sheth H, Hafez T, Glantzounis GK, et al. Glycine maintains mitochondrial activity and bile composition following warm liver ischemia-reperfusion injury. *J Gastroenterol Hepatol*. 2011; 26:194. [PubMed: 21175814]

FIG. 1.

Ex Vivo normothermic machine perfusion system. The perfusion setup was a dual re-circulating circuit that consists of a centrifugal pump, heat exchanger, membrane oxygenator, bubble trap, venous reservoir, and organ chamber. Inflow perfusate was divided into two lines: the arterial and the portal branch. Outflow perfusate collected from the organ chamber was returned to the centrifugal pump. The entire system was kept in a laminar hood.

FIG. 2.

Liver enzymes release during normothermic machine perfusion. At the start of perfusion, perfusate ALT levels detected in the WI-60 group were markedly higher compared with the base level ($t = -5$ min) and the level in WI-0 group [$**P < 0.05$ versus $t = -5$ min (base level); $*P < 0.05$ versus $t = 0$ (WI-0 group)]. The ALT levels in the WI-60 group were elevated slightly after 1 h of perfusion and then remained stable during the rest of 3 h. There was no significant change in ALP levels observed during the perfusion.

FIG. 3. Bile production during normothermic machine perfusion. Livers from the WI-0 control group displayed a constant rate of bile production during 4-h perfusion, whereas the WI-60 group initially showed low bile production within first hour of perfusion and then picked up to the similar rate as WI-0 group during the next 3 h (**versus* wit = 0 group at 1 h perfusion time).

FIG. 4. Oxygen consumption during normothermic machine perfusion. Livers from the WI-60 group showed a slightly higher oxygen consumption rate comparing to WI-0 group, but did not reach the statistical significance ($P > 0.05$ at 1–4 h perfusion time).

FIG. 5.

Changes of tissue ATP levels during warm ischemia, cold ischemia, and normothermic machine perfusion period. In the WI-60 group, the total ATP level sharply decreased to one-third of the original level, and nearly undetectable at the end of 2 h of cold storage; 4 h NMP restored the ATP levels about 80% of initial level.

FIG. 6.

Histologic changes of liver at different stages. (A) Normal liver before warm ischemia and cold storage; (B) liver after 60 min warm ischemia and 2 h cold storage; (C) liver after 4 h normothermic machine perfusion; (D) Liver gross appearance during 4 h normothermic machine perfusion. Warm ischemia resulted in prominent hepatocyte steatotic degeneration evidenced by numerous lipid vacuoles accumulation in the hepatocyte's cytoplasm in HE staining of liver tissue. Some hepatocyte necrosis and apoptosis could also be observed (A) and (B); 4 h NMP almost completely reversed these pathologic changes (C). Some dark patchy areas were visible on the surface of the liver parenchyma at beginning of perfusion. During perfusion, these areas gradually started to disappear. At the end of the 4 h perfusion period, the aspect of the liver was homogeneous and resembled the physiologic appearance (D).