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Development of isolated swine "working heart model" with parabiotic circulation

Padronização de modelo de coração isolado "working heart" com circulação parabiótica

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Abstract

Objective: **To develop an isolated working heart model with parabiotic circulation in swine and to verify its stability and possibility for allowing effective measurements of hemodynamic and metabolic data.**

Methods: **This model was developed during an association study of cardiolegia agents. Eighteen experiments were performed, each with a support animal and a donor animal. Donor animal heart was perfused as isolated working heart with parabiotic circulation from the support animal. The isolated heart underwent regional ischemia by interventricular artery clamping, followed by global ischemia. During reperfusion in a isolated heart in working state at 30, 60, and 90 minutes, contractility indices such as elastance, preload recruitable stroke work index, and metabolic data were acquired.**

Results: **Support animals were kept stable throughout the procedures without use of blood transfusions or vasoactive drugs. Variables such as pH, oxygen partial pressure and hematocrit were kept stable and within physiologic ranges. The isolated heart was perfused adequately throughout the experiment. All hemodynamic and metabolic data proposed were adequately measured in the isolated heart in working state.**

Conclusion: **This isolated swine "working heart" model was kept stable throughout the experiments with no administration of vasoactive drugs, and it allowed adequate measurements of metabolic and hemodynamic data.**

Descriptors: **Heart/surgery. Models, animal. Swine. Cardioplegic solutions. Cardiac surgical procedures. Myocardial reperfusion/methods.**

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Resumo

Objetivo: **Desenvolver modelo de coração isolado de suíno "working heart" sob suporte por circulação parabiótica e verificar se o mesmo é estável e se possibilitou de forma efetiva a mensuração dos dados propostos.**

Métodos: **O modelo foi padronizado durante preparação para estudo de associação de agente à solução cardioplégica. Foram realizados 18 experimentos com um animal suporte e um animal doador em cada. O coração do animal doador foi perfundido como coração isolado pelo animal suporte em modo de execução de trabalho ("coração ejetante"). O coração isolado foi submetido à isquemia regional por pinçamento da artéria interventricular anterior seguido de isquemia global. Durante reperfusão, com o coração ejetante (em modo "working heart"), aos 30, 60 e 90 minutos foram medidos parâmetros hemodinâmicos de contratilidade e metabólicos, obtendo-se assim a elastância máxima (Emáx), o trabalho sistólico pré-recrutável (PRSW), rigidez do ventrículo (EDPRV), fluxo coronariano, consumo de oxigênio e dosagens de lactato e glicose.**

Resultados: **Os animais suporte ficaram estáveis durante todo o experimento. O pH, a pressão parcial de oxigênio e o hematócrito foram mantidos estáveis e dentro da faixa fisiológica. O coração isolado foi perfundido de forma adequada durante todo o experimento. Os dados hemodinâmicos e metabólicos propostos puderam ser mensurados adequadamente e sempre com o coração ejetante, em modo de execução de trabalho ("working heart").**

Conclusão: **O modelo de coração isolado desenvolvido tipo "working heart" se manteve estável durante todo o experimento, sem a administração de drogas cardiotônicas e possibilitou a mensuração de todos os dados propostos de forma efetiva com o coração executando trabalho.**

Descritores: **Coração/cirurgia. Modelos animais. Suínos. Soluções cardioplégicas. Procedimentos cirúrgicos cardíacos. Reperfusão miocárdica/métodos.**

INTRODUCTION

Since Langendorff the isolated heart models have been used successfully for the study of hearts in mammals [1]. These types of models allow for the study of mechanical properties of the heart and coronary flow, and metabolic study of the isolated organ [2]. The primary isolated heart models were advanced with the use of parabiotic circulation by Heymans and Kochmann [3]. In such a model, a support animal is used to keep the isolated organ perfused and, despite the fact that these heart models require more complex preparations than the preparations with solutions crystalloids, they allow the model to have a greater closeness to the physiological situation [4].

In Brazil, the standardization of isolated heart model with parabiotic circulation in pigs has been described, which showed to be a stable model, allowing for the performance of procedures and study of the isolated organ under different conditions [4]. The pig's heart presents anatomical and physiological characteristics that allow its successful use as a model for comparison with the human heart [5].

