Cytochrome P450 2E1 Predicts Liver Retrieval from Donation After Circulatory Death Using Air-Ventilated Normothermic Machine Perfusion

Ji-Hua Shi (✉ jihuashi@zzu.edu.cn)

First Affiliated Hospital of Zhengzhou University

Dong-Jing Yang

First Affiliated Hospital of Zhengzhou University

Qiang Jin

Shanghai University of Traditional Chinese Medicine

Nuo Cheng

First Affiliated Hospital of Zhengzhou University

Yuan-Bin Shi

First Affiliated Hospital of Zhengzhou University

Yang Bai

First Affiliated Hospital of Zhengzhou University

Dong-Sheng Yu

First Affiliated Hospital of Zhengzhou University

Wen-Zhi Guo

First Affiliated Hospital of Zhengzhou University

Guang-Bo Ge

Shanghai University of Traditional Chinese Medicine

Shui-Jun Zhang

First Affiliated Hospital of Zhengzhou University

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Abstract

The optimal oxygen concentration is unclear for normothermic machine perfusion (NMP) of livers from donation after circulatory death donors (DCD). Our purposes were to investigate the effect of air-ventilated NMP on liver retrieval from DCD rats, and to analyze the underlying mechanism. Normothermic liver perfusion was performed using the NMP system with either air ventilation or oxygen ventilation for 2h in the rat liver following warm ischemia and cold ischemia preservation. Proteomics and metabolomics were used to reveal the significant molecular networks. The bioinformation analysis was validated by administering peroxisome proliferator activator receptor- γ (PPAR γ) antagonist and agonist via *ex vivo* perfusion circuit in the air-ventilated NMP. Results showed that air-ventilated NMP conferred a better functional retrieval and a less inflammatory response in the rat DCD liver; integrated proteomics and metabolomics analysis indicated that intrahepatic docosapentaenoic acid (DPA) downregulation and upregulation of cytochrome P450 2E1 (CYP2E1) expression and activity were associated with DCD liver retrieval with air-ventilated NMP; PPAR γ antagonist worsened liver function under air-oxygenated NMP whereas PPAR γ agonist played the opposite role. In conclusion, air-ventilated NMP confers a better liver retrieval from DCD rats through the DAP-PPAR γ -CYP2E1 axis; CYP2E1 activity provides a biomarker of liver retrieval from DCD.

1. Introduction

Normothermic machine perfusion (NMP) could deliver oxygen and recreate the physiological environment of oxygenation and cellular metabolism. This might minimize liver ischemia followed by reperfusion injury, help to estimate liver graft functionality and benefit liver retrieval before graft implantation, especially for organs from donation after circulatory death (DCD) ^{1,2}. Thus, NMP has theoretical advantages over static cold storage (SCS) in benefiting liver transplantation by utilization of DCD donors. Animal transplant models and clinical data have suggested that the use of NMP might improve organ utilization and posttransplant outcomes ³. The mechanisms underlying the benefits of NMP are not completely understood.

The most commonly used approach for applying NMP in clinics is the replacement of cold preservation prior to graft implantation. Experimental models have demonstrated that warm and cold liver ischemia followed by reperfusion injury, that is named ischemia-reperfusion injury, is characterized by oxidative tissue injury and activation of the inflammatory immune response either *in situ* at implantation or during NMP ^{4,5}. Initial dysfunction in mitochondria and endoplasmic reticulum from warm ischemia from DCD may induce a lower threshold of reperfusion injury in liver grafts compared with donors after brain death (DBD) ⁶. Our previous study demonstrated that endoplasmic reticulum stress is highly activated in liver retrieval from DCD rats, and that administration of acting transcript factor 6 agonist under NMP improves liver retrieval ⁷.

