Rev Bras Cir Cardiovasc 2008; 23(2): 224-234

Trimetazidine as cardioplegia addictive without pre-treatment does not improve myocardial protection: study in a swine working heart model

Trimetazidina como aditivo em solução cardioplégica sem pré-tratamento não traz proteção adicional ao miocárdio isquêmico: estudo em modelo suíno de coração isolado

Lindemberg da Mota SILVEIRA FILHO[1], Orlando PETRUCCI JR[2], Marcio Roberto do CARMO[3], Pedro Paulo Martins de OLIVEIRA[4], Karlos Alexandre Sousa VILARINHO[5], Reinaldo Wilson VIEIRA[6], Domingo Marcolino BRAILE[7]

RBCCV 44205-978

Abstract

Objective: **The aim of this study is to verify in an isolated working heart swine model if the acute administration of trimetazidine to cardioplegia, without pre=treatment improves heart performance.**

Methods: **Eighteen pairs of swines were used in this working heart model, divided into three groups (n = 6) that underwent regional and global ischemia. Each group was selected to a different treatment: St Thomas cardioplegia (ST), St Thomas enriched with trimetazidine (TMZ) and control group (Co). Data was collected during reperfusion period at 30, 60 and 90 minutes. Hemodinamic parameters such as elastance contractility index (Emax), preload recruitable stroke work relationship (PRSW) and heart "stiffness" (EDPVR) were measured. Other data included coronary flow, lactate, oxygen and glucose consumption. Results were statistically analyzed.**

Results: **All contractility data were not significantly different among three groups. Lactate became constantly higher according to time uniformly in all three groups. Coronary flow, glucose consumption and oxygen consumption presented large variations during time periods**

1. Master Degree in Surgery. Assistance physician of the Discipline of Cardiac Surgery of the Faculty of Medical Sciences – State University of Campinas
2. PhD in Surgery. Assistance professor. Phd of Discipline of Cardiac surgery. FCM UNICAMP.
3. Perfusionist. Discipline of Cardiac surgery. FCM UNICAMP.
4. Master Degree in Surgery. Assistance physician of the Discipline of Cardiac surgery. FCM UNICAMP.
5. Assistance physician of the Discipline of Cardiac surgery. FCM UNICAMP.
6. PhD Professor. Discipline of Cardiac surgery. FCM UNICAMP.
7. Full Professor of the São José do Rio Preto Medical School (FAMERP) and UNICAMP; Editor of the Brazilian Journal of Cardiovascular Surgery.

This study was carried out at Discipline of Cardiac Surgery , Surgery Department – Faculty of Medical Sciences – State University of Campinas, Campinas, SP, Brazil.

Address for correspondence: Lindemberg Mota Silveira. Rua Barreto Leme 2022/ 101 - Cambuí - Campinas – SP - Brasil - CEP 13025-085
Fone: (55-19) 3255-2792.
E-mail address: lindembergms@uol.com.br

Article receibe on October 14th , 2007
Article accepted on January 7th, 2008

but according to treatments showed no statistical differences in all three groups. Left ventricle final weight was significantly lower in trimetazidine group compared to both other groups.

Conclusion: **Administration of trimetazidine enhanced cardioplegia, without pre-treatment, showed no hemodinamic or metabolic improvement in swine isolated working heart model.**

Descriptors: **Trimetazidine. Models, animal. Heart. Cardioplegic solutions.**

Resumo

Objetivo**: Verificar, em modelo experimental de coração isolado de suínos, se a associação da trimetazidina à solução cardioplégica promove melhora no desempenho do coração.**

Métodos: **O modelo experimental utilizou suínos Large-White, com coração isolado perfundido por suporte de outro animal em modo de execução de trabalho ("working heart state"). Foram divididos em três grupos (n = 6), submetidos a isquemia regional seguida de isquemia global, que recebiam um dos três tratamentos: solução St Thomas (ST), solução St Thomas acrescida de trimetazidina (TMZ) e grupo controle (Co). Durante período de reperfusão, aos 30, 60 e 90 minutos, foram medidos parâmetros hemodinâmicos de contratilidade e metabólicos, obtendo-se assim a elastância máxima (Emáx), o índice de trabalho sistólico pré-recrutável (PRSW), "dureza" do ventrículo (EDPRV), fluxo coronariano, consumo de oxigênio e dosagens de lactato e glicose. Os resultados foram analisados estatisticamente.**

Resultados: **Em relação aos parâmetros hemodinâmicos de contratilidade, não houve diferença estatística significante entre os três grupos. Houve produção crescente de lactato nos três grupos de forma uniforme quanto maior o tempo de reperfusão. O fluxo coronariano, o consumo de oxigênio e o consumo de glicose tiveram grande variação entre os diferentes tempos medidos, mas sem diferença entre os três tratamentos. O peso final do ventrículo esquerdo foi significativamente menor no grupo trimetazidina (TMZ) do que nos demais.**

Conclusão: **A administração da trimetazidina associada como adjuvante à solução cardioplégica, sem pré-tratamento, não demonstrou benefício hemodinâmico ou metabólico em modelo experimental "working heart" de coração isolado em porcos.**

Descritores: **Trimetazidina. Modelos animais. Coração. Soluções cardioplégicas.**

INTRODUCTION

Since the beginning of the use of CABG in cardiac surgery, several drugs have been associated to cardioplegic solutions aiming to promote resistance to ischemia and improvement in operations outcomes. Metabolic manipulation agents, such as the trimetazidine that due to its mode of action may offer interesting option for promoting resistance to ischemia in hearts undergone CPB [1].

