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Comprehensive Evaluation of a Donated After Circulatory Death (DCD) Donor Liver Model in Minipigs

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Graft livers from donors after death from circulatory disease (DCD) often suffer injury from severe ischemia or hypoxia before resection. Thus, earlier evaluation of these livers is critical for the survival of recipients.

Material/Methods: In our study, 18 minipigs, as DCD donor liver models, were evenly divided into a warm ischemia time (WIT) group and a hypoxia plus ischemia group. Another 18 minipigs served as recipients and were implanted with the donor livers of the DCD models. miR-122 levels and hepatic function were examined before and after liver transplantation.

Results: Results indicated that increased miR-122 levels appeared in the early stages of ischemia and hypoxia. Increases in ALT and GGT levels occurred earlier than changes in TBil.

Conclusions: The expression levels of miR-122 in donor liver might play a role in the evaluation of organ injury. Changes in donor liver functions were more sensitive to ischemia than hypoxia in this established porcine DCD model.

MeSH Keywords: Liver Transplantation • Swine, Miniature • Warm Ischemia

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Background

Hepatic transplantation is a treatment option for end-stage liver disease and acute liver failure; however, the availability of donor livers is a major limitation [1,2]. In the early 1990s, attempts were made to expand the donor pool by using livers donated after circulatory death (DCD), albeit with less favorable outcomes compared with using livers donated after brain death (DBD) [1]. Compared with using DBD organs, implanting DCD livers significantly increases serious postoperative complications, such as primary non-function (PNF), hepatic artery thrombosis (HAT), and inflammatory bowel disease (IBD), because DCD is associated with thermal damage caused by complex ischemia and hypoxia during cardiac arrest [3,4]. Therefore, efforts have been made to increase recipient survival by reducing warm and cold ischemia time in DCD donor livers [5]. With DCD liver transplantation becoming more common, quick and accurate assessment of donor liver quality before transplant is essential.

At present, the primary means of evaluating the quality of DCD donor livers are warm and cold ischemia time, the degree of steatosis in biopsy specimens, and the clinical experience of transplant surgeons [6,7]. An effective method for determining the degree of liver damage and predicting the prognosis of a DCD donor liver is lacking, and development of a sensitive and objective evaluation index remains an important goal.

miR-122 is a liver-specific microRNA that is completely conserved in all vertebrates [8–10]. It is the most abundantly expressed microRNA in the liver, making up more than 50% of the total microRNA in that organ. miR-122 regulates diverse liver functions, maintains systemic homeostasis [11,12], and suppresses the expression of non-hepatic genes in the adult liver. miR-122 also is believe to be involved in maintaining homeostasis of cholesterol and triglyceride metabolism, as well as systemic iron levels [13,14]. Studies have shown that miR-122 is released from damaged hepatocytes early after liver injury, suggesting that it might be a biomarker for liver injury. Indeed, recent studies showed that serum miR-122 levels were increased in patients with cirrhosis only when liver injury was present [15–17]. The major reason for tissue damage during liver transplantation is ischemia-reperfusion injury, and studies in mice and rats have demonstrated that the elevation of serum miR-122 levels correlates with hepatic ischemia-reperfusion injury [18]. In rats, pretreatment with crocin reduced hepatic ischemia-reperfusion injury, and also decreased miR-122 levels [19]. Importantly, Farid et al. reported that hepatocyte-derived microRNAs (HD microRNAs) could serve as serum biomarkers of hepatic injury and rejection after liver transplantation [20]. Those findings suggest that miR-122 could be useful as an indicator of liver injury. Moreover, serum specimens are easier to take than liver biopsy specimens and do not damage liver tissue.

In the present study, we established a porcine DCD model to mimic Maastricht category III non-heart-beating donation (NHBD) [21,22]. We investigated the effects of warm ischemia and hypoxia time on the quality of the donor liver by assessing tissue histopathology and graft liver function. We analyzed miR-122 levels in these donor tissues to determine whether they could serve as an objective index to predict survival of the implanted liver.

Material and Methods

Animals and study design

Minipigs, male or female, 4 months old and weighing 35±3.5 kg, were provided by the Experimental Animal Center of Shenyang Agricultural University. All animal experiments were performed according to the Guidelines for Care and Use of Animals in Research approved by the First Hospital of China Medical University. The study was approved by the Ethics Committee of China Medical University, and all the operations, pain management, and euthanasia procedures were proved by the committee (approved protocol number 2010-E-01). The minipigs were kept in a moderate environment with food and water ad libitum.