Constant oxygenation during machine perfusion is of special interest in the context of graft retrieval. Oxygen in the perfusate may help the fast recovery of mitochondrial function following liver cold

preservation. Meanwhile, the availability of oxygen, especially at high concentrations of oxygen may foster formation of reactive oxygen species (ROS). This is of even greater concern under re-oxygenation conditions where the perfusion ROS-mediated injury occurs. This ROS-mediated injury potentially restricts organ function after transplantation. Against this background, different concentrations of oxygen draw attention to yield the positive effects of active oxygenation while avoiding adverse effects⁸. Application of hypothermic machine perfusion (HMP) 100% oxygen for kidney grafts from DCD resulted in a better function compared to HMP with no oxygen or air⁹. So far, it remains unclear what oxygen concentration is optimal for NMP.

Through our pilot experiment based on the established NMP model in rats⁷, the arterial oxygen-ventilated NMP system was demonstrated to create an oxygen pressure of over 400mmHg in the artery ($\text{PaO}_2 > 400\text{mmHg}$), which agrees with the most reported NMP systems⁴, whereas arterial air-ventilated NMP could produce near-to-physiological oxygenation ($\text{PaO}_2 = 90\sim 200\text{ mmHg}$). Thus, our primary purpose in the current study was to compare the effect of air-ventilated NMP with oxygen-ventilated NMP on liver retrieval from DCD rats before implantation. Based on the findings, the further purpose was to investigate the mechanism of liver retrieval using an arterial air-ventilated NMP system on liver retrieval through an integrative proteomics and metabolomics analysis.

2. Methods

2.1 Animal and study design

The animal experiment was approved by the Animal Ethics Committee of Zhengzhou University, Zhengzhou, China (No.2019-KY-019). All methods were carried out in accordance with relevant guidelines and regulations along with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines statement. The Sprague-Dawley male rats weighing 320–350g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All rats were housed under specific pathogen-free conditions at room temperature with a 12/12 h light/dark cycle, and were allowed free access to chow and water before surgical procedures.

Based on the accepted concept that oxygenated NMP would benefit liver graft retrieval^{1,3}, we designed the experimental groups (Table 1) to compare the effects of near-to-physiological oxygenation (PaO_2 : 90~200 mmHg) and hyperoxygenation ($\text{PaO}_2 > 400\text{mmHg}$) of the perfusate on liver graft retrieval: ☐Group control (n = 6), healthy livers without ischemia; ☐Group hyperoxygenated NMP (n = 6), DCD livers exposed to 30 min of *in situ* warm ischemia and 8h cold ischemia preservation followed by 2h NMP with oxygen ventilation; and ☐Group air-oxygenated NMP (n = 6), DCD livers exposed to 30 min of *in situ* warm ischemia and 8 h cold ischemia preservation followed by 2 h NMP with air ventilation.

Table 1
Design of animal experiment (N=30)

Group		Harvested liver	Normothermic machine perfusion (NMP)	Perfusate
Control	N=6	healthy livers without ischemia	-	-
hyperoxygenated NMP	N=6	30 min of <i>in situ</i> warm ischemia followed by 8h cold ischemia preservation	2h NMP with oxygen ventilation	-
air-oxygenated NMP	N=6	30 min of <i>in situ</i> warm ischemia followed by 8h cold ischemia preservation	2h NMP with air ventilation	-
DMSO	N=4	30 min of <i>in situ</i> warm ischemia followed by 8h cold ischemia preservation	2h NMP with air ventilation	DMSO
Rosiglitazone	N=4	30 min of <i>in situ</i> warm ischemia followed by 8h cold ischemia preservation	2h NMP with air ventilation	Rosiglitazone
GW9662	N=4	30 min of <i>in situ</i> warm ischemia followed by 8h cold ischemia preservation	2h NMP with air ventilation	GW9662