The trimetazidine is one (1,2,3,4 trimethoxybenzyl) piperazine, an a*nti-ischemic* agent without direct hemodynamic effect [2]. Its mechanism of action allows to inhibit the mitochondrial 3-ketoacyl-CoA thiolase, increasing the glucose oxidation in heart, promoting metabolic deviation for production of ATP from beta-oxidation of fatty acids for glucose consumption [3]. In situations of ischemia, the glucose breaking may be more desirable because the resynthesis of ATP demands less oxygen consumption, whilst the production of ATP (due to fatty acids) consumes more oxygen and induces accumulation of hydrogen ions and the increase of intracellular calcium ions [4,5].

Patients undergone cardiac operations have been treated with trimetazidine in order to improve the performance in the trans- and postoperative periods, with different results [6-8]. However, the reports have described the use of trimetazidine over three weeks before the operation. The effect of association of trimetazidine only as adjuvant to cardioplegic solution, without pre-treatment, is not totally understood. The aim of this study is to verify in a working heart swine model if the acute administration of trimetazidine - associated to cardioplegic solutions without pre-treatment – offers hemodynamic or metabolic benefit to the heart.

METHODS

18 experiments were performed using female Large-White pigs and in each experiment was used one animal support weighing around 40 kg and one donor animal weighing around 10 kg. The animals were treated according to technical norms and international norms of animal experimentation (COBEA, 1991) [9] and the experiments were approved by the university's Ethics Committee of Animal Experimentation.

Animal support

The animals support in each experiment were fasted for 6 hours before procedure. Each one received atropine (1mg) and ketamine (25 mg/kg) intramuscularly and after it was weighed and sent to operation room, where it was anesthetized by venous access of auricular pavillion, with 12,5 μg/kg of fentanyl-chloride and 15mg/kg of pentobarbital. Each animal also underwent orotracheal

intubation and it was administered 8mg of pancuronium bromide (dose that was repeated when required) and maintained in controlled ventilation with current volume of 10 ml/kg and inspired oxygen fraction (FiO_2) of 100%.

The animal was positioned in horizontal dorsal decubitus and placed on a Claude Bernard drip in order to perform the following procedures:

- Access to the left jugular vein for infusion of crystalloid solution and drugs;
- Dissection of the left common carotid artery and its catheterization with 5mm polyethylene catheter for mensuration of median arterial pressure;
- Administration of heparin 500 IU/kg intravenously and isolation of jugular vein and the right common carotid artery, individually cannulated with ¼ inch (internal diameter) polyethylene catheters for venous return and aspiration of arterial blood toward the perfusion system.

Animal donor

Animal donors in each experiment underwent the same preoperative care and anesthetic monitoring of the animals support. They also underwent pressure mechanic ventilation with 100% oxygen.

Each animal was positioned in horizontal dorsal decubitus and placed on a Claude Bernard drip in order to perform the following procedures:

Animal doador

- Median thoracotomy using longitudinal sternotomy, pleural and pericardial opening. Administration of 500 IU/kg of intravenous heparin;
- Placing of four piezoelectric crystals (TRX6 Sonometrics - London, Ontario - Canada), in the posterior and anterior epicardial walls, in the base and apex of the left ventricle fixed with 4.0 polypropylene wire;
- Placing of "abbocath" catheter (18) in the apex of the left ventricle for measurement of its pressure and fixation with 4.0 polypropylene wire.

Perfusion and monitoring system

To perform mensuration in the donor heart and then in all measures taken with the isolated working heart in the perfusion system, the devices used in this procedure were obtained with funding from Fundação de Amparo á Pesquisa do Estado de São Paulo (FAPESP) as follows:

1. Doppler Fluxometer T206 (Transonic Systems Inc., Ithaca, New York, EUA);
2. Monitor with four pressure channels (PCA-4, Sonometrics, London, Ontário, Canadá);
3. Set of piezoelectric crystals (four) (TRX6 Sonometrics, London, Ontario, Canadá);
4. Piezoelectric crystals Synchronizer (TRX6 Sonometrics, London, Ontário, Canadá);
5. CardioSoft software 3.1.2 (Sonometrics, London, Ontário, Canadá);

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

Preparation of the isolated working heart

The heart of the donor animal was removed with the left ventricle catheter and the piezoelectric crystals were fixed to it. The aorta was clamped, both vena cava were connected and the pulmonary veins were incised and the heart was totally removed after incision in the ascending aorta and in the pulmonary artery. The count of the ischemia time was started. In table, the aorta was cannulated maintaining the cannula above the aortic valve and coronary arteries ostium. The left atrium was totally exposed and the pumonary artery ostia were resectioned and the left atrium above the mitral valve was cannulated in order to avoid regurgitation. The pulmonary artery was cannulated and the cannula was inserted up to the right ventricular cavity. These three cannulas were fixed with polyester wires and connected to the line pressure.

The cannulas and the isolated working heart were fixed and positioned in a support device where they were maintained throughout the experiment. They were connected to the afferent tubes and the reperfusion was initiated interrupting the ischemic time.

Isolated working heart perperfusion

In the first 20 minutes of reperfusion, the isolated working heart was defibrillated when necessary and reperfused in passive mode until its development of satisfactory contractility. In passive mode, the blood aspirated from the carotid artery by roller pump was sent to the cannula in the isolated working heart aorta. This blood was drained by the cannula placed in the right ventricle (RV) by pulmonary artery and collected in cardiotomy reservoir, or drained by the left atrium cannula to another cardiotomy reservoir. Once collected in these reservoirs, the blood returned to the animal support by the roller pump. During this period of passive perfusion, the perfusion pressure of the isolated working heart aorta was monitored by the pressure lines and the flow of this perfusion was

measured by Doppler Fluxometer in the cannula placed in the aorta. The coronary flow was obtained by Doppler Fluxometer in the RV cannula, that collected the effluent from the isolated working heart.