A total of 36 minipigs were used in the study, with half evenly assigned into 3 warm ischemia experiment groups and half evenly assigned into 3 hypoxia plus ischemia experiment groups. The 6 minipigs in each subgroup were further evenly and randomly divided into donors and recipients (3 in each subgroup). In the 3 warm ischemia subgroups, donor minipigs were pretreated with warm ischemia times of 10, 20, and 30 min (WIT 10, 20, and 30 min). In the 3 hypoxia plus WIT subgroups, donor minipigs received hypoxia 15 min + 20 min WIT, hypoxia 30 min + 20 min WIT, and hypoxia 60 min + 20 min WIT. All minipigs (donors and recipients) were fasted for 24 h before the experiment. Resected normal livers from recipient minipigs were used as controls.

Establishment of the DCD model

The organ procurement and transplantation procedures were performed as described previously [23,24]. The DCD model was established to mimic Maastricht category III of NHBD, waiting for cardiac death. Donor minipigs were anesthetized as follows: first, anesthesia was induced with an intramuscular injection containing a mixture of ketamine (5 mg/kg) and chlorpromazine (6 mg/kg); second, succinylcholine for muscle relaxation and fentanyl for analgesia were delivered i.v. during the operation; and third, inhaled isoflurane was combined with i.v. drops of propofol for anesthesia. Midazolam (0.5 mg/Kg i.m.) was used as premedication. A peripheral ear vein was cannulated and

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anesthesia was induced with propofol (5 mg/Kg i.v.). Intubation was achieved with a 6.5 cuffed endotracheal tube and ventilation set at volume-controlled mode (8 mL/Kg). Anesthesia was maintained with isoflurane 2% and continuous analgesia with sufentanil (2 μg/Kg/min i.v.). The pigs were immobilized with cisatracurium besylate (0.2 mg/Kg followed by 0.1 mg/Kg/h i.v.). Ventilator settings were: 2 L of O_2 flow/min and an air flow rate 0.5 L/min. Capacity control mode was PEEP 2–4 cm H_2O, respiratory rate was 15–20 times/min, and tidal air volume was 8–10 mL/kg. Blood pressure was continuously monitored.

During the primary operation, venous blood samples were obtained and an abdominal median incision (from the xiphoid to the pubic symphysis) was made to obtain liver tissue biopsy specimens. Then, the hepatic artery and portal vein, as well as the superior and inferior vena cava and abdominal aorta, were exposed to prepare for arterial perfusion. Pancuronium bromide was given i.v. at 60–100 ug/kg before ventilation was turned off; heparin was then administered i.v. at 3 mg/kg. Ischemia time started when the average arterial pressure dropped below 60 mmHg. Each donor was given a 5-min non-contact time after the heart stopped. Liver tissues were biopsied at various time points after ischemia was initiated. Hypothermal perfusion was performed using portal vein intubation after biopsy with Ringer's lactate solution kept at 4°C and drained through the inferior vena cava. Liver procurement started after 1000 mL of PBS was perfused through both the aorta and portal vein. Before transplantation, donor livers were kept at 4°C in UW (University of Wisconsin) solution.

Establishment of the DCD model with hypoxemia

Donor minipigs were prepared as described for the DCD model, but ventilation was adjusted so that oxygen saturation for donors was maintained at 70–80% for 15, 30, and 60 min before ventilation was turned off. Then, ischemia was induced for 20 min before the livers were resected as described previously.

Transplantation

Recipient minipigs were anesthetized in the same way as that described for the donors. After adequate exposure of the superior and inferior vena cava, portal vein, common hepatic artery, and common bile duct, the recipient hepatectomy was performed after pre-blockage of the hepatic veins, during which blood pressure was maintained above 50 mmHg. The liver was resected with portal vein and then inferior vena cava and supra vena cava. To connect the donor liver, vascular anastomosis started with the superior vena cava, then the portal vein, and finally the inferior vena cava and aorta. A drainage tube was inserted into the bile duct for biliary drainage. The abdominal wall was completely closed after the final biopsy was taken. Dizoxin (5 mg/4–8 h) was used for postoperative analgesia.

Histopathology analysis of donor livers

Liver biopsy samples (0.2-cm-thick) taken from the right lobe were fixed in 4% paraformaldehyde solution for 12–24 h before they were imbedded. Serial sections were made and HE staining was performed using standard procedures. The histopathology of each sample was analyzed using a light microscope.

