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Original Article

Optimized rat models of donation after cardiac death for bench to bed translation

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Abstract: Objective: The number of livers transplanted from donation after cardiac death (DCD) donors has significantly increased in recent years. However, there is still controversy regarding the optimal DCD model to mimic the clinical situation. Here, three different DCD models were compared, with the aim of determining the optimal DCD model. Methods: Three groups of rats were prepared as follows: apical clipping (AC, n=6); potassium chloride injection (PCI, n=6); diaphragmatic cutting (DC, n=6). All animals underwent carotid artery catheterization to monitor blood pressure. Electrocardiography (ECG) was performed to determine the death of animals. After cardiac death, serum was collected to measure liver function [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)], and liver tissue was preserved by fixing in 10% neutral formalin for subsequent histological study (histopathology). Fresh liver samples were collected and stored at -80°C for further analysis [malondialdehyde (MDA) and myeloperoxidase (MPO)]. Results: ECG and blood pressure measurement showed that the time to cardiac arrest induction with AC was longer than that with DC and PCI, accompanied by higher ALT, AST, and LDH levels and severe congestion. DC resulted in a longer time to cardiac arrest induction compared with PCI, and the same level of liver injury shown by ALT, AST, and LDH levels and HE staining. No significant differences in MDA and MPO levels were observed between the three groups. Conclusion: PCI and DC results in less liver injury than AC, which corresponds to the non-heart-beating donation classification of Maastricht III and V, respectively. DC is more suitable for bench to bed translation research in terms of clinical practice.

Keywords: Donation after cardiac death, liver transplantation, animal model, bench to bed

Introduction

"Non-heart-beating donor" currently has the international term "donors after circulatory death" (DCD) and is an alternative source of donor organs. The number of livers transplanted from DCD has significantly increased in recent years, and represents almost 50% of all deceased donation [1]. However, DCD is associated with a high incidence of primary non-function or dysfunction, especially biliary complications [2-4].

Growing experience with DCD donors has resulted in researchers returning to basic scientific research to improve donor selection criteria and minimize ischemia reperfusion injury (IRI) [5]. Several DCD models were previously established for scientific research, however, there is still controversy regarding the optimal DCD model to mimic the clinical situation, which can further affect the validation of experimental data. Here, three of the most common DCD models (apical clipping, potassium chloride injection, diaphragmatic cutting) were compared, with the aim of identifying the ideal DCD model for bench to bed translation.

Materials and methods

Models and groups

Male Sprague Dawley rats weighing 250 to 350 g were anesthetized by 4% chloral hydra-