In the next step, in order to evaluate the effect of the selected targets on liver retrieval from the above step, three more groups were designed: ①Group DMSO (n = 4), DCD livers exposed to 30 min of *in situ* warm ischemia and 8h cold ischemia preservation followed by 2h air-oxygenated NMP with DMSO in the perfusate; ②Group Rosiglitazone (n = 4), DCD livers exposed to 30 min of *in situ* warm ischemia and 8h cold ischemia preservation followed by 2h air-oxygenated NMP with the peroxisome proliferator activator receptor- γ (PPAR γ)-specific agonist Rosiglitazone in the perfusate; and ③Group GW9662 (n = 4), DCD livers exposed to 30 min of *in situ* warm ischemia and 8h cold ischemia preservation followed by 2 h air-oxygenated NMP with the PPAR γ -specific antagonist GW9662 in the perfusate (presented in Table 1). The selective PPAR- γ agonist Rosiglitazone or PPAR γ antagonist GW9662 was dissolved in perfusate with a final concentration of 10 μ g/mL and 0.3 mg/kg iv, respectively ^{10,11}.

2.2 Rat DCD model and *Ex vivo* liver NMP system

The rat DCD model and the *ex situ* liver perfusion system were modified from a previous study ⁴ and performed as published before ⁷. In brief, the DCD model was employed with induction of cardiac arrest due to incision of the diaphragm without prior heparinization in rats. After 30 min of warm ischemia, livers were flushed *in situ* with 0~4 °C heparin saline through both the hepatic artery and portal vein, harvested and then preserved in 0~4 °C UW solution for 8h before NMP.

The *ex situ* liver perfusion had perfusate comprised of rat red blood cells that recirculated through both hepatic artery and portal vein of liver grafts by means of a peristaltic pump (BT-100CA, Jieheng Pump

Ltd, Chongqing, China). The heat exchanger and bath thermostat maintained the temperature within the system at 38°C. Liver grafts in the organ perfusion chamber were perfused for 2 h with a constant portal vein perfusion pressure of 8 - 10 mmHg and hepatic artery pressure of 90 - 100 mmHg. The oxygenator in the circulation of the hepatic artery was regulated with mechanical ventilation (Harvard Inspira Advanced Safety Ventilator, Pressure Controlled MAI 55-7059, Holliston, USA) and gassed with air or 100% oxygen through a hollow fiber dialyzer (SpectrumLabs, Rancho Dominguez, CA, USA).

2.3 Sample collection and analysis

Perfusate samples for blood gas analysis were withdrawn from the cannula placed in the hepatic artery at the three-way stopcock and analyzed using an automatic blood gas analyzer (GEM Premier 4000, Instrumentation Laboratory Co., Lexington, USA). Perfusate samples were taken from the portal vein cannula for measurements of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using standard biochemical methods and tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) using an enzyme-linked immunosorbent assay (ELISA). The viability of the preserved livers was evaluated by changes in ALT (Δ ALT=postperfusion ALT level – preperfusion ALT level) and AST (Δ AST=postperfusion AST level – preperfusion AST level) in the perfusate.

Liver biopsies obtained from the perfused liver were immediately snap-frozen with liquid nitrogen and stored at – 80°C for the further detection of PPAR γ and cytochrome P450 2E1 (CYP2E1), fixed with 10% buffered formalin for further morphological evaluation by performing hematoxylin/eosin (HE) staining, and kept at 0~4°C for assessment of superoxide dismutase (SOD) activity, malondialdehyde (MDA) level and CYP2E1 activity.

2.4 Proteomics and metabolomics-based detection and its integrative analysis

The snap-frozen fresh liver tissues from control, hyperoxygenated NMP and air-oxygenated NMP groups were allocated for the simultaneous detection of mass spectrometry-based proteomics and metabolomics for detecting biomarker candidates.

