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Comparative experimental study of myocardial protection with crystalloid solutions for heart transplantation

Estudo comparativo experimental da proteção miocárdica com soluções cristalóides para transplante cardíaco

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Abstract

Background: **There is a growing need to improve myocardial protection, which will lead to better performance of cardiac operations and reduce morbidity and mortality. Therefore, the objective of this study was to compare the efficacy of myocardial protection solution using both intracellular and extracellular crystalloid type regarding the performance of the electrical conduction system, left ventricular contractility and edema, after being subjected to ischemic arrest and reperfusion.**

Methods: **Hearts isolated from male Wistar (n=32) rats were prepared using Langendorff method and randomly divided equally into four groups according the cardioprotective solutions used Krebs-Henseleit-Buffer (KHB), Bretschneider-HTK (HTK), St. Thomas-1 (STH-1) and Celsior (CEL). After stabilization with KHB at 37°C, baseline values (control) were collected for heart rate (HR), left ventricle systolic pressure (LVSP), maximum first derivate of rise left ventricular pressure (+dP/dt), maximum first derivate of fall left ventricular pressure (-dP/dt) and coronary flow (CF). The hearts were then perfused at 10°C for 5 min and kept for 2 h in static ischemia at 20°C in each cardioprotective solution. Data evaluation was done using analysis of variance in completely randomized One-Way ANOVA and Tukey's test for multiple comparisons. The level of statistical significance chosen was *P*<0.05.**

Results: **HR was restored with all the solutions used. The evaluation of left ventricular contractility (LVSP, +dP/dt and -dP/dt) showed that treatment with CEL solution was better compared to other solutions. When analyzing the CF, the HTK solution showed better protection against edema.**

Conclusion: **Despite the cardioprotective crystalloid solutions studied are not fully able to suppress the deleterious effects of ischemia and reperfusion in the rat heart, the CEL solution had significantly higher results followed by HTK>KHB>STH-1.**

Keywords: **Heart arrest, induced. Myocardial reperfusion injury. Cardioplegic solutions. Ventricular function, left. Rats, Wistar.**

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Abbreviations, acronyms & symbols	
ABTO	Brazilian Association for Organ Transplant / Associação Brasileira de Transplante de Órgãos
CEL	Celsior solution
CF	coronary flow
COBEA	Brazilian College of Animal Experimentation / Colégio Brasileiro de Experimentação Animal
+dP/d*t*	peak positive of the first derivative of left ventricular pressure
-dP/d*t*	peak negative of the first derivative of left ventricular pressure
HR	heart rate
HTK	Bretschneider-HTK solution
KHB	Krebs-Henseleit-Buffer solution
LVSP	left ventricle systolic pressure
STH-1	St. Thomas No. 1 solution
STH-2	St. Thomas No. 2 solution

Resumo

Introdução: **Existe crescente necessidade de aprimorar a proteção miocárdica, para melhor desempenho das operações cardíacas e diminuição da morbimortalidade. Portanto, o objetivo deste estudo foi comparar a eficácia da proteção miocárdica usando tanto solução cristaloide tipo intracelular como extracelular quanto ao desempenho do sistema de condução elétrica, contratilidade do ventrículo esquerdo e edema, após parada isquêmica e posterior reperfusão.**

Métodos: **Corações isolados de ratos Wistar foram montados em Langendorff e aleatoriamente divididos em quatro grupos. de acordo com as soluções cardioprotetoras utilizadas Krebs-Henseleit-Buffer (KHB), Bretschneider-HTK (HTK), St. Thomas-1(STH-1) e Celsior (CEL). Após a estabilização com KHB a 37°C, valores basais (controle) foram coletados para frequência cardíaca (FC), pressão sistólica do ventrículo esquerdo (PSVE), derivada máxima de aumento da pressão ventricular esquerda (+dP/dt), derivada máxima de queda da pressão ventricular esquerda (-dP/dt) e fluxo coronariano (FCo). Os corações foram então perfundidos a 10°C por 5 min e mantidos por 2 h em isquemia estática a 20°C em cada solução cardioprotetora. Avaliação dos dados foi por análise de variância inteiramente casualizados em One-Way ANOVA e teste de Tukey para comparações múltiplas. O nível de significância estatística escolhido foi *P*<0,05.**