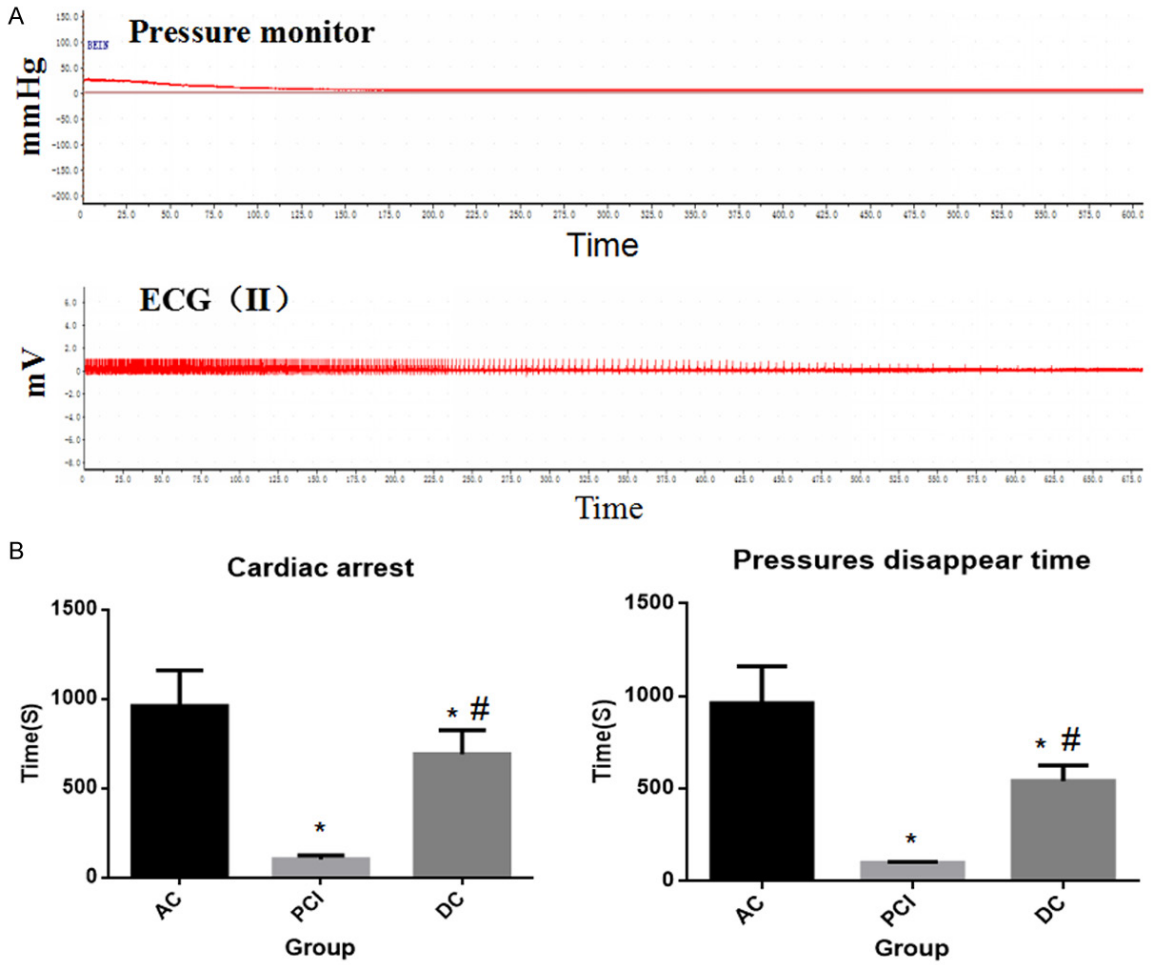

Figure 1. A. Examples of electrocardiography and pressure monitor; B. Statistical results for three groups. *Represents $P<0.05$ compared to group AC; while # represents $P<0.05$ compared to group PCI.

te anesthesia (Shanghai No. 1 Biochemical & Pharmaceutical, Shanghai, China). Systemic heparinization (50 U per animal) was accomplished by injection via the superficial dorsal vein of the penis. Cardiac arrest was induced by the three methods as follows: Group 1: Apical clipping group (AC, n=6, a vessel clamp was used for apical clipping after laparotomy until cardiac arrest). Group 2: Potassium chloride injection group (PCI, n=6, cardiac arrest was induced by intravenous injection of 10% potassium chloride solution (2 mmol/kg). Group 3: Diaphragmatic cutting group (DC, n=6, an incision was made in the diaphragm to induce hypotension and hypoxia, and finally cardiac arrest). Following induction of cardiac arrest, the liver was immediately harvested as described previously [6] and perfused with histidine-tryptophan-ketoglutarate solution (HTK). All procedures in this study were approved by the Ethics Committee for the Use of Experimental Animals in Zhejiang University.

Determination of cardiac arrest and warm ischemia time

All animals underwent carotid artery catheterization to monitor blood pressure. Electrocardiography (ECG) was performed to determine cardiac arrest.

Sample collection

After perfusion of the liver with HTK, blood from the portal vein was collected to measure liver function (ALT, AST and LDH). In addition, liver tissue was preserved by fixing in 10% neutral formalin for subsequent histopathology. Fresh liver samples were collected and stored in -80°C for further analysis (MDA, MPO).