Models of isolated injecting hearts that have parameters that are evaluated with the heart working ("working state") are used to create experimental conditions that are closer to the actual clinical situation [6]. A similar isolated swine heart model with parabiotic circulation that was stable and reproducible, and that is able to allow for the broad study of hemodynamic and metabolic variables of the heart in working state, may be even more similar to human physiological conditions.

Therefore, the aim of this study is to develop an isolated ejecting pig heart model ("working heart") maintained under support by parabiotic circulation, and to verify if that is, in fact, stable enough to be maintained, and if it effectively allows for the measurement of the proposed data.

METHODS

The model was standardized during pilot study with the goal of future studies with intervention. In this case, the supplementary study aimed to test the effect of adding agents to cardioplegic solution. Altogether, 18 experiments were performed using female Large-White pigs. In each experiment, a support animal (weighing approximately 40 kg) and a donor animal (weighing approximately 10 kg) were used. The animals were treated according to international rights standards and techniques for research with animals [7], and the experiments were approved by the Ethics Committee on Animal Experiments of the university.

Support animal

The support animal in each experiment was fasted for 6 hours before the procedure. Each animal received 1mg of atropine and 25mg/kg of ketamine intramuscularly, and was then weighed and sent to the operating room where the animal was anesthetized with 12.5 μg/kg fentanyl hydrochloride and 15 mg/kg of intravenous pentobarbital. The animal was then submitted to orotracheal intubation, and 8mg of pancuronium bromide was administered. This dose was repeated throughout the experiment when necessary. The animal was placed in controlled ventilation with current volume of 10 ml/kg and fraction of inspired oxygen (FiO2) of 100%.

The animal was positioned in dorsal decubitus position in a Claude Bernard drip, and the following procedures were performed:

- Access to the left jugular vein for infusion of crystalloid volume and drugs;

- Dissection and catheterization of the left common carotid artery with 5mm polyethylene catheter to measure the mean blood pressure;

- Intravenous administration of 500 IU/kg heparin and isolation of the jugular vein and the right common carotid artery, individually cannulated with polyethylene catheters with ¼ inch internal diameter for venous return and aspiration of arterial blood to the perfusion system (Figure 1).

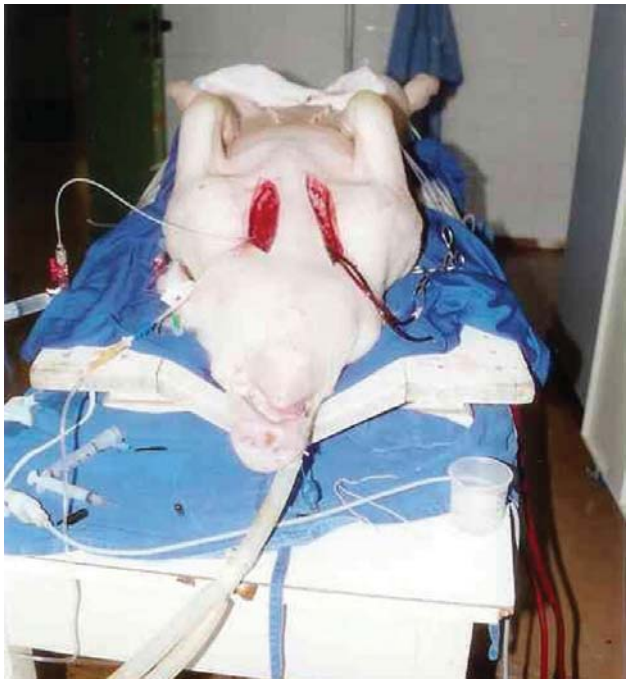

Fig.1- Overview of the animal support in dorsal decubitus position, with cannulated vascular access. Bilateral cervicotomy. Catheters in internal jugular vein and left carotid artery for continuously monitoring of mean arterial pressure (MAP). Cannulation of the jugular vein and right carotid artery with ¼ inch tubes. Orotracheal intubation and mechanical ventilation with FiO2 at 100% and 10 ml/kg of current volume

Donor animal

Donor animals in each experiment were submitted to the same preoperative care and anesthetic monitoring of the support animals. They were submitted to mechanical ventilation pressure with 100% oxygen.

Each animal was placed in dorsal decubitus position in Claude Bernard drip, and the following procedures were performed:

Thoracotomy by median longitudinal sternotomy, pleuras and pericardium opening. Administration of 500IU/kg of intravenous heparin.