Total RNA extraction and miRNA quantification

Liver tissue samples were placed in Trizol reagent (Dalian TaKaRa, Inc., Dalian) immediately after biopsy, and total RNA was extracted using the standard Trizol method according to the manufacturer's specifications. RNA was dissolved in DEPC water and kept at –80°C until further analysis.

First-strand cDNA for microRNA was synthesized using the Mir-X miRNA First-Strand Synthesis Kit (Dalian TaKaRa, Inc., Dalian) at the reaction condition specified by the manufacturer's manual. miR-122 and U6 qRT-PCR were carried out using the SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (Dalian TaKaRa, Inc., Dalian), with the miR-122-specific primer (5'-TGGAGTGTGACAATGGTGTTTGT-3') for miR-122 and U6-specific primers for U6. Relative quantification for the starting amount of miR-122 was calculated with ΔΔCt method.

Serum examination of liver function

Levels of alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and total bilirubin (TBil) in serum were measured standard clinical laboratory procedures.

Statistical analysis

Data are presented as mean ± standard deviation (mean ±SD) and were analyzed with ANOVA for multiple comparisons. Statistical significance was defined as $P<0.05$. Data were analyzed with SPSS13.0 statistical software (IBM SPSS Statistics for Windows, Version 13.0, IBM Corp., Armonk, NY)

Result

No apparent pathology changes were found in donor livers pretreated with WIT and hypoxia

We first assessed the effects of warm ischemia injury at different time points by examining histopathological changes in donor livers. As seen in Figure 1A, liver tissue structure and staining are clear. There is slight blurring in Figure 1C and clear blurring in Figure 1D, suggesting that there was slight edema in liver tissue pretreated with WIT of 20 and 30 min, respectively (Figure 1). We then assessed the impact of hypoxia at different time points

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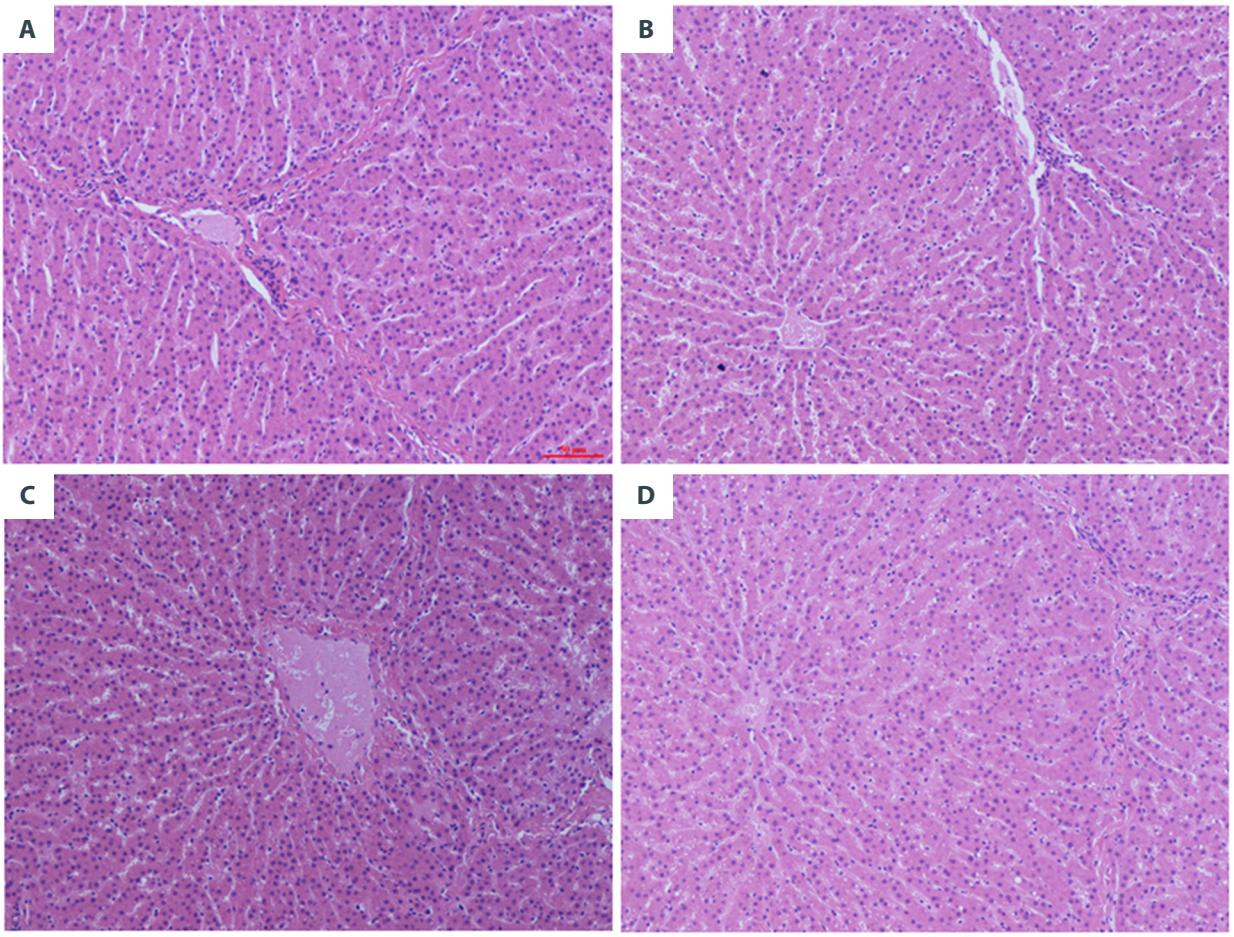

