Non heart beating organ donor. New experimental model in pigs[1]

Doador de coração parado. Novo modelo experimental em suínos

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ABSTRACT

PURPOSE: To describe technical aspects of a new experimental model that simulates a non heart beating organ donor.

METHODS: Landrace pigs were operated on and cardiac arrest was obtained by means of myocardial infarction and interruption of ventilator support.

RESULTS: Mean cardiac frequency, systolic and diastolic blood pressure levels, central venous pressure, oxygen saturation and concentration of expired CO_2 dropout occurred at seven minutes after cardiac arrest.

CONCLUSION: The procedure was easily reproduced and a homogeneous circulatory failure could de obtained by the end of seven minutes. The model is suitable for further studies regarding abdominal organ transplantation.

Key words: Transplantation. Models, Animal. Experimental Development. Heart Arrest. Swine.

RESUMO

OBJETIVO: Descrever os aspectos técnicos de um novo modelo experimental que simula um doador de órgãos após a parada cardíaca.

MÉTODOS: Suínos da raça Landrace foram operados e a parada cardíaca foi obtida por meio de infarto do miocárdio e interrupção do suporte ventilatório.

RESULTADOS: Freqüência cardíaca, pressão arterial sistólica e diastólica, pressão venosa central, saturação de oxigênio e concentração parcial de CO_2 são consistentes com falência hemodinâmica ao final de sete minutos.

CONCLUSÕES: O procedimento foi facilmente executado e uma falência circulatória pode ser obtida ao final de sete minutos. Este modelo é adequado para estudos posteriores com respeito a preservação e tranplantes de órgãos abdominais.

Descritores: Transplante. Modelos Animais. Desenvolvimento Experimental. Parada Cardíaca. Suínos.

Introduction

Liver transplantation is the treatment of choice for terminal liver failure. The most important issue regarding this modality is the gap between an insufficient pool of organ donors and the increasing number of potential recipients[1,3].

Several alternatives are considered to attenuate this discrepancy, including the use of marginal donors. Therefore, organs from non heart beating donors (NHBD) can be suitable for transplantation, regardless of a worse first year graft survival outcome[1,2,4,9]. Contemporary aspects of organ donation after cardiac death have stimulated the development of experimental models aiming the improvement of preservation and organ dysfunction[2,6,7].

There are several methods of simulating a NHBD condition. Interestingly, there is not a standard technique available and the means of promoting the cardiac arrest may contribute to worsen organ preservation[2-4,6]. Thus, new experimental models of non heart beating donating will be useful to control research bias and allow further studies on organ transplantation.

This paper aims the description of a new experimental model of donation after cardiac death.

Methods

The sample consisted of twelve male Landrace pigs, weighing between 30 and 50kg, provided by the Poultry Industry (EVZ/UFG). The animals were kept under standard laboratory conditions and fasted for 24 hours before surgery.

This study was approved by the Ethics Committee in Research of the Clinics Hospital Faculty of Medicine Federal University of the Goias (Protocol 042/2011). All procedures were performed at the Laboratory of Experimental Surgery and Anesthesiology, School of Veterinary and Animal Science, Federal University of Goias (EVZ/UFG), according to international principles of animal research and in compliance with Federal Law Nº. 11.794, of October 8, 2008 and Decree No. 6689 of July 15, 2009 which regulates Law 11794 governing the use of animal experimentation in Brazil. All surgeries were supervised by a veterinarian.

Procedures

Before general anesthesia, all animals received intramuscular azaperone (2 $mg.kg^{-1}$). After 10 minutes, animal received intramuscular ketamine (10 $mg.kg^{-1}$), midazolam (0.5 $mg.kg^{-1}$) and meperidine (4 $mg.kg^{-1}$). The marginal ear vein was catheterized and anesthesia was induced with iv propofol (5-10 $mg.kg^{-1}$). The internal carotid artery and both jugular veins were dissected and isolated. Lines were placed for for blood pressure measurement, blood sampling and fluids administration (Figure 1).

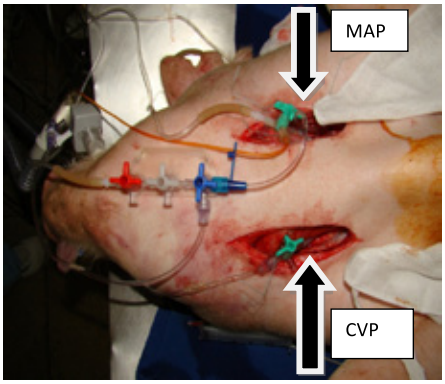

FIGURE 1 - Instrumentation of swine by dissection and catheterization of the jugular veins and both internal and external carotid arteries. **MAP** - Mean Arterial Pressure; **CVP** - Central Venous Pressure.