Untargeted proteomics, protein identification and quantification by liquid chromatography-tandem mass spectrometry (LC-MS/MS) were performed as previously¹². In brief, the peptides were enriched by prewashed antibody beads (PTM Biolabs, Hangzhou, China), followed by analysis with tandem-mass-tag labeling combined with LC-MS/MS on the tissue samples. The resulting MS/MS data were processed using the engine software MaxQuant (version 1.5.2.22, Computational Systems Biochemistry, Martinsried, Germany) and uploaded to Proteomics identifications database (PRIDE) under the project accession number PXD028641. A differentially expressed protein was considered significant if $P < 0.05$ and FOLD >1.2.

Quantitative metabolomics was conducted on the XploreMET platform (Metabo-Profile Biotechnology, Shanghai, China) according to the previously published method¹³. Briefly, after the quality control, each specimen was introduced for GC-TOFMS analysis on a time-of-flight mass spectrometry (GC-TOF/MS)

system (Pegasus) with an Agilent 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). The raw data from GC-TOF/MS were processed with XploreMET 3.0, and each data set was converted to comparable data for further statistical analysis ($P < 0.05$ and FOLD > 1.5 as significant difference).

The ratio of two adjacent metabolites from a known metabolic relation network was calculated for the analysis of individual metabolic alterations. The Z-score heat map was applied for data visualization of the entire relationship. A multivariate regression model with the O2PLS method and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were applied to integrate proteomics data with metabolomics data. Integrative analysis and coregulated pathways by Spearman correlation analysis were constructed to elucidate the impact of oxygen concentration on DCD liver graft retrieval with NMP.

Cytoscape/MetScape-based analysis was used to visualize and interpret the interaction network between proteins and metabolites.

2.5 HE staining and morphological assessment of liver injury

Liver tissues were fixed in 4% paraformaldehyde in phosphate-buffered, embedded in paraffin wax, and stored at 4°C. HE staining was performed, and histological analysis was evaluated according to Suzuki histological criteria by the pathologist as report before ¹⁴.

2.6 Western blot analysis

Protein extracts of liver tissue were prepared and separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene difluoride (PVDF) membrane by electroblotting, and processed for Western blot analysis as previously described ¹⁴. Primary antibodies used were against PPAR γ (1:1000, 16643-1-AP, Proteintech, Wuhan, China), CYP2E1 (1:1000, 19937-1-AP, Proteintech, Wuhan, China), and GAPDH (1:5000, 60004-1-Ig, Proteintech, Wuhan, China). Immunoreactivities were visualized by secondary horseradish peroxidase-conjugated rabbit antibody (1:2000, SA00001-2, Proteintech, Wuhan, China), mouse antibody (1:2000, SA00001-1, Proteintech, Wuhan, China), and the ECL Western Blotting Substrate (Solarbio Life Sciences, Beijing, China) according to the manufacturer's instruction of KPL Protein Detector Western Blot Kit (SeraCare Life Sciences, Milford, USA).

2.7 Fluorescent probes for selectively sensing activity

Based on the ratiometric fluorescent probes for selectively sensing activity of CYP2E1, liver S9 fraction was prepared from the perfused livers to detect levels of the P450-catalyzed drug metabolizing enzyme activities ¹⁵.

2.8 Determination of SOD activity and MDA level

Detection of the SOD activity at 550 nm and quantification of MDA content were performed using a WST-8 assay kit and thiobarbituric acid assay according to the manufacturer's instructions (S0131 and S0103,

Beyotime Biotechnology, Haimen, China). Results were obtained using a Multiscan FC plate reader with SkanIt software (Thermo Fischer Scientific, Waltham, USA).

2.9 Enzyme-Linked Immunosorbent Assay

Inflammatory cytokine (TNF- α and IL-6) levels in the liver were measured according to the manufacturer's protocols (KE20001, Proteintech, Wuhan, China; EK0412, Boster Biological Technology, Wuhan, China).

2.10 Statistical analysis

Values are given as means with a standard deviation (SD). Differences between the two groups were analyzed by using two-tailed unpaired Student's test, and differences between more than two groups were analyzed by one-way ANOVA. The statistical tests were employed by using SPSS version 21.0 (IBM, Armonk, New York, USA). A probability level of less than 5 per cent ($P < 0.05$) was considered statistically significant.