After 20 minutes of passive reperfusion, the perfusion was converted to "working heart state". The flow from the animal support toward the isolated working heart's aorta was interrupted and the aspirated blood was sent to cardiotomy reservoir – working as a preload reservoir. From this reservoir, the blood was sent to the cannulated left atrium of the isolated working heart. The left atrium pressure was measured. The blood from the left ventricle was pumped from the isolated working heart through the aortic cannula toward an arterial reservoir. The pressure perfusion was measured and then achieving at the same time the necessary pre- and afterloads for the isolated working heart. The pressure in the aorta and left atrium were measured by the pressure lines connected to the cannulas and the pressure in the left ventricle was measured by 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 it was aspirated by the roller pumps and reinfused to the animal support to be oxygenated. The blood from the coronary sinus, correspondent to the coronary flow was drained from the right ventricle to the third reservoir and reinfused to the animal support (Figure 1).

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

Methodology

After 30 minutes of reperfusion (20 minutes of "passive reperfusion" followed by reperfusion in "working heart" mode),the stabilization data of the model were measured. After this, the infusion was converted again to a passive mode and the anterior interventricular artery was clamped and thus maintained for 30 minutes in order to induce regional ischemia. During this period, often occurred ventricular arrhythmias that were not treated (Figure 2). Then, the forceps of the anterior interventricular artery was removed and the perfusion was interrupted for 90 minutes. The models were randomly selected and divided into three groups: the isolated working hearts from the group 1 received (in every 30 minutes of this period) 100 ml of St. Thomas cardioplegic solution at 4ºC; the group 2 received the same dose of St Thomas with 10^5 molar (M) solution of trimetazidine (TMZ) and the group 3 was the Control Group (Co), without receiving cardioplegic solution.

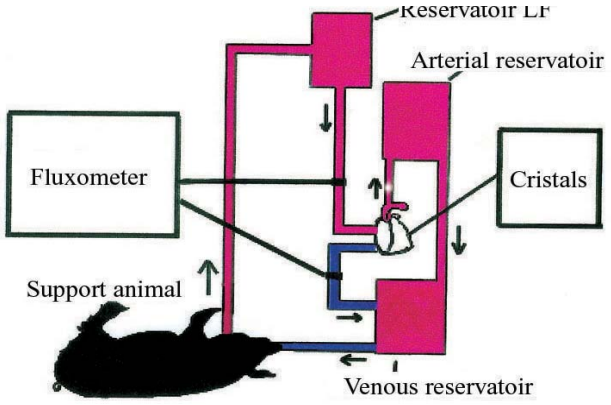

Fig.1- Isolated perfused working heart. The blood was aspirated from the support animal into the LA reservoir, which worked as preload and, from this reservoir to the isolated heart, from which it was ejected in the aorta into the arterial reservoir. The blood returned to a venous reservoir along with the effluent blood from the coronary sinus, drained by cannula in the pulmonary artery. From this reservoir, the blood was reinfused to the support animal. Height variations in the LA and arterial reservoirs corresponded to variations of pre- and postload, respectively. Under monitoring by the pressure lines in the LA, aorta, and LV by the piezoelectric crystals and the fluxometers.

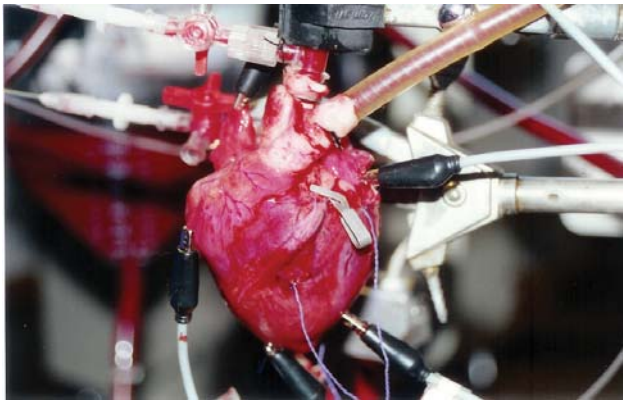

Fig.2 – Isolated heart during induction of regional ischemia. Cannula in RV to collect the effluent from the coronary sinus. Piezoelectric crystals. Mini forceps on anterior interventricular artery. Pressure line in the aortic root, measuring the coronary perfusion pressure. Line pressure in LA, measuring the pressure corresponding to preload. Probe for electrocardiographic record.

At the end of 90 minutes, the isolated working heart perfusion was restarted, (initially in passive mode) during 20 minutes. During this phase, the heart was defibrillated with shocks of 5 J as often as was necessary in order to maintain a satisfactory contractility. The total number of defibrillations were recorded. After 20 minutes, the isolated working heart perfusion was converted to the "working heart" mode and was determined pre- and afterload. The model was maintained in this working state until the end of the experiment. After 30 minutes from the start of reperfusion, the measurements were performed with the help of equipment already mentioned. The measurements were taken again at 60 and 90 minutes of reperfusion.

During the whole procedure, arterial gasometry examinations and sodium, potassium and hematocrit dosage of the animal support were periodically performed. The animal support was anesthetized and maintained with mean blood pressure around 80 mmHg, to receive the venous return of its blood by circuit and crystalloid replacement. 8.4% sodium bicarbonate was administered when needed in order to avoid acidosis in the animal support. At no time any cardiotonic agent was used.