General anesthesia was maintained with isoflurane and continuous infusion of fentanyl (10 $\mu g.Kg^{-1}$) and atracurium (0.4 $mg.kg^{-1}$/ hour). We performed intermittent positive pressure ventilation to maintain normocapnia (35-45 mmHg).

Surgical protocol

The surgical approach was performed by wide median thoracoabdominal incision (Figure 2). Heparin (500 IU-kg) was administered in bolus five minutes before cardioplegia. Cardiac arrest was obtained by *en block* suture of both coronary arteries and interruption of ventilatory support (Figures 3 A and B). Absence of arterial pulse wave and the presence of either fine ventricular fibrillation or isoelectric line were the criteria to consider the animal as a donor after cardiac death. At this point, euthanasia was obtained by means of exsanguination under general anesthesia. The carcasses were properly incinerated, at the Department of Pathology (EVZ/UFG).

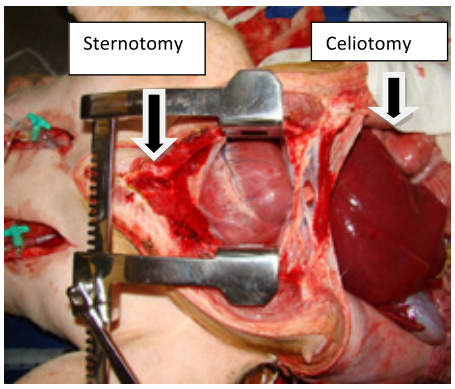

FIGURE 2 - Thoracoabdominal surgical approach.

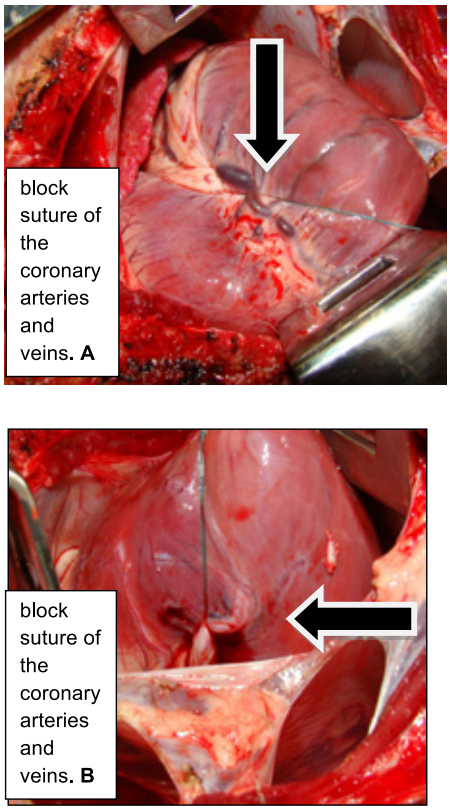

FIGURE 3 - Method of cardioplegia block suture of the coronary arteries and veins before (**A**) and after (**B**).

The studied variables included heart rate, systolic, mean and diastolic blood pressure, central venous pressure, pulse oximetry and CO_2 concentration at the end of expiration throughout the surgical procedure.

Statistical analyses

Data were expressed as mean values, reporting hemodynamic and gasometrical parameters during the surgical procedures.

Results

Concentration of expired CO2 remained near 50 mmHg during the first three minutes after cardiac infarction. An abrupt increase was noticed after this point, reaching 75 mmHg by the end of seven minutes (Figure 4).

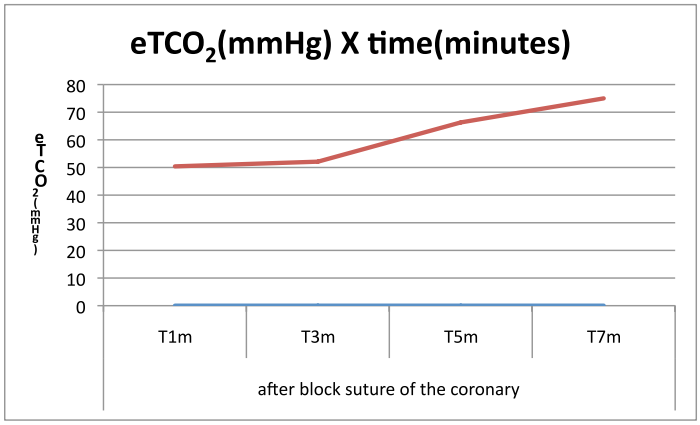

FIGURE 4 - The concentration of CO_2 in expired gas in relation to time in minutes.