3. Results

3.1 Air-oxygenated NMP confers a better functional retrieval and a less inflammatory response of rat DCD liver after static cold preservation

To compare the degree of liver retrieval following air-ventilated NMP and oxygen-ventilated NMP, aminotransferases in perfusate, HE staining, SOD activity, MDA level and inflammation parameters of the perfused liver were investigated.

Histological staining showed that microvesicular steatosis with hepatocyte ballooning, inflammatory cell infiltration, sinusoidal dilatation and congestion indexed with Suzuki score were significantly reduced following air-oxygenated NMP in comparison with that of hyperoxygenated NMP (0.750 ± 0.167 vs 1.583 ± 0.319) ($t = 6.061$, $P = 0.001$) (Figure 1A and 1B).

The function parameters, ALT and AST levels in the perfusate showed an increasing trend during NMP. The elevations of ALT and AST following air-ventilated NMP were significantly lower than those of the NMP group ($t = 4.925$ and 2.985 , $P = 0.008$ and 0.041) (Figure 1C).

SOD activity and MDA level of liver tissues were 4.8 ± 2.1 U/mg and 8.4 ± 2.6 μ mol/mg following oxygen-ventilated NMP, and 8.9 ± 2.2 U/mg and 2.7 ± 0.6 μ mol/mg following air-ventilated NMP, respectively, with a significant change ($t = 3.244$ and 5.1527 , $P = 0.009$ and 0.003) (Figure 1D). TNF- α and IL-6 in the liver following oxygen-ventilated NMP were 99.7 ± 12.1 pg/mg and 1217.3 ± 390.9 pg/mg, and TNF- α and IL-6 following air-ventilated NMP were 83.9 ± 8.5 pg/mg and 521.6 ± 221.2 pg/mg with significant changes ($t = 2.510$ and 3.835 , $P = 0.031$ and 0.003) (Figure 1E).

3.2 Integrative proteomics and metabolomics analysis reveals differentially expressed proteins and metabolites of the rat DCD liver between hyperoxygenated NMP and air-oxygenated NMP

The proteomics analysis identified 120 differential expressed proteins with 33 upregulated proteins and 87 downregulated proteins between hyperoxygenated NMP and air-oxygenated NMP (Figure 2A). The metabolomics analysis revealed that five differential expressed metabolites were identified (Figure 2A).

Based on the data of the proteomics and metabolomics platforms, KEGG pathway analysis found that three differentially expressed proteins were highly connected with the four identified metabolites. Among them, docosapentaenoic acid (DPA) and CYP2E1 were enriched in the pathway of biosynthesis of unsaturated fatty acids (Figure 2B). A heatmap with two-dimensional hierarchical clustering illustrated that DPA was correlated with the selected CYP2E1 protein (Figure 2C). The metabolite–protein interaction network provides a global view of potentially functional relationships between metabolites and connected proteins in Figure 2D.

3.3 Intrahepatic changes of DPA, PPAR γ , and CYP2E1 are associated with DCD liver retrieval with NMP

The role of DPA has been confirmed as a PPAR γ agonist in the previous study ¹⁶. In addition, PPAR γ agonists could reduce the expression and activity of CYP2E1 ¹⁷. In the current study, we detected the changes in DPA, PPAR γ and CYP2E1 as well as the function retrieval under air-ventilated NMP. In comparison with hyperoxygenated NMP, the quantitative metabolomics analysis showed that DPA expression in the air-oxygenated NMP was downregulated by gas chromatography-mass spectroscopy ($t = 3.776$, $P = 0.009$, Figure 3A), whereas the untargeted proteomics analysis showed that CYP2E1 expression was upregulated ($t = 2.839$, $P = 0.030$, Figure 3A). The expression of PPAR γ and CYP2E1 were evidenced by Western blot, and CYP2E1 expression was higher in the air-oxygenated NMP ($t = 2.613$, $P = 0.019$, Figure 3B and 3C).