Measurements obtained with isolated working heart in working state mode

During the isolated working heart (in working state mode) were measured:

- Cardiac output and volume of the left ventricle (LV) through synchronized piezoelectric crystals, fixed to the epicardium and connected to TRX6 synchronizer;
- LV pressure (by pressure line);
- Pressure of the left atrium (by pressure line) corresponding to the offered pre-load;
- Pressure in the aortic root (by pressure line);
- Aortic and coronary flow by the Doppler Fluxometers;
- Electrocardiographic recording;
- Gasometry in the effluent blood and the animal support, thus allowing to determine the oxygen volume and consumption of the isolated working heart;
- Glycemia and lactate of the effluent blood from the isolated working heart and the animal support;
- Plotting of the volume-pressure curves (by sonic synchronization of the piezoelectric crystals performed by the software);
- Maximum elastance (Emáx) – relation between the systolic pressure and the ventricular volume;
- Preload-recruitable stroke work (PRSW) – linear relationship between systolic work and end-diastolic volume;
- Exponencial regression between the diastolic pressure and end-diastolic volume (EDPRV - "*end diastolic pressure volume relationship*"), allowing the measurement of the ventricular rigidity;

These last three parameters were obtained from the analysis of volume and pressure curves, allowed by the software in records accomplished with pre-load variation. All the above measurements were taken in determined periods and recorded by the software CardioSoft 3.1.2 (Sonometrics, London, Ontario, Canada).

The oxygen consumption was obtained by means of the difference between the arterial blood sample from the animal support and the blood collected from the coronary sinus in the right ventricle cannula, according to the formula: MVO2 = 0.1 X Coronary flow / Final Weight of the left ventricle X [(SO2a - SO2v) X 1.34 X Hb / 100 + 0.03 X 7,5 X (pO2a - pO2v)], where MVO2 and consumption of oxygen (expressed by 100g of myocardium, SO2a and SO2v) are the oxygen saturation (in %) from the animal support and the coronary sinus of the isolated working heart respectively; Hb and the hemoglobin volume (in g/L) and pO2a and pO2v are the partial pressures of oxygen from the animal support and the coronary sinus of the isolated working heart, respectively [10].

After measurements at 90 minutes of reperfusion, the experiment was interrupted with euthanasia of the animal support, with a lethal dose of potassium chloride. The isolated heart was dissected by removing of the right ventricle wall, atria and great vessels and then, weighing [11]. Samples of the left ventricle on the area submitted to regional ischemia were sent to histological analysis.

Statistical analysis

The results are shown as mean ± standard error of the mean. For comparison among groups in different periods, analysis of variance (ANOVA) associated with Bonferroni test was used for differences identification. A p d" 0.05 value was considered significant. The tests were performed using the statistical package for Windows Graphpad Prism (Graphpad Software, San Diego, California).

RESULTS

The animals support were maintained hemodynamically stable throughout the experiment without differences in relation to the partial pressure of oxygen and the pH. The hematocrit was lower in the group St Thomas than the others, but without significant variation along the experiment (Figure 3).

In the period of reperfusion, after 30 minutes of regional ischemia, followed by 90 minutes of global ischemia, the Control Group and the group St Thomas presented average of five defibrillations. The group Trimetazidine presented average of one defibrillation, but not demonstrated statistically significant difference. The final wet weight of the left ventricle showed statistically significant difference (p d" 0.05) between the group Trimetazidine and the others,

being lesser the final wet weight of the left ventricle in the group Trimetazidine. There was no difference between St. Thomas and Control groups.

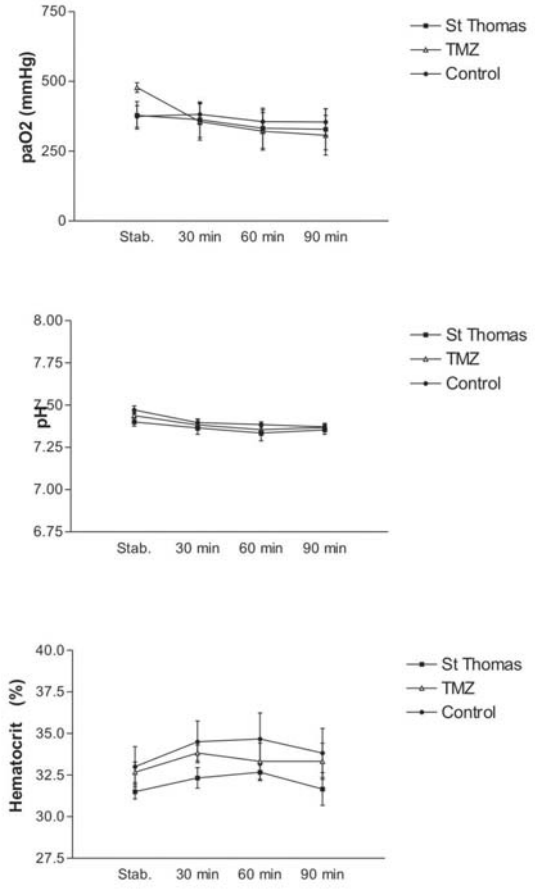

Fig. 3 - Oxygen partial pressure (pO_2a) (above), pH (middle) and hematocrit (below) of the support animals throughout the procedure. Data presented as mean and two standard errors of the mean (SEM) (n = 6) in each group.