Placing of four TRX6 piezoelectric crystals from Sonometrics - London, Ontario - Canada, in the epicardium of the posterior and anterior walls at the base and the apex of the left ventricle, fixed with 4.0 polypropylene string.

Insertion of 18 "abbocath" catheter in the apex of the left ventricle to measure its pressure, and fixation with 4.0 polypropylene string.

Perfusion and monitoring system

To perform the measurements in the donor heart, and in all measurements taken with the isolated heart on the perfusion system, the following devices were used:

- Doppler Fluxometer T206 (Transonic Systems Inc., Ithaca, New York, USA);
- Monitor with four pressure channels (PCA-4, Sonometrics, London, Ontario, Canada);
- Set of piezoelectric crystals (four), (TRX6 Sonometrics, London, Ontario, Canada);
- Piezoelectric crystals synchronizer (TRX6 Sonometrics, London, Ontario, Canada);
- Software CardioSoft 3.1.2 of Sonometrics, London, Ontario, Canada.

The system used to perfuse the donor animal's isolated heart (as soon as it was removed from the animal) was composed of polyvinyl tubes with ¼ internal diameter inch, Y connectors (¼ x ¼ x ¼) cardiotomy reservoirs, heart lung machine with DeBakey rollers manufactured by Braile Biomédica (Sao Jose do Rio Preto - SP), polyethylene catheters with 15mm internal diameter for pressure lines and infant venous cannulas connected to three-way stopcock.

Isolated heart preparation

The donor animal's heart was excised with the left ventricle catheter and the piezoelectric crystals were attached to it. The aorta was clamped, both vena cava were connected, the pulmonary veins were incised, and the heart was completely withdrawn after an incision in the ascending aorta and pulmonary artery. Ischemia time count was initiated. On the table, the aorta was cannulated, maintaining the cannula above the aortic valve and the coronary arteries ostia. The left atrium was exposed and the pulmonary arteries ostia were resected; the left atrium was cannulated above the mitral valve to avoid regurgitation. The pulmonary artery was cannulated and the cannula was introduced into the right ventricle cavity. These three cannulas were fixed with polyester string and connected to the pressure lines.

The cannulas and the isolated heart were fixed and

positioned in a support unit, where they were kept throughout the experiment. They were connected to the afferent and efferent tubes and reperfusion was initiated, interrupting the ischemic time.

Isolated heart reperfusion

During the first 20 minutes of reperfusion, the isolated heart was defibrillated when needed and reperfused in passive mode until there was stabilization of monitored variables. In passive mode, the blood aspirated from the carotid artery by roller pump was forwarded to the cannula in the isolated heart aorta. This blood was drained by the cannula placed in the right ventricle (RV) through the pulmonary artery and collected in cardiotomy reservoir or drained through the left atrium cannula to another cardiotomy reservoir. Once inside these reservoirs, the blood was returned to the support animal through the roller pumps. During this period of passive perfusion, the perfusion pressure of the isolated heart aorta was monitored by the pressure lines, and flow of this perfusion was measured by a Doppler fluxometer in the aorta cannula. The coronary flow was obtained by a Doppler fluxometer in the RV cannula that collected the effluent from the isolated heart (Figure 2).

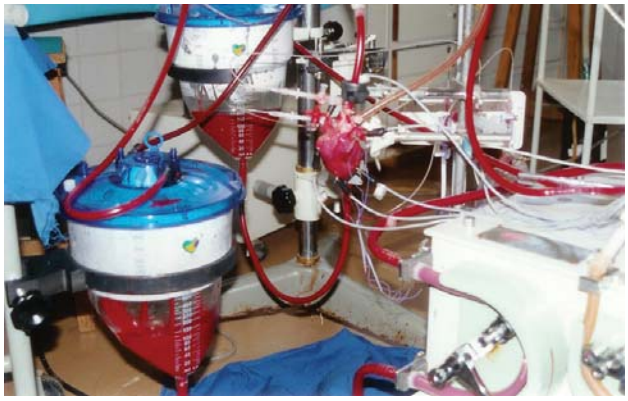

Fig.2 – Overview of the perfused isolated heart. Isolated heart with metallic mini forceps for induction of regional ischemia. LA reservoir working as preload reservoir. Venous reservoir from which the blood was reinfused toward the animal support. Afferent line of the roller pumps from the animal support. Piezoelectric crystals wires