The change in intrahepatic CYP2E1 activity was further detected by the fluorescent probe. The results showed that the CYP2E1 activity of air-oxygenated NMP with 66.438 ± 4.215 pmol/min/mg protein was higher than that of oxygen-oxygenated NMP with 52.479 ± 2.115 pmol/min/mg protein ($t = 2.960$, $P = 0.014$), and showed no significant difference with control with 69.180 ± 3.686 pmol/min/mg protein ($t = 0.479$, $P = 0.643$) (Figure 3D). The above mentioned results demonstrated that the restoration of expression and activity of CYP2E1, due to downregulation of DPA and PPAR γ ^{16,17}, was correlated with liver function, and therefore CYP2E1 might be indicated as a biomarker of DCD liver retrieval during NMP.

3.4 Targeting PPAR γ might affect liver function during DCD liver retrieval with air-oxygenated NMP

Our findings demonstrated that DPA and PPAR γ might participate in the liver retrieval with NMP. PPAR γ agonists have been shown to protect against liver injury^{18,19}. Thus, during DCD liver retrieval with NMP, targeting PPAR γ may theoretically improve liver function.

To test the function of PPAR- γ during DCD liver retrieval, administration of PPAR- γ agonist and antagonist in the *ex vivo* perfusion circuit was employed in the air-oxygenated NMP system. In comparison with administration of DMSO and PPAR- γ agonist Rosiglitazone in the *ex vivo* perfusion circuit, administration of PPAR- γ antagonist GW9962 worsened liver injury and inhibited retrieval of DCD liver under NMP, as indicated by HE staining and Suzuki criteria score ($F = 8.714$, $P = 0.017$, Figure 4A and 4B), ALT ($F = 22.896$, $P = 0.002$, Figure 4C) and AST in the perfuse ($F = 5.431$, $P = 0.004$, Figure 4C), MDA level and SOD activity ($F = 5.990$ and 6.894 , $P = 0.018$ and 0.002 , Figure 4D) and TNF α and IL-6 levels by ELISA ($F = 6.426$ and 12.602 , $P = 0.018$ and 0.002 , Figure 4E). The above results demonstrate that PPAR γ agonist via the NMP perfusion circuit benefits DCD liver retrieval.

4. Discussion

In some experienced transplant centers, static cold preservation and NMP with subsequent transplantation are becoming the valid practice for DCD grafts²⁰. NMP could simulate the physiological environment of cellular metabolism for liver retrieval through oxygen delivery, and simultaneously NMP may initiate mitochondrial oxygenation, and trigger the cascade of inflammation and injury^{4,5}. It remains unclear whether there is a consensus on the NMP protocol for graft retrieval from the DCD liver.

After we compared the effect of NMP with air ventilation (PaO₂ in the perfusate= 90~200 mmHg) and oxygen ventilation (PaO₂>400 mmHg) on liver retrieval in a rat DCD model, the results demonstrated that air-ventilated NMP was associated with a superior liver morphological change, a better functional retrieval and a less inflammatory response of the rat DCD liver with static cold preservation. Based on the findings, the integrative proteomics and metabolomics analysis was further performed to investigate the mechanism of air-ventilated NMP on DCD liver retrieval with the rat NMP model⁷. The integrative proteomics and metabolomics analysis revealed that the downexpressed DPA and upregulated CYP2E1 were functionally correlated with liver retrieval with air-ventilated NMP.