Figure 1. Representative donor liver tissue sections after WITs of 0 (**A**), 10 (**B**), 20 (**C**), and 30 (**D**) min (H&E staining with 200× magnification).

plus 20 min of ischemia injury on donor liver tissues. Figure 2A shows liver tissue pretreated with only WIT 20 min without hypoxia damage. Still, blurring is increased from slight to mild to severe (Figures 2B–2D), corresponding to pretreatment with 15, 30, and 60 min hypoxia plus WIT 20 min, respectively (Figure 2).

miR-122 expression is increased earlier

miR-122 expression in donor liver tissue after ischemia injury was compared with expression in control liver tissue using qRT-PCR. As shown in Figure 3A, miR-122 levels were increased 1.73-, 2.08-, and 2.92-fold in response to 10, 20, and 30 min, respectively, of ischemia injury compared with control conditions. miR-122 levels were not increased in livers pretreated with 15 min hypoxia plus WIT 20 min, but they were increased in livers pretreated with 30 and 60 min hypoxia plus WIT 20 min (Figure 3B). The elevated miR-122 levels in donor livers pretreated with different durations of warm ischemia demonstrated that changes in miR-122 levels occur earlier than histopathological changes.

Parameters of liver function after transplantation

Serum samples were collected from recipients 1, 3, 6, 12, and 24 h after liver transplant. Serum ALT, GGT, and TBil concentrations were assessed. There were some noteworthy findings. First, serum ALT and GGT concentrations were significantly increased and corresponded to postsurgical time, with WIT and with hypoxia plus WIT (Tables 1–4). Second, liver function damage seemed more severe with WIT 30 min than with hypoxia 60 min plus WIT 20 min; for example, at 1 h after surgery, ALT concentration was 577.33±164.51 U/L in the WIT 30 min group and 307.00±13.11 U/L in the hypoxia 60 min plus WIT 20 min group (Tables 1, 3). Third, 3 recipients implanted with DCD liver pretreated with WIT 30 min died within 6 h of surgery, while 3 recipients implanted with DCD liver pretreated with hypoxia 60 min plus WIT 20 min died within 24 h of surgery (Tables 1–6). Finally, in recipients implanted with livers pretreated in WIT 10 min, serum TBil concentrations were not changed at any time point after surgery. However, serum TBil concentrations changed in a postsurgical