After 20 minutes of passive reperfusion, the perfusion was converted to "working state." The flow from the support animal to the isolated heart aorta was interrupted, and the blood was drained and forwarded to the cardiotomy reservoir and, from this (acting as a preload reservoir), it was forwarded to the cannulated left atrium of the isolated heart. The left atrium pressure was measured. The blood forwarded to the left ventricle was pumped to the isolated heart by the aortic cannula into an arterial reservoir. The perfusion aortic pressure was measured, thus obtaining – at the same time - the pre- and afterloads needed for the isolated heart. The aorta and left atrium pressures were measured by the pressure lines connected to the cannulas, and the left ventricle pressure was measured by the pressure lines connected to the left ventricle catheter. These pressure lines were connected to the monitor (PCA-4, Sonometrics, London, Ontario, Canada). The blood that came to the arterial reservoir returned to a third cardiotomy reservoir and from this was aspirated by the roller pumps and reinfused to the support animal to be oxygenated. The effluent blood from the coronary sinus, corresponding to the coronary flow, was drained from the right ventricle to the third reservoir and from this reinfused to the support animal (Figure 3).

During the reperfusion in "working state", the isolated heart was reperfused in working state. The aortic and coronary flows were measured with Doppler fluxometers placed in the aortic and right ventricle cannula, respectively, and connected to console (T206, Transonic Systems Inc., Ithaca, New York, USA). The piezoelectric crystals of the left ventricle were connected to a synchronizer (TRX6, Sonometrics, London, Ontario, Canada) to calculate the left ventricle volume and to obtain the volume-pressure curves and their respective contractility indexes.

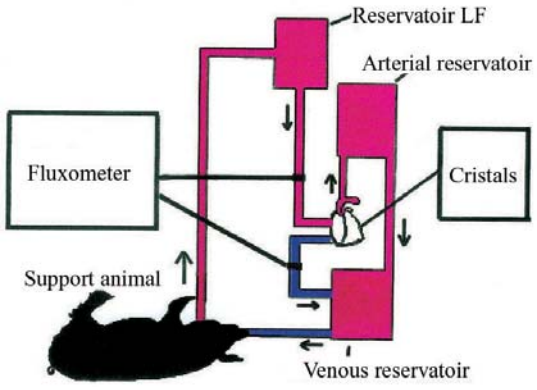

Fig.3 - Outline of the perfusion system. In "working heart" mode, the blood aspirated from the animal support was forwarded to the LA reservoir that worked as a preload. From this reservoir the blood was forwarded to the isolated heart from where it was ejected in the aorta toward the arterial reservoir. The blood returned to a venous reservoir along with the effluent blood from the coronary sinus and, drained by cannula in the pulmonary artery. From this reservoir it was reinfused in the animal support. Variations in height of the LA and arterial reservoirs refferred to variations of pre- and afterload, respectively. Under monitoring by the pressure lines in the LA, in the aorta and in the LV (by the piezoelectric crystals for obtaining ventricular volume) and by the fluxometers for measurement of cardiac output and coronary flow

Monitoring performed "in situ" and in isolated heart in working state

With the animal donor heart "in situ," during and after reperfusion of the isolated heart in working state, the following levels were measured:

- Cardiac output and LV volume using synchronized piezoelectric crystals, fixed to the epicardium and connected to the TRX6 synchronizer;
- LV pressure (using pressure line);
- Left atrium pressure (using pressure line) corresponding to the offered preload;
- Aortic root pressure (using pressure line);
- Aortic and coronary flow using Doppler fluxometers;
- Electrocardiographic recording;
- Gasometry in effluent blood from the isoated heart and the support animal, thus making it possible to determine the volume and oxygen consumption of the isolated heart;
- Glycemia and lactate dosage of the effluent blood from the isolated heart and the support animal;
- Volume-pressure curves (using the sonic synchronization of piezoelectric crystals performed by the software) during periods in which preload variations were provoked;
- Maximum elastance (Emáx) - Relation between the systolic pressure and ventricular volume. Myocardial contractility index corresponding to the slope of the axis composed of different points of the volume-pressure relantionship obtained at the end-systole in different preload conditions [8];
- Pre-recrutable systolic work (PRSW) - Linear relationship between systolic work and end-diastolic volume;
- End diastolic pressure volume relationship (EDPRV), allowing for measurement of ventricle rigidity.