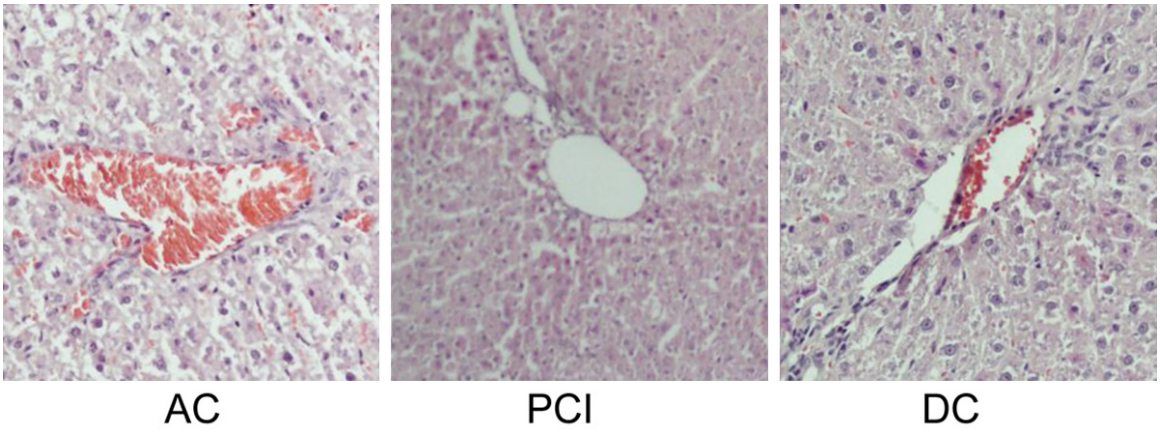

Figure 2. HE staining in the three groups.

Histopathologic examination

Liver tissue was fixed at 4°C for 24 h before deparaffinization and hydration, then examined with hematoxylin and eosin staining using our standard protocol [7].

Liver function

Blood samples were centrifuged at 3,000× g for 10 min at 4°C to collect sera for liver function tests using a Hitachi 7600 automatic analyzer (Hitachi, Tokyo, Japan).

Levels of lipid peroxidation and myeloperoxidase activity

Malondialdehyde (MDA), which reflects the level of lipid peroxidation in liver tissue, was determined using an MDA assay kit following the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The activity of myeloperoxidase (MPO), which is a marker of polymorphonuclear neutrophil (PMN) infiltration, was measured using a MPO assay kit (Nanjing Jiancheng Bioengineering Institute) in accordance with the instructions. All assays were detected using a Beckman Coulter DU-800 Spectrophotometer (USA).

Results

Period of cardiac arrest

DCD requires the confirmation of cardiac death prior to organ procurement and during this process the graft is subject to warm ischemia. ECG is widely used to confirm cardiac death and a pressure monitor is used to measure the time to pressure disappearance, and can be used to calculate warm ischemia time. ECG showed that time to cardiac death induction in the AC group was longest among the three groups (vs DC, P=0.021; vs PCI, P<0.001), while time to cardiac arrest induction was longer in the DC group than that in the PCI group (P<0.001). The pressure monitor showed similar trends: time to pressure disappearance in the AC group was longest among the three groups (vs DC, P= 0.039; vs PCI, P<0.001), while time to pressure disappearance in the DC group was longer than that in the PCI group (P=0.003) (**Figure 1**).

HE staining

Graft IRI was determined by HE staining according to histopathological assessment based on the degree of sinusoidal congestion and necrosis. The HE results were in line with the time to cardiac death induction and time to pressure disappearance. The AC group showed severe congestion compared with the DC and PCI group. However, there was no obvious necrosis in the three groups (**Figure 2**).

Liver function

In terms of liver function, higher ALT, AST, and LDH levels and a higher glucose level were found in the AC group. The ALT level in the AC group was highest among the three groups (vs DC, P=0.011; vs PCI, P=0.008), while the ALT level was higher in the DC group than that in the PCI group (P=0.015). The AST level in the AC group was highest among the three groups (vs DC, P=0.045; vs PCI, P=0.11), while no statisti-