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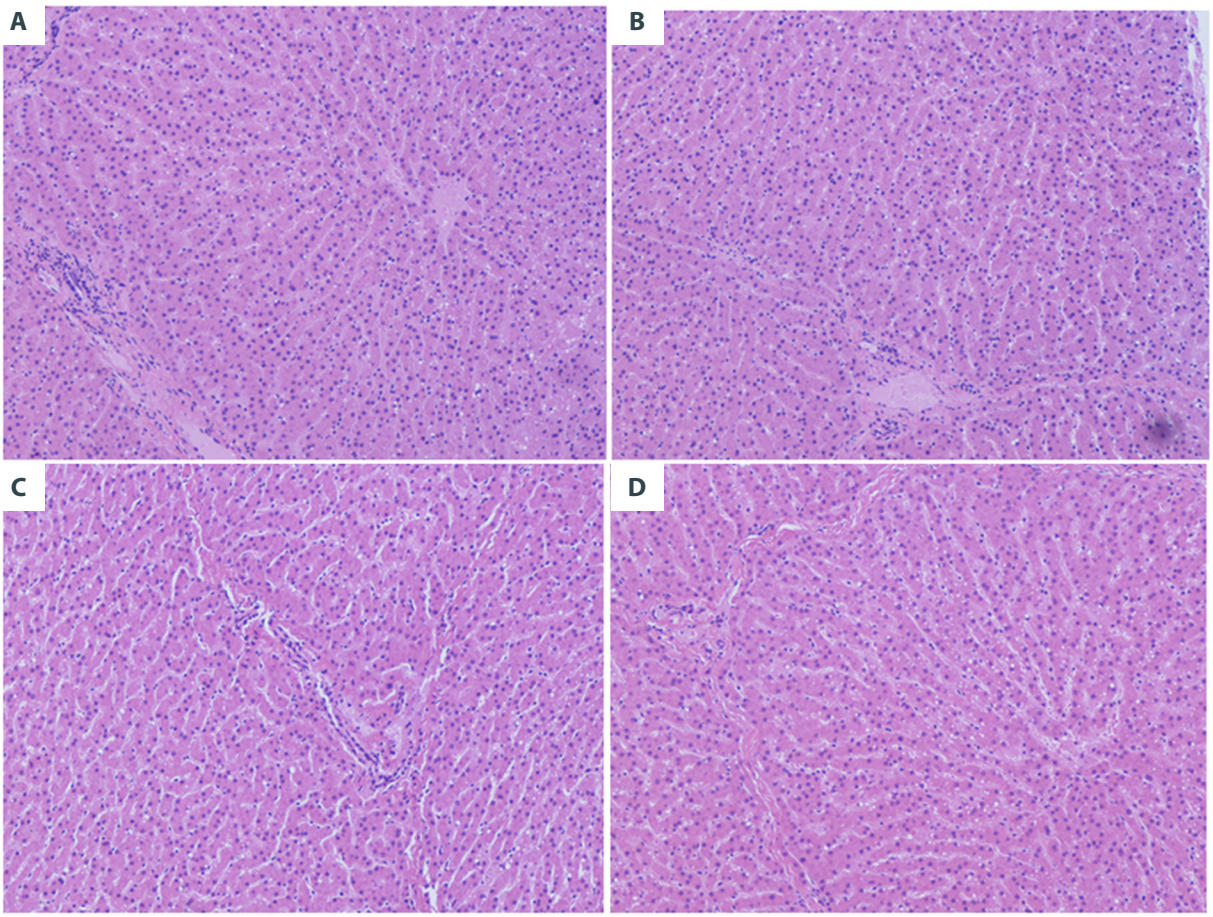

Figure 2. Representative donor liver tissue sections after hypoxia times of 0 (**A**), 15 (**B**), 30 (**C**), and 60 (**D**) min plus WIT 20 min (H&E staining with 200× magnification).

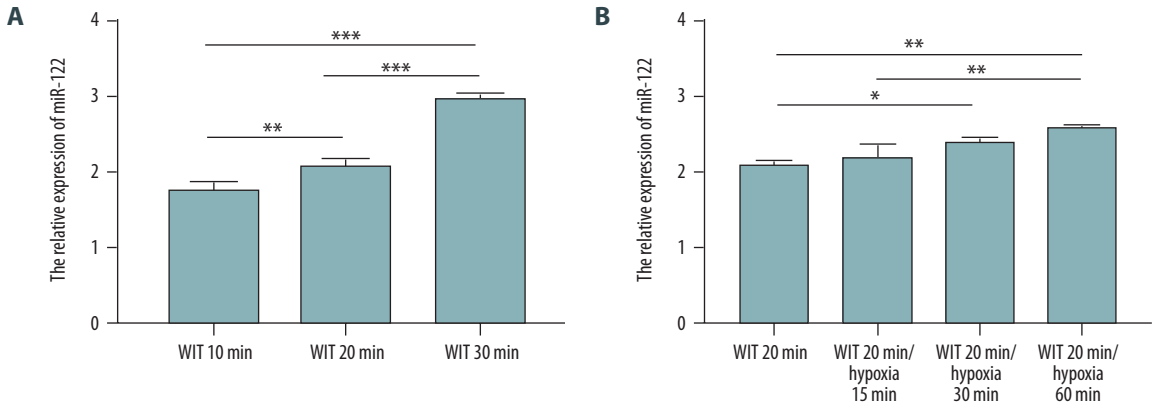

Figure 3. Relative miR-122 levels in donor livers *vs.* normal controls after warm ischemia injury alone (**A**) and after different durations of hypoxia plus WIT 20 min (**B**) (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

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Table 1. Serum ALT(U/L) changes in recipient pigs before and after implantation of DCD liver pretreated with different WIT (min) (n=3/each group).