Resultados: **Houve recuperação da FC com todas as soluções utilizadas. A avaliação da contratilidade ventricular esquerda (PSVE, +dP/dt e -dP/dt) demonstrou que o tratamento com a solução CEL foi melhor em comparação às outras soluções. Ao analisar o CF, a solução HTK indicou melhor proteção contra edema.**

Conclusão: **Apesar das soluções cristaloides cardioprotetoras estudadas não serem capazes de suprimir os efeitos deletérios da isquemia e reperfusão no coração de ratos, a solução CEL apresentou resultado superior seguido por HTK>KHB>STH-1.**

Descritores: **Parada cardíaca induzida. Traumatismo por reperfusão miocárdica. Soluções cardioplégicas. Função ventricular esquerda. Ratos Wistar.**

INTRODUCTION

Currently, most heart surgeries are performed with anoxic arrest induced by using different cardioplegic solutions, suggesting the lack of a gold standard for myocardial protection [1]. Procedures with short period of ischemia, preservation is simpler. However, procedures where long ischemic periods are common, myocardial viability may be compromised by the current methods of myocardial preservation [2]. Thus, establishing satisfactory method of preservation is critical to ensure success in procedures with prolonged ischemic time, particularly in cardiac transplantation, which can also lead to expanding the pool of donors [2].

Due to the shortage of donated hearts, selection criteria are under constant review in order to increase the number of marginal donors [3,4]. Nevertheless, studies in the field of myocardial protection have great relevance for the advancement of heart transplantation [1]. Prolonged myocardial ischemia is an independent risk factor for early and late survival of the patient [4].

The crystalloid cardioplegic solutions were initially idealized in order to depolarize the cell membrane. Thus, their initial formulations were basically ionic. The progress of research on myocardial protection showed the need for additives in the solutions to expand their effectiveness. The main actions of additives aimed at removal of free radicals, providing nutrients, prevention of intracellular acidosis and stabilization of cell membranes to minimize swelling [5].

Studies on myocardial protection with additives in the solutions associated with hypothermia, demonstrated improved contractile function after long periods of ischemia [6]. Hypothermia protects cellular energy metabolism acting improving the resistance to ischemia in cardioplegic cardiac arrest [7]. The increase in the ratio between supply and energy demand during ischemia is generally attributed to hypothermic protection. The hypothermia also combats oxidative stress induced by ischemia and reperfusion [8].

There is still growing need to further investigate and improve heart preservation methods, thus improving performance of cardiac operations, reducing morbidity, increasing the donor pool, and extending its indications and benefits [4].

The objective of this study was to compare the efficacy of myocardial protection solution using both intracellular

and extracellular crystalloid type regarding the performance of the electrical conduction system, left ventricular contractility and edema, after being subjected to ischemic arrest and reperfusion.

METHODS

The Brazilian College of Animal Experimentation (COBEA) and the Ethics Committee of the Fundação Cardiovascular São Francisco de Assis, Belo Horizonte, Minas Gerais, Brazil, approved all experiments. All experiments used an isolated isovolumetrically contracting rat heart. Male Wistar albino rats (n=32), 310 to 320 g, were anesthetized by intraperitoneal injection of a mixture of ketamine (50 mg/kg) and xilazine (10 mg/kg). After the chest was opened, heparin (500 IU) was injected into the left atrium. An aortic cannula filled with perfusate was rapidly inserted into the aorta, and retrograde perfusion was started with an oxygenated Krebs-Henseleit buffer at 37°C and maintained at a constant pressure of 100 mmHg in a single-pass way by the Langendorff technique [9]. The pulmonary artery was incised to allow outflow of the perfusate. A latex balloon was placed in the left ventricle and connected to a pressure transducer line. The balloon was inflated with water to create a diastolic pressure of 7 to 9 mmHg. The hearts were beating spontaneously at an average rate of 300 beats/min. After 15 min of perfusion at 37°C with KHB solution for stabilization, we collected the values considered baseline (control) for the following parameters: heart rate (HR) to evaluate the electrical conduction system; left ventricle systolic pressure (LVSP), the maximum rate of rise in left ventricular (+d*P*/d*t*), the maximum rate of fall in left ventricular (-d*P*/d*t*) pressures to evaluate the ventricular contractility and coronary flow (CF) to evaluate the edema.