Mean cardiac frequency of 250 beats per minute (bpm) came to a near 140 bpm after three minutes of cardiac infarction and was undetectable by the end of seven minutes (Figure 5).

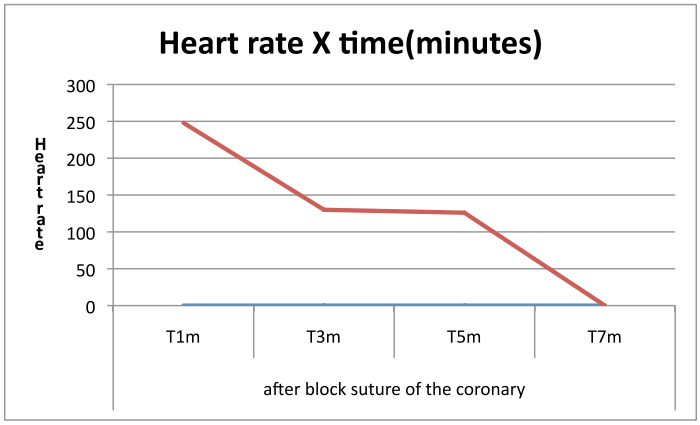

FIGURE5 - The heart rate versus time in minutes.

Systolic and diastolic blood pressure levels showed a drop from 75x26 mmHg to 65x22 mmHg (mean: 40 mmHg) during the initial three minutes of surgical protocol. Both blood pressure levels were undetectable by the end of seven minutes (Figures 6 to 8).

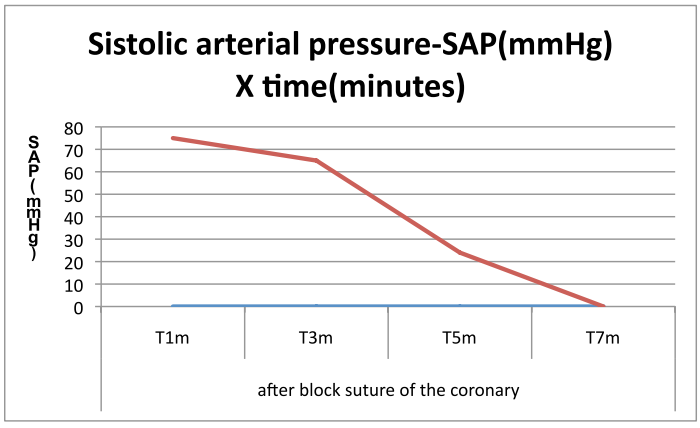

FIGURE 6 – The systolic arterial pressure to time in minutes.

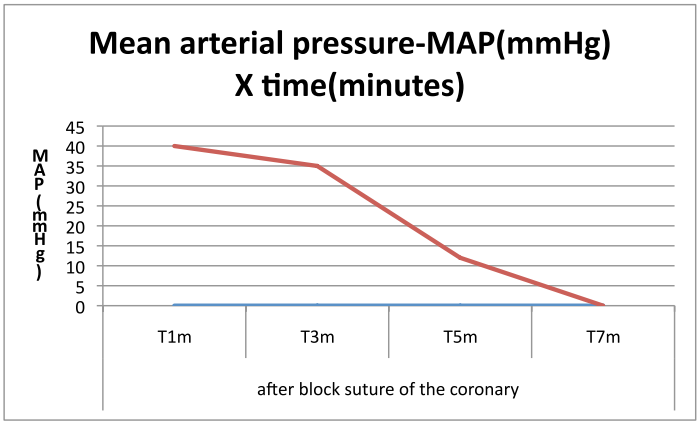

FIGURE 7 – The mean arterial pressure to time in minutes.

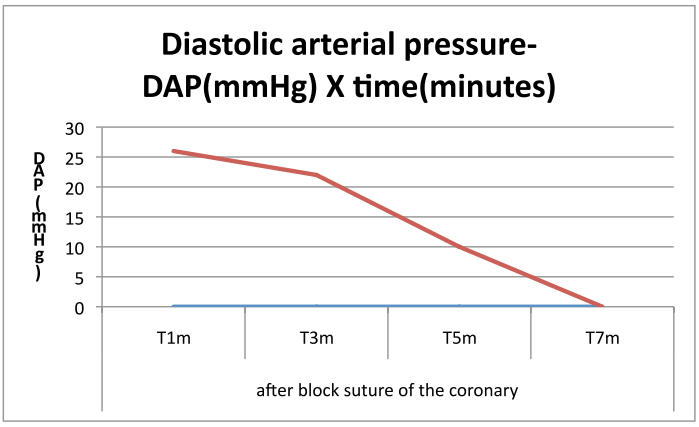

FIGURE 8 – The diastolic arterial pressure to time in minutes.