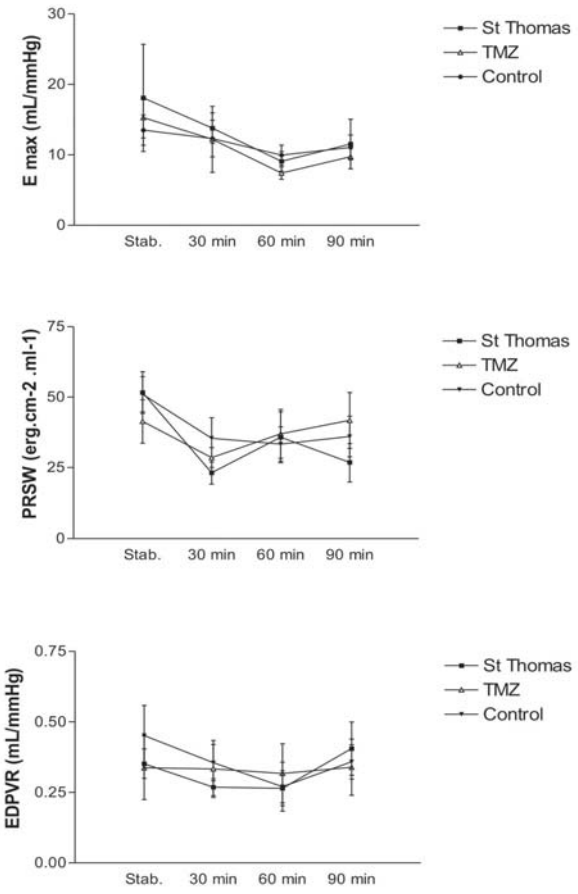

Fig. 4 – Contractility indexes. Maximum elastance(above), PRSW (middle), "rigidity" or "hardness" of the ventricle (EDPVR) (below) of isolated hearts. Stabilization measurements and at 30, 60 and 90 minutes of reperfusion in "working heart" mode after periods of regional and global ischemia. Data presented as mean and two standard errors of the mean (SEM) (n = 6) in each group.

The contractility indexes (less dependent on external parameters, such as pre- and afterload and cardiac frequency) are shown in Figure 4 [12]. The maximum elastance presented an important decrease of estabilization measurements at 30, 60 and 90 minutes of reperfusion.

However, there was no statistically significant difference between the three groups. The Preload-recruitable stroke work (PRSW) showed no statistically significant difference between the three groups, and "EDPVR" related to the "hardness" or "inflexibility" of heart, remained stable and with no significant statistical difference between the three groups.

The coronary flow presented great dispersion in the results of St Thomas and Control groups, whilst presented more regular in the group Trimetazidine, however, there was no statistically significant difference between the three groups.

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

The oxygen consumption was obtained according to the formula described above and there was no statistically significant difference among the three groups. The consumption of glucose at 30 minutes of reperfusion showed statistically significant difference between the Control group and the group Trimetazidine (p d" 0.05), but this difference was not verified at 60 and 90 minutes of reperfusion among the three groups (Figure 5).

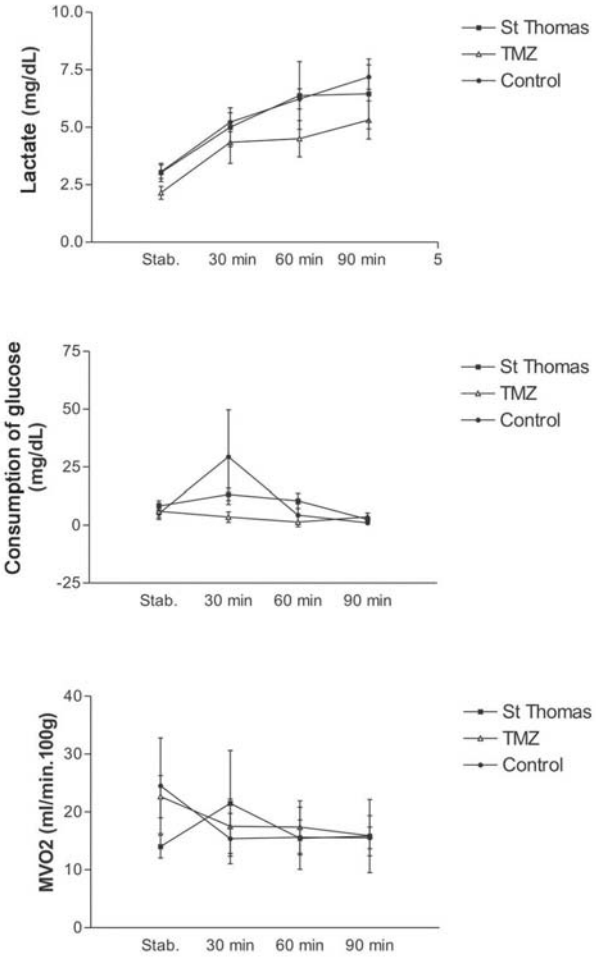

Fig. 5 -Metabolic data. Effluent lactate from the isolated heart (above), glucose consumption (middle) and oxygen consumption (below) of the isolated heart. Stabilization measurements and at 30, 60 and 90 minutes of reperfusion in "working heart" mode after periods of regional and global ischemia. Data presented as mean and two standard errors of the mean (SEM) (n = 6) in each group.

DISCUSSION

Trimetazidine has been used with general acceptance in Cardiology for the symptomatic control of angina [2.13]. In 2003, Marzilli and Klein [14] published metanalysis comparing the effectiveness of trimetazidine with the monotherapy, beta blockers or calcium channel blockers, with good results for the treatment of symptomatic angina. The trimetazidine is a drug without direct hemodynamic effect [15]. The justification for its use may be its ability to inhibit the mitochondrial 3-ketoacyl thiolase, increasing the glucose oxidation in the heart due to deviation of the metabolism of fatty acids oxidation for glucose consumption [2].

The human heart produces ATP from the substrate that may be more available - such as glucose or fatty acids - but in normal conditions his energy is based on almost 90% in the fatty acids metabolism [16.17]. In ischemia, gradually increases the importance of glucose in the production of ATPs. According to Stanley [5.18], the number of "mols" of the ATP produced by "mol" of consumed oxygen is 12% higher (from the glucose oxidation) than that produced from the fatty acids.

Trimetazidine, acting as agent of metabolic manipulation, may be able to deflect the energy production from fatty acids to promote glucose and lactate consumption, producing high-energy phosphate with lower oxygen consumption [19]. This mechanism may be able to minimize the ischemic dysfunction and improve the heart performance.