The hearts were randomly divided equally into four groups, as follows: Group 1 were treated with Krebs-Henseleit (KHB) solution (Research Laboratory of Fundação Cardiovascular São Francisco de Assis, Belo Horizonte, MG, Brazil), Group 2 with Bretschneider-HTK (HTK) solution (Dr. Franz Köhler Chemie GMBH - Germany), Group 3 with St. Thomas No. 1 (STH-1) solution (Braile Biomédica Industry, Sâo Paulo, SP, Brazil), and Group 4 with Celsior (CEL) solution (Genzyme Polyclonals S.A.S., France).

The hearts were then perfused with their respective cardioprotective solutions for 5 min at 10°C and kept for 2 h in static ischemia at 20°C. Subsequently, the hearts were reperfused with KHB at 37°C for 60 min and data were collected every 5 min. Data evaluation was based on analysis of variance in completely randomized One-Way ANOVA and Tukey's test for multiple comparisons. The criterion for significance was $P<0.05$ for all comparisons.

RESULTS

To evaluate myocardial protection, we first compared the effect of the solutions on HR. Figure 1 shows the trend in HR of the solutions used in the experiment at 10°C, compared with the control, represented by a ratio of 1.0 (basal HR). CEL and KHB solutions provided a more stable HR throughout the length of the experiment. On the other hand, use of HTK and STH-1 solutions initially resulted in lower HR, which increased after 15 min and 30 min, respectively, and stabilized at a similar HR compared to the other solutions. These results indicated that all four solutions were able to recover the HR.

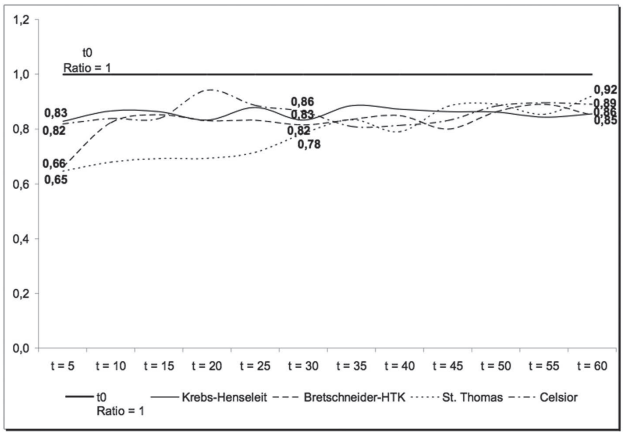

Fig. 1 - Heart rate (HR), according to the solution. Reperfused hearts were monitored for 60 min after treatment with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas No. 1, and Celsior. Baseline was calculated after stabilization and prior to treatment

Left ventricular contractility was represented by the corresponding hemodynamic variables LVSP, (+d*P*/d*t*), and (-d*P*/d*t*) (Figures 2 to 4). These variables show similar trends with the different solutions. CEL solution was more stable and with higher rates compared to the other solutions. With the HTK solution, rates increased constantly throughout the 60 min period, and were higher compared to STH-1 and KHB. KHB solution resulted in higher rates for all variables compared to STH-1, but was still lower than HTK at LVSP and (-d*P*/d*t*). Despite these differences, KHB reached approximately the same rate of (+d*P*/d*t*) after 40 min, compared to HTK. The contractile performance of STH-1 was lower than the other solutions. Here, the data show that treatment with CEL is superior to the others solutions.