	WIT 10 min	WIT 20 min	WIT 30 min
Pre-surgery	44.00±3.61	45.00±2.65	46.33±3.79
Post-surgery 1 h	40.67±0.58	64.33±8.33	577.33±64.57*,**,a
Post-surgery 3 h	89±8.66a,b	111.33±9.29a,b	623.21±89.51*,**,a
Post-surgery 6 h	86.67±7.51a,b	102±15.72*,a,b	All death
Post-surgery 12 h	105.00±1.73a,b,c,d	150.67±9.71*,a,b,c,d	All death
Post-surgery 24 h	145.00±6.93a,b,c,d,e	194.33±10.12*,a,b,c,d,e	All death

All superscript signs represented P<0.05 or less; * compared with WIT 10 min; ** compared with WIT 20 min; [a] compared with pre-surgery; [b] compared with post-surgery 1 h; [c] compared with post-surgery 3 h; [d] compared with post-surgery 6 h; [e] compared with post-surgery 12 h.

Table 2. Serum GGT (U/L)changes in recipient pigs before and after implantation of DCD liver pretreated with different WIT (min) (n=3/each group).

	WIT 10 min	WIT 20 min	WIT 30 min
Pre-surgery	40.05±1.53	41.33±2.31	41.12±1.03
Post-surgery 1 h	32.00±1.73	67.33±4.04*	133.33±16.99*,**,a
Post-surgery 3 h	84.00±6.93a,b	137.33±13.32*,a,b	243.12±22.31*,**,a,b
Post-surgery 6 h	56.33±2.89b,c	119.00±9.17*,a,b	All death
Post-surgery 12 h	38.67±16.74c	131.67±13.58*,a,b	All death
Post-surgery 24 h	68.67±1.15a,b,e	219.00±15.00*,a,b,c,d,e	All death

All superscript signs represented P<0.05 or less; * compared with WIT 10 min; ** compared with WIT 20 min; [a] compared with pre-surgery; [b] compared with post-surgery 1 h; [c] compared with post-surgery 3 h; [d] compared with post-surgery 6 h; [e] compared with post-surgery 12 h.

Table 3. Serum ALT (U/L) changes in recipient pigs before and after implantation of DCD liver pretreated with different hypoxia time plus WIT 20 min (n=3/each group).

	Hypoxia 15 min plus WIT 20 min	Hypoxia 30 min plus WIT 20 min	Hypoxia 60 min plus WIT 20 min
Pre-surgery	43.67±4.16	44.07±3.00	44.21±3.5
Post-surgery 1 h	68.66±1.53a	115.33±14.19*,a	307.00±13.11*,**,a
Post-surgery 3 h	121.00±7.55a,b	185.00±10.54*,a,b	544.33±10.79*,**,a,b
Post-surgery 6 h	140.33±7.77a,b	187.67±7.57a,b	844.33±64.01*,**,a,b,c
Post-surgery 12 h	176.00±7.94a,b,c,d	263.33±9.15*,a,b,c,d	799.00±53.81*,**,a,b,c
Post-surgery 24 h	232.00±10.44a,b,c,d,e	294.33±9.07*,a,b,c,d,e	All death

All superscript signs represented P<0.05 or less; * compared with WIT 10 min; ** compared with WIT 20 min; [a] compared with pre-surgery; [b] compared with post-surgery 1 h; [c] compared with post-surgery 3 h; [d] compared with post-surgery 6 h; [e] compared with post-surgery 12 h.

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Table 4. Serum GGT (U/L) changes in recipient pigs before and after implantation of DCD liver pretreated with different hypoxia time plus WIT 20 min (n=3/each group).