These last three parameters were obtained by the analysis of volume-pressure curves made possible by the software in the records made with preload variation. All the measurements above were taken during specific periods and recorded by the software CardioSoft 3.1.2 from Sonometrics.

The oxygen consumption was calculated as the difference between the arterial blood sample from the support animal and the blood collected from the coronary sinus in the right ventricle cannula according to the following formula:

$MVO2 = 0.1 \text{ X coronary flow / Final weight of the left ventricle X } ((SO_{2a} - SO_{2v}) \times 1{,}34 \times Hb/100 + 0.03 \times 7{,}5 \times (pO_{2a} - pO_{2v}))$,

where MVO_2 and oxygen consumption of oxygen express per 100g of myocardium, SO_{2a} and SO_{2v} are the oxygen saturation (in %) of the support animal and the coronary sinus of the isolated heart, respectively; Hb and hemoglobin volume (in g/L) and pO_{2a} and pO_{2v} are the partial oxygen pressure from the support animal and the coronary sinus from the isolated heart, respectively [9].

After performing measurements at 90 minutes of reperfusion, the experiment was interrupted with euthanasia of the support animal, with a lethal dose of potassium chloride. The isolated heart was dissected, and the right ventricle wall, the atria and great vessels were removed; the rest was then weighed [10]. Left ventricle samples around the area submitted to regional ischemia were sent for histological analysis.

Measurements compared between the groups with heart "in situ" and isolated heart

- Maximum elastance;
- "EDPVR";
- Preload recruitable stroke work (PRSW).

Measurements compared to only the isolated heart

- Lactate dosage;
- Oxygen consumption;
- Glucose consumption;
- Wet end weight of the left ventricle;
- Number of defibrillations.

Methodology and data acquisition

With the apparatus installed in the donor animal's heart with it still "in situ," the hemodynamic data extracted from volume-pressure curves were obtained by a tractioning of cardiac tape applied to the inferior vena cava, to promote preload variation. After the excision of the donor heart, and after placing it on the support apparatus after 30 minutes of reperfusion (20 minutes of "passive reperfusion" followed by "working heart" reperfusion), the data of stabilixation of the model were measured. Next, the perfusion was converted again to passive mode, and the anterior interventricular artery was kept clamped for 30 minutes to induce regional ischemia. During this period, ventricular arrhythmias often occurred that were not treated. Then, the forceps of the anterior interventricular artery were removed, and the perfusion was interrupted for 90 minutes. The models were randomly selected and divided into three groups: Group 1 received St. Thomas cardioplegic solution (ST) every 30 minutes over this period, Group 2 received the same dose of St Thomas with trimetazidine (TMZ), and Group 3 was the Control group (Co), which did not receive cardioplegic solution.

At the end of 90 minutes, the perfusion of the isolated heart was restarted, initially in passive mode for 20 minutes. During this phase, the heart was defibrillated with shocks at 5J as often as was needed to maintain a satisfactory

contractility, and the total number of defibrillations were recorded. After 20 minutes, the perfusion of the isolated heart was converted to ejecting heart model ("working heart") under specific pre- and afterload. The model was kept in this working mode until the end of the experiment. Thirty minutes after the start of reperfusion, the measurements were performed with the use of the previously mentioned equipment. The records of variations of the volume-pressure curves were obtained while the height of the preload reservoir was manually changed; this maneuver was similar to the tractioning of the inferior vena cava performed in the heart "in situ." The measures were performed again at 60 and 90 minutes into reperfusion (Figure 4).