Because the occurrence of edema, which is a negative factor in the recovery of the heart, the CF was considered in the corresponding hemodynamics variables dynamics. All treatments showed a downward trend (Figure 5). However, treatment with HTK solution produced higher

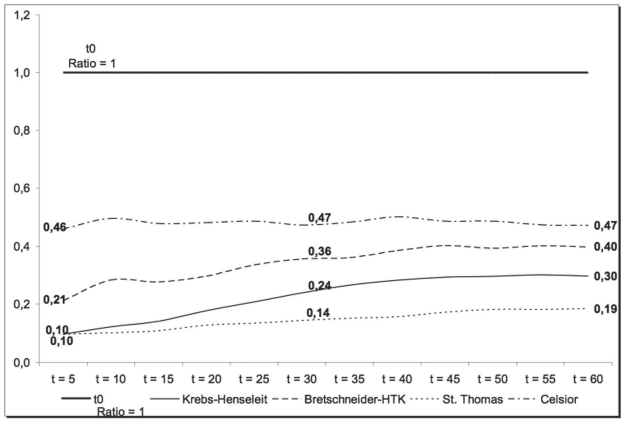

Fig. 2 - Left ventricle systolic pressure (LVSP) according to the solution. Reperfused hearts were monitored for 60 min with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas No. 1, and Celsior. Baseline was calculated after stabilization and prior to treatment

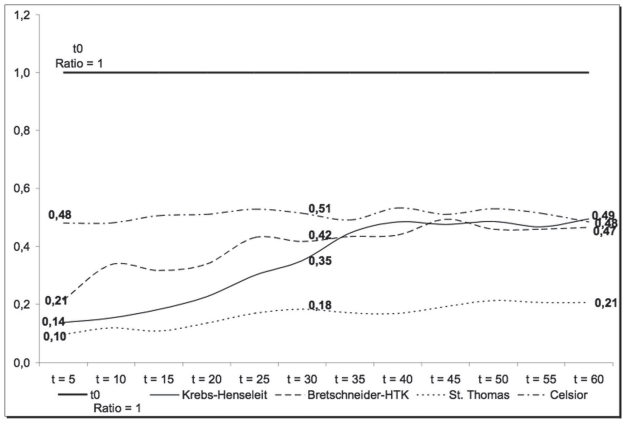

Fig. 3 - Maximum rate of rise in left ventricular pressure (+dP/dt), according to the solution. Reperfused hearts were monitored for 60 min with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas No. 1, and Celsior. Baseline was calculated after stabilization and prior to treatment

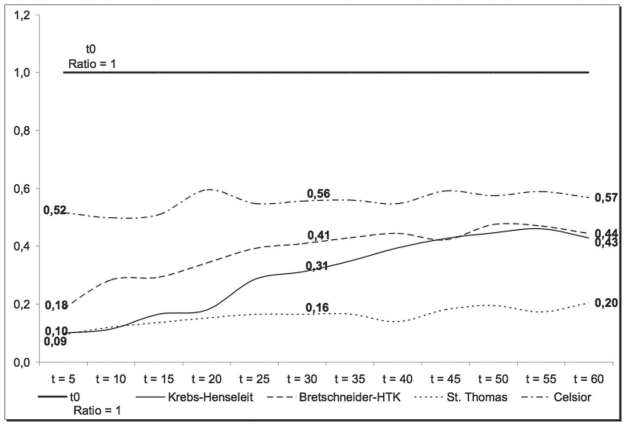

Fig. 4 - Maximum rate of fall in left ventricular pressure (-dP/dt), according to the solution. Reperfused hearts were monitored for 60 min with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas No. 1, and Celsior. Baseline was calculated after stabilization and prior to treatment

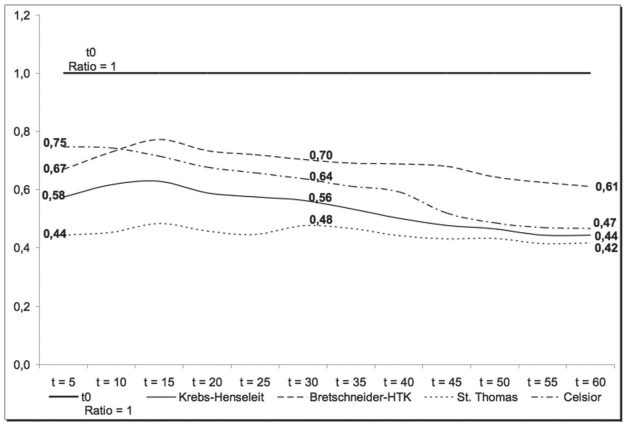

Fig. 5 - Coronary flow (CF) according to the solution. Reperfused hearts were monitored for 60 min after treatment with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas No. 1, and Celsior. Baseline was calculated after stabilization and prior to treatment

flow values compared to the others. Moreover, these treatments indicated a decreasing order of efficiency: HTK>CEL>KHB>STH-1. Together, these results indicate that performance on CF maintenance is time-dependent. However, use of HTK suggests better protection against development of tissue edema.