The function of the metabolite DPA has been confirmed as a PPAR γ agonist in the previous study¹⁶. In addition, CYP2E1 has been shown to be transcriptionally and functionally linked to PPAR γ and liver function, and PPAR γ agonist can reduce CYP2E1 expression and activity¹⁷, potentially protecting in liver injury^{18,19}. Thus, air-ventilated NMP benefits DCD liver retrieval, which might be mediated through the DPA-PPAR γ axis. Furthermore, in our present study, PPAR γ agonist Rosiglitazone improved liver function in the *ex vivo* liver NMP system whereas GW9962 functioned the opposite, indicating that PPAR γ could be regarded as a therapeutic target against liver injury during liver retrieval from DCD.

To assess the graft viability of perfused livers for transplantation, a couple of perfusate parameters and bile parameters before implantation have been developed²⁰. Expression and activity of liver cytochrome

P450 isozymes were significantly changed after ischemia/reperfusion injury, indicating their use as biomarkers of function retrieval during liver injury ^{21,22}. CYP2E1 is part of the cytochrome P450 family of drug-metabolizing enzymes in the liver. The CYP2E1 protein level was positively associated with high inflammation scores through ROS-induced lipid peroxidation ^{23,24}. Notably, CYP2E1 activities and concentration were gradually decreased during cold preservation and reperfusion ²⁵. Our research group constructed the ratiometric fluorescent probes for selectively sensing the activity of cytochrome P450, including CYP2E1, 1A2, 2J2, 3A4 and 3A5 ^{15,26,27}. In the present study, DCD liver retrieval with air-ventilated NMP was found to be correlated with the upregulated expression and activity of CYP2E1. Thus, intrahepatic expression and activity of CYP2E1 would be indicated as a biomarker to monitor DCD liver graft function during NMP. In addition, the fluorescence probe is an easy and feasible method to evaluate the intrahepatic CYP2E1 activity and DCD liver retrieval.

There are still some limitations to the study should be kept in mind. Based on the previous animal study and clinical practices, NMP was select to 120 min, possibly reflecting the liver retrieval to some extent effects ²⁸. A couple of biological parameters, including lactate clearance and bile production, are suggested to access the graft viability in the clinic. With the limitation of the detection of the current study in rats, assessment of clinically known parameters ^{20,29}, especially bile parameters, cannot be drawn and compared from these data. Additionally, results from the *ex vivo* reperfusion model could represent preliminary data and still require confirmation in appropriate transplantation models in future studies.

5. Conclusions

The results of the present study showed that air-ventilated NMP can simulate a near-to-physical oxygen pressure through artery perfusion, and thus confer better liver retrieval from DCD rats following ischemia-reperfusion injury. The integrative proteomics and metabolomics analysis and the further functional tests confirmed that the DAP (PPAR γ agonist)-PPAR γ -CYP2E1 axis might provide promising biomarkers and therapeutic targets, and thus predict and improve liver graft quality from DCD donors.

Abbreviations

CYP2E1, cytochrome P450 2E1;

DAP, docosapentaenoic acid;

DBD, donors after brain death;

DCD, donation after cardiac death;

HE, hematoxylin/eosin;

HMP, hypothermic machine perfusion;

IHC, immunohistochemistry;

MDA, malondialdehyde;

NMP, normothermic machine perfusion;

PPAR γ , peroxisome proliferator activator receptor- γ ;

SCS, static cold storage;

SOD, superoxide dismutase

Declarations

Author Contributions

Authors J.-H. S., D.-J. Y. and Q. J. contributed equally to this article.

J.-H. S., D.-J. Y., N. C., and Q. J. have full access to all the data in the study and take responsibility for the data analysis. S.-J. Z., G.-B. G., W.-Z. G. and J.-H. S. designed and supervised the study. J.-H. S., D.-J. Y., N. C., Y.-B. S., Y. B., and D.-S. Y. performed the experiments and analyzed the data. S.-J. Z. and J.-H. S. obtained the funding. J.-H. S. prepared the figs and wrote the manuscript. S.-J. Z., G.-B. G., W.-Z. G. and J.-H. S. provide administrative, technical, or material support.