The largest series with the use of trimetazidine in surgical patients were obtained from pre-treatment. Fabiani et al. [6] published serie in which 19 patients orally treated with trimetazidine over three weeks underwent elective CABG. An improvement in the index of left ventricular systolic work was found comparing to placebo, without finding of survival differences.

Vedrinne et al. [7] evaluated the contractile function by intraoperative echocardiography of patients undergone elective CABG who received trimetazidine in the preoperative. The echocardiographic measurements of the fractional area and the percentage of systolic thickening were analyzed and no differences were found in relation to the group treated with placebo. Tünerir et al. [8] randomized 30 patients who were orally treated with 60mg of Trimetazidine or placebo over three weeks before CABG and they obtained lower levels of T troponin with Trimetazidine without any statistically significant difference in hemodynamic function of survival.

Experimental models of isolated working heart for investigation of different treatments are widely used. Rodrigues et al. [20] studied the effect of intermittent administration of cardioplegic solution in hypothermia or

normothermia in a model of acutely ischemic isolated working rabbit heart. This same group [21], achieving to evidence effects of addition of glutamate and aspartate in intermittent cardioplegic solution did not find significant differences between the two treatments.

The animal model used in this study was developed with the purpose of stablishment of greater similarity with clinical situation in which the acutely ischemic heart may need to be operated. The clamping of the anterior interventricular artery of the animal - in order to characterize in the model a situation of acute ischemia with the heart soon to receive treatment - was a strategy very similar to that described by Horsley et al. [22] in 1993. Thus, the aim was to promote an appropriate situation to evaluate the effect of acute administration of trimetazidine associated to cardioplegic solution in acute ischemic heart to be operated.

The model used in this study allowed the measurement of the data with the heart in "working heart state". The achievement of data in this mode (and not during simple passive reperfusion of the organ) allowed the study of the proposed treatment in condition more similar to the physiological condition.

The contractility indexes compared herein were those less dependent on external factors, obtained from the volume-pressure curves. According to Grossman et al. [23], the maximum elastance (Emáx) is an index relatively (but not completely) independent of momentary pre- and afterload. It corresponds to the curve obtained by the association of several points that reffering to the end of systole, obtained in following volume-pressure curves (obtained by means of the partial occlusion and the time of the inferior vena cava. In this study, the variation that allowed measurement of this data was obtained with the displacement of the reservoir reffering to the pre-load.

The maximum elastance demonstrates the capacity of the ventricle to create pressure when the intraventricular volume is high. Ventricles that demonstrate good contractile capacity will increase the pressure when distended, while ventricles with worst function will show less tolerance to this distension [12]. The pre-recruitable systolic work (PRSW) possibly may be the contractility index less dependent on other parameters within physiological values [24.25]. In these two data related to the systolic function was not shown significant improvement with the association of trimetazidine.

The EDPVR ("end diastolic pressure volume relationship") is an exponential regression between diastolic pressure and the end-diastolic volume obtained from pressure-volume curves. The EDPVR implies on the ventricular volume-pressure relantioship at the end of diastole. It provides, thus, the rigidity or the "hardness" of the ventricle [25].

Petrucci Jr. [12], in isolated working heart model "not working heart", found improvement of the elastance in the group treated with St Thomas in relation to the Control Group at 60 and 90 minutes of reperfusion. In relation to the other hemodynamic parameters studied by this author, the blood mean was better compared to both St Thomas as the Control group at all times, but without difference between these two other groups. In this study, in order to respond if the trimetazidine could offer improvement when combined with cardioplegic solution was used only the crystalloid mean Thus, possible benefit that was verified may be indeed attributable to trimetazidine. The lack of improvement in hemodynamic behavior with the use of any method of myocardial protection in relation to the Control group possibly may be attributed to the refinement of this model to include measured data when in "working heart state" mode.

The sample size was selected from studies of Petrucci Jr. [12.26] using parabiotic circulation in pigs. The methodology related to the cross circulation and the isolated heart perfusion was developed from these studies, adding the steps that allowed the achievement of data in "working heart", due the already described reasons.

The lack of contractility improvement with association of trimetazidine observed in this study meets the surgical experience with the pretreatment with trimetazidine, in which little improvement was observed and, when found, did not bring benefit to the patients survival. Despite these studies were performed in operated patients and the verified hemodynamic data were not independent of external parameters, the hemodynamic results were similar [6-8].

According to the mechanism of action of the trimetazidine, may be expected that the possible benefit caused by this agent should be initially verified as an improvement of the metabolic parameters.

Allibardi et al. [27], in an experimental isolated rat heart model perfused with 10-6 M trimetazidine, verified improvement in acidosis and in the lactate production during reperfusion and improvement in the levels of tissue high-energy phosphate. Several authors have contributed in the understanding of the benefits related to trimetazidine and its capacity to reduce releasing of lactate, promoting less acidosis, glucose consumption and decrease of the oxygen consumption [19.28]. Fabiani et al. [6] verified significantly lower levels of malondialdehyde extracted from the coronary sinus from patients undergone CABG at 20 minutes of reperfusion. These findings strongly suggest trimetazidine's capacity to increase the lactate consumption in the myocardium and to reduce the damage of ischemia and reperfusion during heart surgery .

The total number of defibrillations were lower in the group Trimetazidine, but without statistically significance different. Hoersley et al. [22] in experiments with cardiopulmonary bypass in dogs, verified the improvement in the needed number of defibrillations when they used blood cardioplegia in relation to the Control, but without difference between the crystalloid solution and the Control group. These results were similar to those verified in this study.

The final wet weight of the left ventricle was significantly lower in the group Trimetazidine than in the Control group ($p < 0.05$).