To better evaluate the efficiency of myocardial protection was made a study of multiple comparisons between treatments (Table 1). Table 2 shows the chemical composition of the solutions studied. For HR, only CEL versus HTK were not significantly different. For LVSP, (+d*P*/d*t*), (-d*P*/d*t*) and CF, all comparisons were significantly different. Overall, use of CEL resulted in significant improvement in hemodynamic variable outcome compared to the other solutions.

Table 1. Ratio of hemodynamic variables corresponding (Tukey's test - t60).

Dependent Variable	KHB	HTK	STH-1	CEL
HR	HTK	KHB	*	*
LVSP	*	*	*	*
+dP/dt	CEL	CEL	*	KHB and HTK
-dP/dt	*	*	*	*
CF	*	*	*	*

(*) There was statistical difference (p <0.05) between solutions. There was no statistical difference (P >0.05) only for the solution quoted. KHB - Krebs-Henseleit Buffer; HTK – Bretschneider-HTK; STH-1 – St. Thomas-No.1; CEL – Celsior; HR – Heart rate; LVSP – Left ventricular systolic pressure; (+dP/dt) - Maximum rate of rise of left ventricular pressure during ventricular contraction; (-dP/dt) - Maximum rate of fall of left ventricular pressure during ventricular contraction; CF – Coronary flow

Table 2. Chemical Composition of the solutions studied

Components(mmol/L)	KHB	HTK	STH-1	CEL
Lactobionate	-	-	-	80
Manitol	-	30	-	60
Glutamate	-	-	-	20
á-Ketoglutarate	-	1	-	-
Tryptophan	-	2	-	-
Histidine . HCL.H_2O	-	18	-	-
Histidine	-	180	-	30
Glutatione	-	-	-	3
Na^+	126	15	144	100
Glucose	11.5	-	-	-
K^+	4.8	9	20	15
Mg^{++}	1.2	4	16	13
Ca^{2+}	2.5	0.015	2.2	0.25
$NaHCO_3$	25	-	10	-
Procaine	-	-	1	-
pH	7.4 ± 0.5	7.4-7.45	7.4	7.4 ± 0.2
Osmolality(mOsm/L)	330	310	324	320

KHB - Krebs-Henseleit Buffer; HTK – Bretschneider-HTK; STH-1 – St. Thomas-No.1; CEL – Celsior

DISCUSSION

Clinical investigations on the comparative performance of the cardioplegic solutions offer the greatest difficulties on result interpretation and may bring false judgment. Langendorff system was chosen because it is well standardized in our laboratories about myocardial protection evaluation and is possible also analyzing the direct effects on the heart with systemic interferences exclusion [10].

Hypothermia was adopted in this study because it is a standard strategy of myocardial protection. Cleveland et al. [11] showed that hypothermia is the most important factor in myocardial protection. Studies on myocardial protection with cardioprotective additives, associated with hypothermia, demonstrated improved contractile function after long ischemia periods [6].

Pereda et al. [12] compared the performance of Celsior (CEL) versus St. Thomas No. 2 (STH-2) solutions, as blood cardioplegia, demonstrating that they were not significantly different.

Loganathan et al. [13] analyzed the effects of reperfusion up to 24 hours using Bretschneider-HTK (HTK) solution and modified Bretschneider-HTK (Custodiol-N). The last one improves myocardial and endothelial function during the critical phase of reperfusion after heart transplantation.

Lee et al. [14], contrariwise, found that Bretschneider-HTK (HTK) solution exhibited superior protective effects over CEL against prolonged cold ischemia in a syngeneic rat transplantation model.