Oxygen saturation remained near 100% during the first five minutes, and undetectable by the end of seven minutes of the experimental protocol (Figure 9).

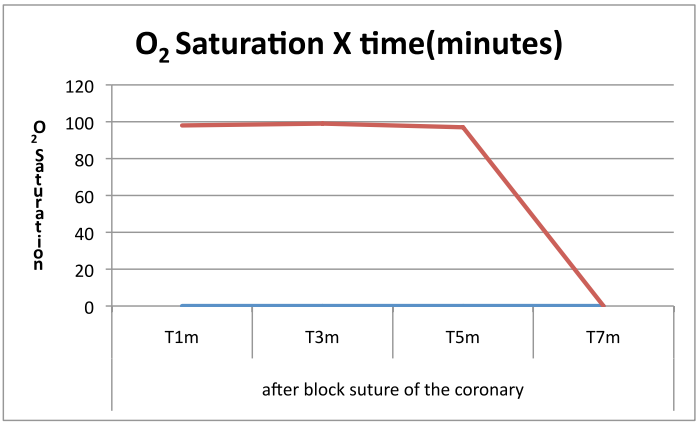

FIGURE 9 – The O_2 saturation to time in minutes.

Central venous pressure levels showed an increase from 2 mmHg to 11 mmHg by the end of seven minutes (Figure 10).

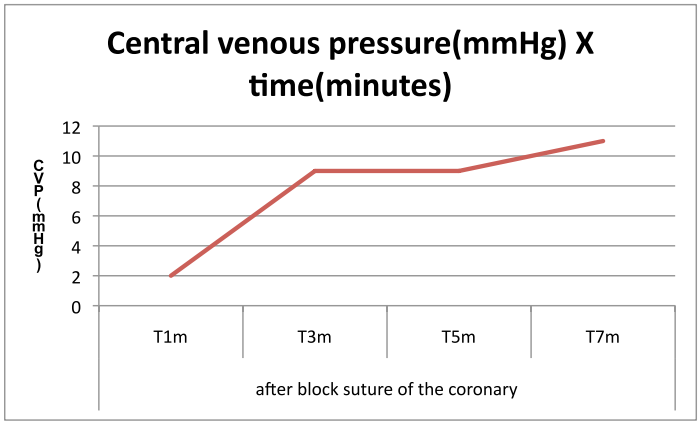

FIGURE 10 – The central venous pressure to time in minutes.

Discussion

This paper presents a new experimental method suitable for studies on organ donation after cardiac death. Our results have shown that the surgical protocol is easily performed and a homogenous circulatory collapse can be obtained by the end of seven minutes. Since no pharmacologic agents were used with the intent of cardiac arrest, we speculate that organ preservation can be achieved with minimal experimental bias. We also believe that the reproducibility of the method may be useful to the rational use of animals in studies of non heart beating organ donation. Economic savings might be another benefit to consider, as well as the usefulness of the method on simulating the Maastricht's category III of NHBD[2,10].

Sato *et al.*[15] presented a study in which cardiorespiratory support was removed to obtain the model of cardiac arrest. The criticisms of this model are the absence of specification of electrocardiographic criteria used to define heart failure and high variability of time between the withdrawal of support and cardiac arrest, which interferes with normothermic ischemia and potentially compromises the analysis of variables and outcomes.

Schön *et al.*[14] describe the exsanguination as a way to obtain the donor heart arrest. The induction of hypovolemic shock in this model directly interferes in the organ to be preserved, since hypovolemia worsens and begins the process of ischemia and reperfusion, making it difficult to validate the proposed results.

Takada *et al.*[11], Kamachi *et al.*[12] and Valero *et al.*[13] are groups that describe the model by administering a lethal dose of potassium chloride. However, there is significant interference to the mechanism of active transport across membranes by K + / Na +, promote cellular changes that can compromise organ preservation.

Our surgical protocol, on the other hand, focuses on the avoidance of strategies that could jeopardize organ preservation

and is currently being used in our laboratory for studies on liver and kidney transplantation.

Conclusions

The method is technically simple and a reproducible circulatory arrest can be obtained by the end of seven minutes. This model is suitable for further studies regarding abdominal organ transplantation.

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Received: December 20, 2011
Review: February 21, 2012
Accepted: March 19, 2012
Conflict of interest: none
Financial source: none

[1]Research performed at Laboratory of Experimental Surgery and Anesthesiology, School of Veterinary and Animal Science, Federal University of Goias (EVZ/UFG), Goiania-GO, Brazil. Part of the thesis Master degree in the Postgraduate Program in Health Sciences.