	Hypoxia 15 min plus WIT 20 min	Hypoxia 30 min plus WIT 20 min	Hypoxia 60 min plus WIT 20 min
Pre-surgery	40.67±1.15	39.67±3.21	39.00±3.61
Post-surgery 1 h	72.00±8.12a	111.03±2.06*,a	115.67±8.50*,a
Post-surgery 3 h	144.33±11.53a,b	238.33±12.50*,a,b	280.00±32.92*,a,b
Post-surgery 6 h	154.67±6.43a,b	219.33±10.21*,a,b	416.67±9.50*,**,a,b,c
Post-surgery 12 h	183.67±8.62a,b,c,d	285.00±11.07*,a,b,c,d	562.33±33.29*,**,a,b,c,d
Post-surgery 24 h	243.33±5.03a,b,c,d,e	336.33±17.90*,a,b,c,d,e	All death

All superscript signs represented P<0.05 or less; * compared with WIT 10 min; ** compared with WIT 20 min; [a] compared with pre-surgery; [b] compared with post-surgery 1 h; [c] compared with post-surgery 3 h; [d] compared with post-surgery 6 h; [e] compared with post-surgery 12 h.

Table 5. Serum TBil (mg/dL)changes in recipient pigs before and after implantation of DCD liver pretreated with different WIT (min) (n=3/each group).

	WIT 10 min	WIT 20 min	WIT 30 min
Pre-surgery	2.17±0.06	2.23±0.51	2.77±0.21
Post-surgery 1 h	2.47±0.40	3.30±0.35	8.53±4.64
Post-surgery 3 h	3.03±0.12	5.33±0.15a,b	12.16±5.51*
Post-surgery 6 h	1.50±0.52c	5.00±0.17*,a,b	All death
Post-surgery 12 h	1.40±0.69c	6.30±0.56*,a,b	All death
Post-surgery 24 h	2.97±0.76d,e	8.40±1.22*,a,b,c,d,e	All death

All superscript signs represented P<0.05 or less; * compared with WIT 10 min; ** compared with WIT 20 min; [a] compared with pre-surgery; [b] compared with post-surgery 1 h; [c] compared with post-surgery 3 h; [d] compared with post-surgery 6 h; [e] compared with post-surgery 12 h.

Table 6. Serum TBil (mg/dL) changes in recipient pigs before and after implantation of DCD liver pretreated with different hypoxia tine plus WIT 20 min (n=3/each group).

	Hypoxia 15 min plus WIT 20 min	Hypoxia 30 min plus WIT 20 min	Hypoxia 60 min plus WIT 20 min
Pre-surgery	2.11±0.10	2.25±0.15	2.40±0.15
Post-surgery 1 h	3.53±0.12a	4.27±0.12a	5.37±0.67*,**,a
Post-surgery 3 h	5.70±0.17a,b	7.67±0.50*,a,b	11.33±1.36*,**,a,b
Post-surgery 6 h	5.17±0.15a,b	7.40±0.53*,a,b	12.60±1.28*,**,a,b
Post-surgery 12 h	6.73±0.12a,b,c,d	9.80±0.20*,a,b,c,d	19.17±1.25*,**,a,b,c,d
Post-surgery 24 h	9.67±0.50a,b,c,d,e	11.47±0.55*,a,b,c,d,e	All death

All superscript signs represented P<0.05 or less; * compared with WIT 10 min; ** compared with WIT 20 min; [a] compared with pre-surgery; [b] compared with post-surgery 1 h; [c] compared with post-surgery 3 h; [d] compared with post-surgery 6 h; [e] compared with post-surgery 12 h.

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time-dependent manner when WITs reached 20 and 30 min, and under the conditions of hypoxia 15, 30, and 60 min plus WIT 20 min (Tables 5, 6).

Death occurred in the recipient minipigs as follows: 3 recipients implanted with livers pretreated with WIT 30 min died within 6 h after surgery, while 3 recipients implanted with livers pretreated with hypoxia 60 min plus WIT 20 min died within 24 h after surgery, suggesting that DCD donor livers are more sensitive to WIT 30 min than to hypoxia 60 min plus WIT 20 min. The remaining recipients died after 48 h following liver transplantation.