The histological analysis showed less edema, both in group Trimetazidine as in the St Thomas in relation to the Control group. These results demonstrated some protection offered by cardioplegic solutions in both groups. The lack of difference verified in the contractility indexes and in the metabolic parameters between the two groups that received myocardial protection and the Control group may be explained due to the collecting data obtained in a "working heart" model. With this refinement, the authors found no hemodynamic or metabolic improvement attributable to cardioplegic solutions compared to Control group. Anyway, this fact did not impair the aiming of this study to find a possible benefit caused by trimetazidine.

The lack of improvement in oxygen and glucose consumption and in the lactate production in the group that received trimetazidine in relation to the other may not be associated to the failure of the drug itself, but due to the way of drug administration. The acute administration without previous use of the drug is verified in a few studies, without reports of experimental study in isolated working heart of the great mammals.

Kober et al. [29] published article approaching acute intracoronary administration of trimetazidine during coronary angioplasty and they found earlier resolution for ST deflection without survival benefit. Beygui [30] in 2000, and Steg et al. [31] in 2001, obtained similar results with acute intravenous administration of trimetazidine. The EMIP-FR [32] multicenter randomized study, developed between 1993 and 1999 with more than 19,000 patients who acutely received intravenous trimetazidine or placebo associated to AMI thrombolysis, did not show improvement in their final purposes of short- and long-term survival increase in patients who received thrombolytics. However, it demonstrated statistically significant improvement in survival for those not thrombolized patients due medical reasons [32].

Grynberg [33], in his comment on publication of the EMIP-FR study, proposes that the absence of verifiable benefit of trimetazidine associated to the thrombolysis may be considered because any benefit of this agent may depend on inhibition of fatty acids beta-oxidation. The uncoupling of glycolysis occurred during ischemia and the excessive production of hydrogen ions by the resulting situation of severe anaerobiosis may cause the obstruction of the trimetazidine (acutely administred) to effectively act the mentioned inibition of fatty acids beta-oxidation.

The lack of improvement with acute administration of trimetazidine associated to cardioplegic solution may be related to the impossibility of this agent to remove free radicals, due the fact that most of the ischemia and reperfusion lesion occur during the reperfusion [28]. The administrated dose was used according to the literature and its infusion at the aortic root ensured the regular administration of the drug to the cardiomyocytes [27]. In the oter hand, the hypotesis of Grynberg for the lack of improvement with the use of acute trimetazidine in the EMIP-FR study may be appropriate to justify the lack of benefits observed in this study.

Experimental studies have demonstrated the importance of free radicals in the lesion during reperfusion [28]. Studies in rats prove the benefit of the pretreatment effect with trimetazidine in isolated working heart models [34-36]. Studies in patients pretreated with pre-trimetazidine undergone CABG show benefit in several parameters. However, the clinical situation of acutely ischemic patient does not allow other approach unless the verification of its effect in exclusively acute use.

Budrikis et al. [10], in studies with cold crystalloid solution with isolated working pig heart model, report the importance of experimental observation over long periods such as 12 hours, in order to verify differences in hemodynamic parameters of ischemia and reperfusion in animal models. Although no tendency has been observed in this study that may assume any change in the obtained results, it is clear that longer periods of observation may always show not easily suspectable results.

CONCLUSION

Therefore, the authors conclude that the acute administration of trimetazidine associated with cardioplegic soluction without pretreatment did not offer hemodynamic or metabolic benefit to the heart in the experimental model used in this study.

REFERENCES

1. Desideri A, Celegon L. Metabolic management of ischemic heart disease: clinical data with trimetazidine. Am J Cardiol. 1998;82(5A):50K-53K.

2. Lavanchy N, Martin J, Rossi A. Anti-ischemic effects of trimetazidine: 31P-NMR spectroscopy in the isolated rat heart. Arch Int Pharmacodyn Ther. 1987;286(1):97-110.

3. Belardinelli R. Trimetazidine and the contractile response of dysfunctional myocardium in ischaemic cardiomiopathy. Rev Port Cardiol. 2000;19(Suppl 5):V35-9.

4. Knight C, Fox K. From antianginal drugs to myocardial cytoprotective agents. Am J Cardiol. 1995;76(6):4B-7B.

5. Stanley WC. Changes in cardiac metabolism: a critical step from stable angina to ischaemic cardiomiopathy. Eur Heart J. 2001;3(suppl):2-7.

6. Fabiani JN, Ponzio O, Emerit I, Massonet-Castel S, Paris M, Chevalier P, et al. Cardioprotective effect of trimetazidine during coronary artery graft surgery. J Cardiovasc Surg. 1992;33(4):486-91.

7. Vedrinne JM, Vedrinne C, Bompard D, Lehot JJ, Boissel JP, Champsaur G. Myocardial protection during coronary artery bypass graft surgery: a randomized, double-blind, placebo-controlled study with trimetazidine. Anaesth Analg. 1996;82(4):712-8.

8. Tünerir B, Colak O, Alatas O, Besogul Y, Kural T, Aslan R. Measurement of troponin T to detect cardioprotective effect of trimetazidine during coronary artery bypass grafting. Ann Thorac Surg. 1999;68(6):2173-6.

9. Colégio Brasileiro de Experimentação Animal. Princípios éticos na experimentação animal. In: São Paulo:Congresso do Colégio Brasileiro de Experimentação Animal; 1991.

10. Budrikis A, Bolys R, Liao Q, Ingemansson R, Sjöberg T, Steen S. Function of adult pig hearts after 2 and 12 hours of cold cardioplegic preservation. Ann Thorac Surg. 1998;66(1):73-8.

11. Brooks WW, Healey NA, Sen S, Conrad CH, Bing OH. Oxygen cost of stress development in hypertrophied and failing hearts from the spontaneously hypertensive rat. Hypertension. 1993;21(1):56-64.