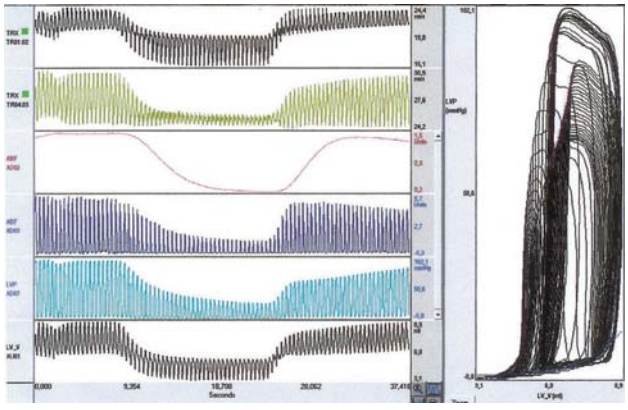

Fig. 4 – View of typical screen of the software used in this study. On the left, the record of monitoring of crystals and the obtained curves. On the right, example of typical volume-pressure curve with pre-load variation. From the variation of these curves were obtained the rates of the studied contractility

Thus, the isolated heart was maintained by the support animal for 240 minutes, divided thus:

- 30 minutes for initial stabilization after removing the heart from the donor animal;
- 30 minutes of induction of regional ischemia;
- 90 minutes of global ischemia and administration of the different treatments;
- 90 minutes of reperfusion.

Periodically throughout the experiment, arterial gasometry exams were performed on the support animal, and sodium, potassium and hematocrit dosages were evaluated. The support animal was kept under anesthesia and with mean arterial pressure at approximately 80 mmHg, receiving the venous return of its blood by the circuit and crystalloid replacement. 8.4% sodium bicarbonate was administered when needed to keep the support animal free from acidosis. At no time was any cardiotonic agent used.

Statistical analysis

The results are shown in mean ± two standard errors of the mean. For comparison between groups at different times, analysis of variance was used (ANOVA), associated with Bonferroni test for identification of differences. A pd"0.05 value was considered significant. The tests were performed using the statistical package for Windows Graphpad Prism (Graphpad Software, San Diego, CA).

RESULTS

The mean blood pressure of the support animals was kept stable with the measurements described and was always above 80 mmHg. The support animals were kept hemodynamically stable throughout the experiment without differences in relation to the oxygen partial pressure and the pH. The hematocrit was lower in the St Thomas group than in the others, but without significant variation throughout the experiment (Figure 5).

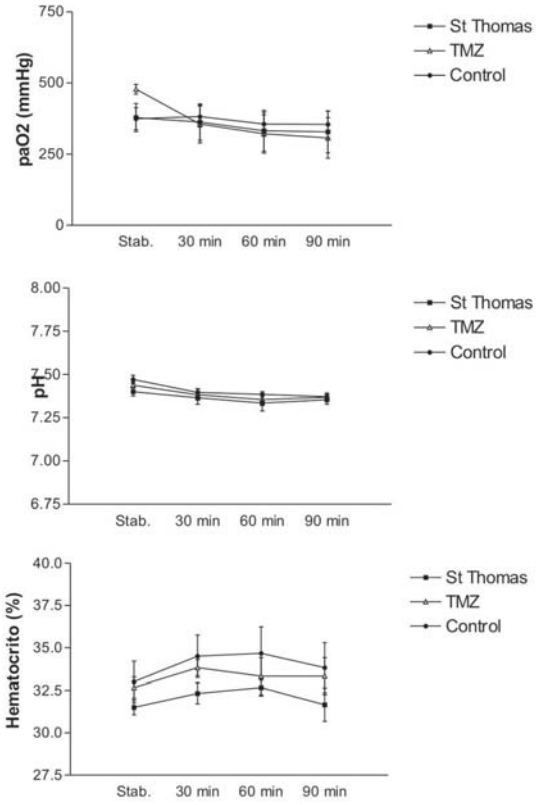

Fig. 5 - Partial pressure of oxygen (above), pH (middle) and hematocrit (below) of the animals support throughout the procedure. Data presented as mean and two standard errors of the mean (SEM) (n=6) in each group. The data show stability of the model from the stabilization up to the 90 minutes of total reperfusion (total period of 180 minutes)

The mean time of ischemia, calculated in the beginning of the excision of the donor animal's heart until the beginning of his reperfusion, was 755±102 seconds.

During reperfusion - after 30 minutes of regional ischemia followed by 90 minutes of global ischemia - the Control group and the St Thomas group showed a median of 5 defibrillations. Trimetazidine group presented a median of one defibrillation, but there were not any statistically significant differences. The final wet weight of the left ventricle showed statistically significant differences (p d" 0.05) between the Trimetazidine group and the others, with a minor final wet weight of the left ventricle of the Trimetazidine group. There was no difference between St. Thomas and Control groups.