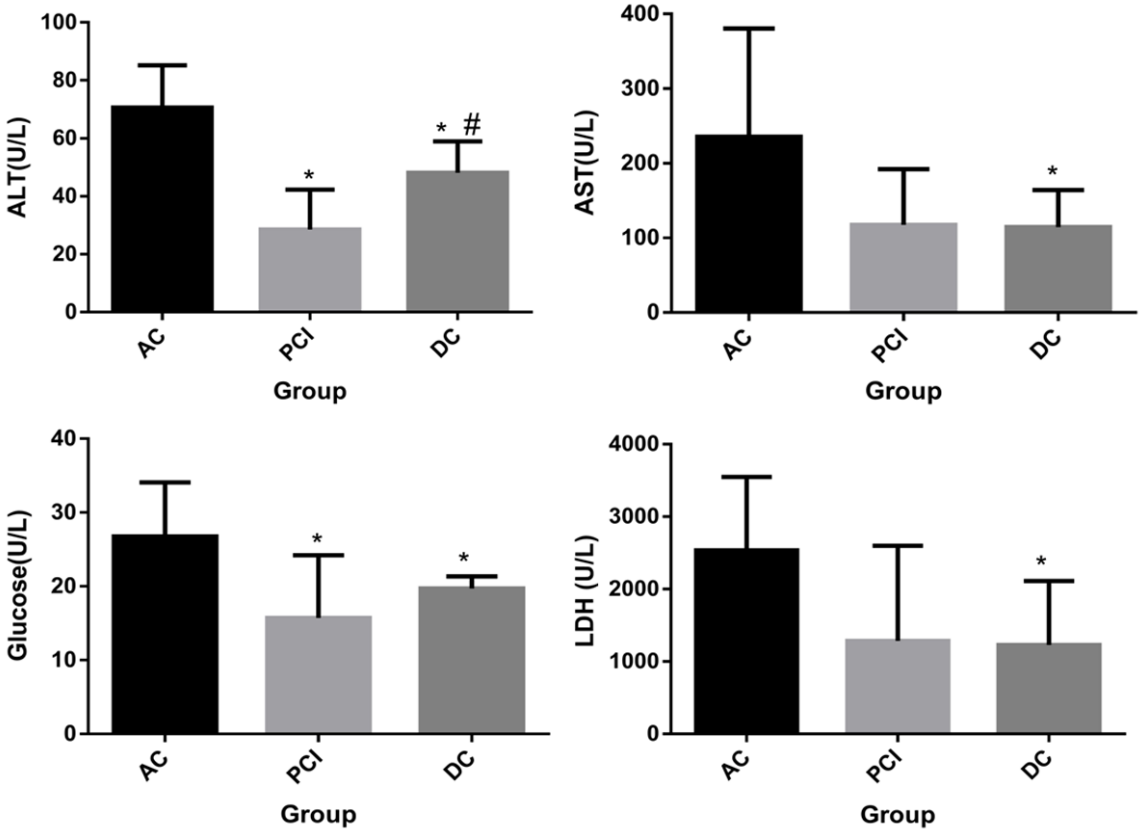

Figure 3. Liver function in the three groups. *Represents $P<0.05$ compared to group AC; while # represents $P<0.05$ compared to group PCI.

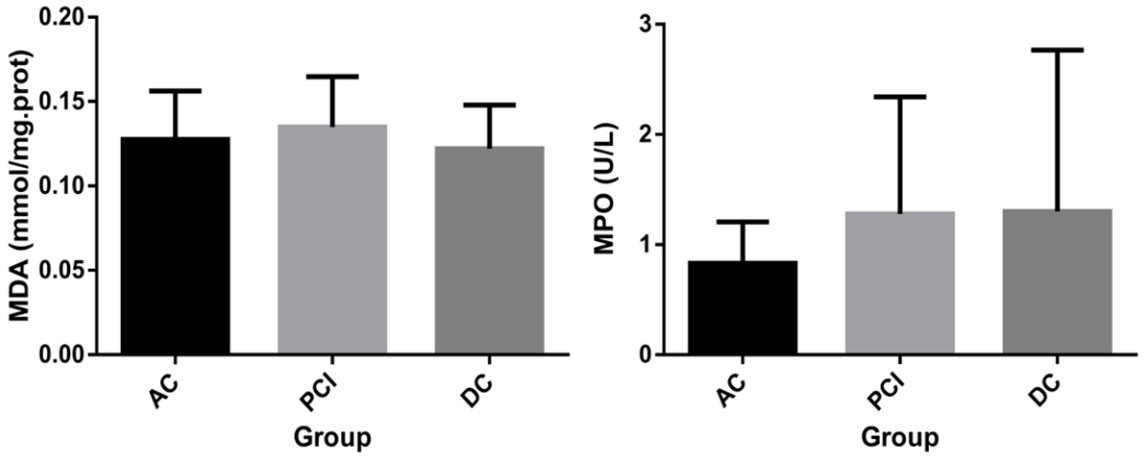

Figure 4. Malondialdehyde and myeloperoxidase levels in the three groups.

cally significant difference between the DC and PCI group was observed. Glucose level in the AC group was highest among the three groups (vs DC, $P=0.032$; vs PCI, $P=0.049$), while no statistically significant difference between the DC and PCI group was observed. LDH level in the AC group was highest among the three groups (vs DC, $P=0.038$), while no statistically significant difference between the DC and PCI group was observed (**Figure 3**).