Discussion

In this study, we successfully established minipig DCD models to simulate the clinical process of warm ischemia and hypoxia in donor livers prior to transplantation. We found that a 1.73-fold increase in miR-122 levels in donor livers was already evident under the condition of WIT 10 min, suggesting that miR-122 levels gradually increased as the WIT was prolonged, which suggests that miR-122 plays a role in the evaluation of liver damage. Previous studies revealed that microRNA profiles in graft preservation solution could be an indicator of ischemic-type biliary lesions in human liver transplantation, with plasma miR-122 levels correlating well with liver injury [25,26]. A recent study showed that increased levels of miR-122 or a high miR-122/miR-222 ratio in graft preservation solution at the end of cold ischemia were predictive of early allograft dysfunction in a porcine DCD model [27]. The investigators also found that increased WIT led to higher levels of miR-122 in graft preservation solution. Our results showed that miR-122 levels were continuously increased with prolonged WIT, consistent with prior studies. More importantly, increased miR-122 levels could be measured immediately after warm ischemia and hypoxia treatment; however, morphology changes could not be detected at the same time in these liver tissues, suggesting the miR-122 level measurements could be an early indicator of the degree of ischemia and hypoxia in these donor livers. It is known that miR-122 is differentially expressed in cells of hematopoietic lineage [9]. Several liver enriched transcription factors, including HNF4α, HNF1A, HNF3A, and HNF3B, are implicated in regulating its transcription [28,29]. Moreover, peroxisome proliferator activated receptor-gamma (PPARγ) and retinoid X receptor alpha (RXRα) are also shown to activate the transcription of miR-122 [30]. Inflammation is reported to induce miR-122 expression and secretion, while Grainyhead-like transcription factor (GRHL2) is also implicated in regulating miR-122 expression [31]. Thus, there are multiple factors that can affect the level of miR-122 in the liver. Many studies have indicated that miR-122 expression abnormalities are associated with viral hepatitis, liver cirrhosis, and liver cancer, as well as acute rejection after transplantation. However, the exact mechanisms of increased miR-122 during WIT are still not clear and require further investigation. Ischemia and hypoxia can both cause liver injury, and our study found that the abnormal increase of miR-122 in the donor liver correlated with the length of ischemia and hypoxia, which provided a sensitive and timely evaluation of donor liver injury.

Circulatory death may lead to multiple complications before death, and hypoxia is one of them. Thus, we investigated the effect of hypoxia of various durations plus WIT 20 min on DCD livers. We found that there was no significant difference between pretreatment with WIT 20 min and with hypoxia 15 min plus WIT 20 min, suggesting that hypoxia for a short period of time has no obvious effect on donor liver injury. However, with prolonged tissue hypoxia, the levels of miR-122 increased; while at the same time, postoperative liver function recovery became more difficult to achieve. These findings suggest that prolonged tissue hypoxia can cause donor liver damage, although it may not be as severe as ischemia. Previous studies have demonstrated that warm ischemia and cold ischemia selectively injure different cell types in the liver; warm ischemia mainly causes hepatocyte damage, whereas cold ischemia primarily injures sinusoidal lining cells [32,33]. In fact, ischemia decreases the supply of oxygen and other nutrients and the metabolic waste eliminated.

Our results show that, after liver implantation, changes in serum ALT and GGT concentrations in recipients occurred earlier than changes in TBil. ALT and GGT levels were significantly increased at 3 h after implantation with DCD livers pretreated with WIT 10 min, and 1 h after implantation with DCD livers pretreated with WIT 20 and 30 min. Moreover, pretreatment with hypoxia plus WIT accelerated liver function damage, with ALT and GGT levels significantly increased 1 h after transplantation.

Notably, changes in serum TBil levels were not as sensitive as changes in serum ALT and GGT in recipients. When donor livers were pretreated with WIT 10 min alone, there were no changes in serum TBil levels in the recipients, even 24 h after liver transplantation. However, significant changes in serum TBil levels could be seen 1 h after liver transplantation in DCD donor livers pretreated with hypoxia plus WIT, indicating that TBil changes after more sensitive to hypoxia than WIT.

Deaths among recipients further demonstrated that DCD donor livers were more sensitive to damage from WIT 30 min than from hypoxia 60 min plus WIT 20 min.

Conclusions

In our minipig DCD liver transplantation models, increased levels of miR-122 appeared in the early stages of ischemia

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and hypoxia in donor livers. Short hypoxia time caused limited injury to donor livers; however, tissue hypoxia time greater than 20 min could damage the quality of the DCD donor liver, although it may not be as prominent as the effect of ischemia injury. Both warm ischemia and hypoxia can cause donor liver loss of function, as seen in the increased levels of ALT and GGT early, and TBil later.

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Conflicts of interest

None.

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