The contractility indexes obtained from the preload variation of the volume-pressure curves were compared between the groups, including data collected "in situ." The three contractility data that were less dependent on external factors showed no statistically significant difference in relation to the values obtained at the moment of stabilization before induction of ischemia or specific treatment. This was observed in the three groups. The maximum elastance showed a significant decrease in stabilization measurements at 30, 60 and 90 minutes of reperfusion. However, there was no statistically significant difference among the three groups. The prerecruitable systolic work (PRSW) and "EDPVR," related with the heart rigidity, remained stable and with no significally statistical difference among the three groups.

The coronary flow presented great dispersion in the results presented in St. Thomas and Control groups, but it presented as more regular in the Trimetazidine group. However, there was no statistically significant difference among the three groups.

The effluent lactate from the coronary sinus, collected from the cannula placed in the right ventricle by the pulmonary artery, presented increase from the time of the stabilization measurements up to 30, 60 and 90 minutes of reperfusion. This increase was gradual and occurred in all three groups without showing statistically significant differences between them. When the obtained lactate level was corrected according the final weight of the left ventricle of each experiment, the obtained results were similar with no significant difference.

The oxygen consumption numbers were obtained according to the formula previously described, and there was no statistically significant difference among the three groups. The glucose consumption at 30 minutes of reperfusion showed statistically significant difference between the Control group and the Trimetazidine group (p d" 0.05), but this difference was not observed at 60 and 90 minutes of reperfusion among the three groups.

The histological analysis was illustratively performed analyzing colored fragments with hematoxylin-eosin. Fragments from Control group (Co) hearts presented a more frequent presence of **swollen** heart cells and a cytoplasmic structure showing signs of cellular lysis. The cell nuclei from the Control group (Co) also showed picnosis more frequently. The fragments from the St Thomas (ST) and trimetazidine (TMZ) groups presented less edema and less cell picnosis. No obvious differences were observed between the St Thomas (ST) and trimetazidine (TMZ) groups.

DISCUSSION

The swine heart is anatomically very similar to human heart, except for the hemiazygos vein that drains to the coronary sinus. The swine heart dimensions are also very similar to that of a human in terms of weight. Its similarity to the human heart in terms of preexisting poorness of collateral coronaries, but also in terms of its great capacity for generation of colleterals by ischemia, make the pig a very appropriate animal for studying the human heart [5].

Models have been used in swine heart "in situ" to measure different parameters of contractility in a proper and reliable way in relation to human anatomy and physiology [11]. Studies in isolated swine heart have been described aiming to mimic the physiological situations found in humans with a model more compatible with the human anatomy without losing the advantages related to the study of isolated heart itself [6], such as the possibility of studying pharmacological and mechanical responses of the isolated heart without involving the responses of different systems of the animal [4.6].

The described model allowed for data acquisition in an isolated heart perfused with blood. To this end, parabiotic circulation was used. The maintenance of the model was over 240 minutes (90 minutes of reperfusion after the isolated heart remained under regional ischemia (30 minutes) followed by global ischemia (90 minutes), which demonstrates the feasibility of the model. In a description of an experimental model of isolated swine heart with parabiotic circulation (not "working heart"), Petrucci Jr et al. [4] obtained a model that was stable for a period close to that which was achieved in this study without the use of homoderivatives of cardiotonic agents.

The hematocrit provided for the three groups remained constant and within this physiological range of Flecknell [12], although it was slightly lower in the St. Thomas (ST) group. The arterial partial pressure of oxygen (pO2a) and the pH also remained stable and within the physiological values during the experiments.

The ischemia time resulting from the removal of the donor animal's heart until the start of reperfusion aiming

the stabilization was compatible with the literature about preparations in mammals [13.14].

Soon after the end of ischemia (and after a period of regional ischemia followed by global ischemia in which groups were treated), the reperfusion of the isolated heart was started in passive mode. By a simple clamping of the tubes, the blood was initially forwarded to the aorta and therefore maintained a pressure of appropriate coronary perfusion, monitored and always close to 70 mmHg. At this phase (called infusion in passive mode), the isolated heart was was drained, and the blood was deflected to the reservoir connected to the left atrium, which was maintained at a level below the isolated heart.