Level of MDA and MPO

MDA is considered an indirect measurement of oxidative damage induced by reactive oxygen species (ROS), while MPO is a marker of polymorphonuclear neutrophil (PMN) infiltration. No significant differences in MDA and MPO levels were observed among the three groups (**Figure 4**), which showed that ROS and PMN infiltration levels were comparable in the three groups.

Discussion

DCD has been proposed as a means of improving the shortage of organs for transplantation worldwide, However, grafts from DCD are associated with inferior clinical outcome and a high discard rate due to poor graft quality [8]. Accurate judgment and improvement in DCD graft quality are essential to maximize recipient benefit and make full use of scarce liver grafts, and to extend the donor pool, which requires further basic scientific and translation research based on ideal animal DCD models which can simulate clinical practice.

Clinically, DCD was categorized by the Maastricht classification in 1995 and modified in 2014 [9] as follows: Category I: the patient is declared "dead on arrival"; Category II: "unsuccessful resuscitation" occurred out or in hospital; Category III: "awaiting cardiac arrest" to declare death, which is the most common situation; Category IV: cardiac arrest during brain death; Category V: "euthanasia" or patients who undergo medically assisted circulatory death. Categories I and II are defined as uncontrolled while categories III, IV, and V are controlled.

According to this classification, the three most frequently used animal DCD models (AC, PCI and DC) were compared in the present study. These three DCD models all belong to the controlled categories, of which DC mimics category III and PCI mimics category V. AC can induce cardiac arrest away from the clinical situation, and external compression of the heart (exogenous tamponade) is seldom experienced in potential donors. According to the results of this study, liver injury in the AC group was severe in terms of AST, ALT, and LDH, and histopathology which secondary to the long warm ischemia time, mainly due to the longest time to cardiac arrest induction and time to pressure disappearance. The evidence shows that prolonged warm ischemia can generate more ROS, induction of apoptosis, and stimulation of the innate and adaptive immune systems, and then induce delayed graft function after surgery, which in turn is associated with poor long-term graft and patient survival [5]. In this respect, AC is not an ideal DCD model for bench to bed translation.

When comparing DC with PCI, although DC results in severe liver injury in terms of liver function and histopathology due to longer time to cardiac arrest induction and longer time to pressure disappearance, it meets the criteria for most cases in clinical practice. The DC model is similar to respirator withdrawal in the clinical setting of Maastricht III donors, while the PCI model is similar to "euthanasia" in the clinical setting of Maastricht V donors. Thus, these two models should be chosen according to actual demand. Considering the clinical reality that "euthanasia" is not popular worldwide, DC may be the more appropriate model in a translational study due to the use of category III DCD donors.

Conclusion

In summary, PCI and DC, which result in reduced liver injury compared with AC, correspond to Maastricht III and V, respectively. In clinical practice, the DC model is more suitable for most cases of bench to bed translation research.

Acknowledgements

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Disclosure of conflict of interest

None.

Authors' contribution

(1) Conceived and designed the study (Shusen Zheng, Lin Zhou, Haiyang Xie); collected the data (Yanfei Zhou, Li Jiang, Meiying Tian,); analyzed and interpreted the data (Jianhui Li, Junjun Jia, Jing Zhang); (2) wrote the manuscript or provided critical revisions important for intellectual content (Jianhui Li, Junjun Jia); and (3) approved the final version of the manuscript (Shusen Zheng, Lin Zhou).

Abbreviations

DCD, Donation after cardiac death; AC, Apical clipping; PCI, Potassium chloride injection; DC, Diaphragmatic cutting; ECG, Electrocardiography; IRI, Ischemia reperfusion injury; HTK, Histidine-tryptophan-ketoglutarate solution; MDA, Malondialdehyde; MPO, Myeloperoxidase; PMN, Polymorphonuclear neutrophil.

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