All authors completed critical revision of the manuscript for important intellectual content.

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Ethics approval

The animal experiment was approved and supervised by the Animal Ethics Committee of Zhengzhou University, Zhengzhou, China (No.2019-KY-019)

Competing interest and consent for publication

The authors declare that there is no conflict of interest regarding the authorship and publication of this paper. All the authors have no interest with any business company.

Data Availability Statement

The data will be available on reasonable request.

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Figures

Figure 1

Air-oxygenated normothermic machine perfusion (NMP) confers a better functional retrieval in rat liver from donation after cardiac death († denotes significant differences between air-oxygenated NMP and hyperoxygenated NMP, $P < 0.05$; $n = 6$) A. Histology (hematoxylin and eosin staining) of the perfused livers (hematoxylin counterstaining, original magnification $\times 200$, and scale bars $50\mu\text{m}$); B. Suzuki sinusoidal injury score of the perfused livers on a scale from 0 to 4; C. Changes in levels of ALT and AST in the NMP

perfusate; D. Relative changes in SOD activity and MDA level in the perfused livers; E. Relative levels of inflammation factors, TNFα and IL-6, in the liver performed using ELISA.

Figure 2

The integrative analysis of proteomics and metabolomics-based detection in rat livers from donation after cardiac death following static cold storage and normothermic machine perfusion (NMP) A. The volcano plot represents the results of the proteomics (left) and metabolomics (right) analysis between air-oxygenated and hyperoxygenated NMP. Red spots represent the upregulated proteins/metabolites; blue spots represent the downregulated proteins/metabolites; and grey spots represent the unchanged proteins/metabolites. The differentially expressed protein (CYP2E1) and metabolite (DPA) were identified

and encircled. B. The KEGG enrichment bubble chart shows the differentially expressed proteins (triangle) and metabolites (bubble) in the corresponding KEGG pathway. The color represents the degree of statistical significance. The size represents the number of the differentially changed proteins/metabolites in the corresponding pathways. The encircled pathways of biosynthesis of unsaturated fatty acids were identified to relate to CYP2E1 and DPA. C. Heatmap of Spearman correlation analysis of significant proteins (y-axis) and metabolites (x-axis). Each color cell on the map corresponds to a Spearman correlation coefficient between the identified proteins (y-axis) and metabolites (x-axis). Red denotes positive correlation, whereas blue denotes negative correlation, and the dark color denotes the value of the Spearman correlation coefficient. The encircled pathway was shown as negatively correlated to relate with P05182 (CYP2E1) and Met183 (DPA). D. The protein-metabolite interaction network provides a visualization of the interaction between functionally related protein (CYP2E1) and metabolite (DPA) identified from proteomics and metabolomics.

Figure 3

Expression of DPA, PPAR γ and CYP2E1 in livers from donation after cardiac death using normothermic machine perfusion (NMP) († denotes significant differences between hyperoxygenated NMP and air-

oxygenated NMP, $P < 0.05$, $n = 6$) A. Relative expression of DPA by metabolomics and CYP2E1 by proteomics; B. Expressions of PPAR γ and CYP2E1 by WB; C. Semiquantification of CYP2E1 by WB. D. CYP2E1 activity quantified using the fluorescent probe

Figure 4

Effect of PPAR γ on functions of livers from donation of cardiac death under air-oxygenated normothermic machine perfusion (NMP) (* denotes significant differences among DMSO, Rosiglitazone and GW6471, $P < 0.05$, $n = 4$) A. Histology (haematoxylin and eosin staining, original magnification $\times 200$, and scale bars $50\mu\text{m}$); B. Suzuki sinusoidal injury scores of the perfused livers on a scale from 0 to 4; C. Changes in levels of ALT and AST in NMP perfusate; D. Relative changes in MDA level and SOD activity in the perfused livers; E. Relative levels of TNF α and IL-6 in the perfusate using ELISA.

Supplementary Files

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