The blood corresponding to the coronary flow at this phase was also drained by the cannula (placed in the right ventricle) to another reservoir. During this period, the isolated heart was defibrillated when needed, and it gradually obtained a satisfactory minimum contractility. After 20 minutes of reperfusion in this way, with change of clamping of the tube from the afferent pathway, the blood aspirated through the rollers pump of the support animal was forwarded to preload reservoir with its already high height, connected to the left atrium. In this way, the isolated heart was infused with the blood taking its "natural" pathway, reaching the left ventricle by the reservoir that corresponded to preload. The infusion was maintained with the isolated heart ejecting blood into the arterial reservoir for the remaining time, and with this reperfusion mode, the data were obtained and stored.

The use of piezoelectric crystals (TRX6 Sonometrics) in the donor animal epicardium and after their placing (isolatedly) allowed the accurate achievement of ventricular volume. No other studies were found regarding isolated hearts and cardioplegic solution additives with the use of similar equipment to which these less dependent contractility parameters could be compared.

During data acquisition "in situ" and in the isolated heart (with the tracnioning cardiac tape applied to the inferior vena cava [15]) and with the change of height of the preload in the reperfusion of the isolated heart, it is possible to obtain volume-pressure curves which, according to their variation, allowed for the calculation of clear contractility indexes.

The maximum elastance is an index relatively independent of momentaneous pre- and afterload [8.15]. This is an index shown by the curve that is obtained from the union of several points corresponding to the end of systole. These points are obtained in the following volume-pressure curves that are obtained with partial and temporary occlusion of the inferior vena cava, which in the isolated heart was obtained with a variation of the height of the preload reservoir. The prerecruitable systolic work (PRSW) is possibly the contractility index that is less dependent on other parameters within physiological values [4.16]. The EDPVR ("end diastolic pressure volume relationship") is an exponential regression between diastolic pressure and end-diastolic pressure volume (also obtained from the variation of the volume-pressure curves [17]). These three index make it possible to use the model to calculate experimental indexes minus variables according to the external parameters such as afterload cardiac frequency.

Araki et al. [18] published standardized model in isolated swine heart "working heart" perfused with crystalloid solution, and they measured the maximum elastance and strength of the ventricle. In this study, the results of the contractility parameters obtained "in situ" were consistent with the results obtained in the stabilized isolated heart, before treatments, emphasize the applicability of this model. This fact was also compatible with the literature [18.19].

The cardiac output may be measured using the volume-pressure curves obtained from the piezoelectric crystals, as well as the pressure monitoring from the left ventricle and by the Doppler Fluxometer placed in efferent cannula from the isolated heart. The coronary flow, similarly, may be measured by Doppler Fluxometer placed in efferent cannula from the right ventricle. During the procurement of hemodynamic data, samples of blood from the support animal and effluent blood from the coronary sinus of the right ventricle cannula in the isolated heart were collected. Thus, it was possible to appropriately measure the effluent lactate, the oxygen consumption and the difference in affluent and effluent glucose in the isolated heart (which made it possible to measure glucose consumption).

The clamping of the interventricular artery from the support animal was performed in order to characterize a situation of acute ischemia in the model with the heart about to receive treatment. This strategy was similar to that described by Horsley et al. [20].

The lack of improvement in hemodynamic behavior in relation to control with the use of any method of myocardial protection could be attributed to the refinement of this model to include the data measured in "working heart" mode.

The final wet weight of the left ventricle was significantly lower in the Trimetazidine group than in the others. When we calculated the corrected weight over the weight of the donor animal, the values of Trimetazidine group were significantly lower in relation to the Control group, without difference in the St Thomas group. Petrucci Jr. [21] found wet weight higher in the Control group than in isolated swine hearts that received crystalloid or blood cardioplegic solution. It is likely that not administrating any solution of myocardial protection to the Control group has promoted greater interstitial edema, which has resulted in an increased final wet weight.

The differences found in the histological analysis, with evidence of minor edema and cell picnosis between the

groups that received St Thomas cardioplegic solution associated or not to Trimetazidine suggest some degree of myocardial protection in these groups in relation to the Control group.

CONCLUSION

The isolated heart of "working heart" model developed remained stable throughout the experiment, without the administration of cardiotonic drugs and allowed us to effectively measure of all the proposed data with the heart in working mode.

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