12. Petrucci Jr O. Estudo comparativo entre solução cardioplégica cristalóide e microcardioplegia sanguínea normotérmica em corações agudamente isquêmicos [Tese de Doutorado]. Campinas:Universidade Estadual de Campinas;2004.

13. Detry JM, Leclercq PJ. Trimetazidine European Multicenter Study versus propranolol in stable angina pectoris: contribution of Holter electrocardiographic ambulatory monitoring. Am J Cardiol. 1995;76(6):8B-11B.

14. Marzilli M, Klein WW. Efficacy and tolerability of trimetazidine in stable angina: a meta-analysis of randomized, double-blind, controlled trials. Coron Artery Dis. 2003;14(2):171-9.

15. Cesar LAM, Dallan LA, Gowdak LHW, Lisboa LAF, Moretti MA. Alternatives for the treatment of angina pectoris: new drugs, external counterpulsation, spinal-cord stimulation for analgesia, therapeutic angiogenisis and transmyocardial revascularization. Rev Soc Cardiol Estado de São Paulo. 2003;13(2):240-53.

16. Braile DM. In: Cardioplegia sanguínea isotérmica anterógrada e retrógrada de baixo volume. São José do Rio Preto;1997.

17. Herrmann JLV, Stefanini E. Drug therapy. Choosing the best schedule. Rev Soc Cardiol Estado de São Paulo. 2003;13(2):196-203.

18. Stanley WC. Cardiac energetics during ischemia and the rationale for metabolic interventions. Coron Artery Dis. 2001;12(Suppl 1):53-7.

19. Pichon G, Rousseau D, Helies C. Control by Trimetazidine of the energy substrate balance in ventricular myocite. J Moll Cell Cardiol. 1999;31:A88.

20. Rodrigues AJ, Vicente WVA, Carneiro JJ, Bassetto S. Cardioplegia sanguínea anterógrada intermitente normotérmica e hipotérmica: estudo comparativo em corações agudamente isquêmicos de coelhos. Rev Bras Cir Cardiovasc. 2000;15(3):238-50.

21. Rodrigues AJ, Sader AA, Vicente WVA, Bassetto S. Cardioplegia sanguínea normotérmica intermitente anterógrada. II. Com e sem aminoácidos: estudo comparativo em coelhos. Rev Bras Cir Cardiovasc. 1997;12(4):340-7.

22. Horsley WS, Whitlark JD, Hall JD, Gott JP, Pan-Chih, Huang AH, et al. Revascularization for acute regional infarct: superior protection with warm blood cardioplegia. Ann Thorac Surg. 1993;56(6):1228-37.

23. Grossman W, Braunwald E, Mann T, McLaurin LP, Green LH. Contractile state of the left ventricle in man as evaluated from end-systolic pressure-volume relations. Circulation. 1977;56(5):845-52.

24. Glower DD, Spratt JA, Snow ND, Kabas JS, Davis JW, Olsen CO, et al. Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. Circulation. 1985;71:994-1009.

25. Guyton RA. A synopsis of cardiac physiology. In: Edmunds LH. Cardiac surgery in the adult. 1st ed. New York:McGraw-Hill Companies;1997. p.59-84.

26. Petrucci Júnior O, Oliveira PP, Carmo MR, Vieira RW, Braile DM. Standardization of an isolated pig heart preparation with parabiotic circulation: methodological considerations. Braz J Med Biol Res. 2003;36(5):649-59.

27. Allibardi S, Chierchia SL, Margonato V, Merati G, Neri G, Dell'Antonio G, et al. Effects of trimetazidine on metabolic and functional recovery of postischemic rat hearts. Cardiovasc Drugs Ther. 1998;12(6):543-9.

28. Kantor PF, Lucien A, Kozak R, Lopaschuk GD. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. Circ Res. 2000;86(5):580-8.

29. Kober G, Buck T, Sievert H, Vallbracht C. Myocardial protection during percutaneous transluminal coronary angioplasty: effects of trimetazidine. Eur Heart J. 1992;13(8):1109-15.

30. Beygui F. Trimetazidine as adjunctive therapy to primary PTCA for acute myocardial infarction. Rev Port Cardiol. 2000;19(Suppl 5):V31-4.

31. Steg PG, Grollier G, Gallay P, Morice M, Karrillon GJ, Benamer H, et al. A randomized double-blind trial of intravenous trimetazidine as adjunctive therapy to primary angioplasty for acute myocardial infarction. Int J Cardiol. 2001;77(2-3):263-73.

32. The European Myocardial Infarction Project-Free Radicals (EMIP-FR) Group. Effect of 48-h intravenous trimetazidine on short- and long-term outcomes of patients with acute myocardial infarction, with and without thrombolitic therapy; A double-blind, placebo-controlled, randomized trial. The EMIP-FR Group. European Myocardial Infarction Project-Free Radicals. Eur Heart J. 2000;21(18):1537-46.

33. Grynberg A. The EMIP-FR Study: the evolution of scientific background as a non-controlled parameter. Eur Heart J. 2001;22(11):975-7.

34. Ikizler M, Erkasap N, Dernek S, Batmaz B, Kural T, Kaygisiz Z. Trimetazidine-induced enhancement of myocardial recovery during reperfusion: a comparative study in diabetic and non-diabetic rat hearts. Arch Med Res. 2006;37(6):700-8.

35. Ikizler M, Dernek S, Sevin B, Kural T. Trimetazidine improves recovery during reperfusion in isolated rat hearts after prolonged ischemia. Anadolu Kardiyol Derg. 2003;3(4):303-8.

36. Onay-Besikci A, Guner S, Arioglu E, Ozakca I, Ozcelikay AT, Altan VM. The effects of chronic trimetazidine treatment on mechanical function and fatty acid oxidation in diabetic rat hearts. Can J Physiol Pharmacol. 2007;85(5):527-35.