The current clinical practice in the Brazilian myocardial protection in cardiac transplantation commonly uses the St. Thomas No. 1 (STH-1) crystalloid solution, extracellular type. Currently, other solutions were added to the therapeutic arsenal of myocardial protection, such as the Celsior solution (CEL), extracellular type, and Bretschneider-HTK solution (HTK), intracellular type (Table 2). Such solutions have been used increasingly in major transplant centers. Supported in the international literature we compared the ventricular performance experimentally using the above solutions in the myocardium of rats subjected to ischemia and reperfusion.

In the present investigation we adopted the absence of cardiac pacing to enhance the intrinsic rhythm of the heart. Additionally, it should be emphasized that the heart's conduction tissue is more sensitive to ischemia [15]. Thus, heart rate is ultimately a variable capable of providing indirect information on the severity of injury caused by ischemia and reperfusion [16]. All solutions provided preservation of the HR, but the results were below the baseline value for this variable. It was observed that after 30 min of reperfusion, all solutions were stable. Note that the STH-1 solution took 30 min for stabilization.

The myocardial contractility was assessed in an integrated manner by the following variables: LVSP, (+d*P*/d*t*), and (-d*P*/d*t*). We observed that the effects of ischemia and reperfusion on the myocardium are extremely deleterious, producing a marked reduction in ventricular performance. We infer that the concentrations of K^+ (15 mmol/L) and Ca^{2+} (0.25 mmol/L) of CEL solution can contribute to a better performance by promoting the depolarizing arrest without contributing to an overload of intracellular calcium during the ischemic period [17,18].

Considering that isolated hearts used in this study had a fixed pressure gradient, essentially the only factor responsible for decreased blood flow would be related to interstitial edema. Therefore, by analyzing the behavior of coronary flow, we aimed to relate it directly to myocardial edema. The results indicate that HTK solution were those that produced the highest flow values. The other solutions used showed a descending order of efficiency in maintaining coronary flow, as follows: CEL>KHB>STH-1. We suggest that each solution has an optimal preservation temperature, where hypothermia can facilitate or interfere with tissue edema, possibly by directly influencing membrane conductive properties in myocardial cells, as well as modifying the permeability of the endothelium [8,19,20]. Moreover, another antagonistic factor to edema development could be related to the osmotic properties of each solution used [18,21]. Relative to osmolarity, these solutions have the following decreasing order: KHB>STH-

1>CEL>HTK. However, we did not observe this same order considering comparative performance. Additionally, Na^+ is also an important variable in this process, and these solutions have the following decreasing order in concentration of this ion: STH-1>KHB>CEL>HTK. The comparative performances between them do not obey this order, indicating that Na^+ is not solely responsible for the participation of edema [21].

This study is part of a line of research that includes endothelial dysfunction and apoptosis using different cardioprotective methods, and has inherent limitations. Perfusion of non-human isolated hearts with solutions without blood produces disturbances in cardiac performance. However, even though the data obtained cannot be translated directly to clinical application, one must consider that comparative studies with animal models have proven effective in research related to myocardial preservation [22,23].

CONCLUSION

Despite the cardioprotective crystalloid solutions studied are not fully able to suppress the deleterious effects of ischemia and reperfusion in the rat heart, the CEL solution had significantly higher results followed by HTK>KHB>STH-1. Other researches are still needed, considering different infusion temperatures and others cardioplegic solutions to extend the cardioprotective methods.

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REFERENCES

1. Demmy TL, Biddle JS, Bennett LE, Walls JT, Schmaltz RA, Curtis JJ. Organ preservation solutions in heart transplantation: patterns of usage and related survival. Transplantation. 1997;63(2):262-9.

2. Hertz MI, Aurora P, Christie JD, Dobbels F, Edwards LB, Kirk R, et al. Scientific Registry of the International Society for Heart and Lung Transplantation: introduction to the 2010 annual reports. J Heart Lung Transplant. 2010;29(10):1083-8.

3. Fiorelli AI, Stolf NA, Pego-Fernandes PM, Oliveira Junior JL, Santos RH, Contreras CA, et al. Recommendations for use of marginal donors in heart transplantation: Brazilian Association of Organs Transplantation guideline. Transplant Proc. 2011;43(1):211-5.

4. Jeevanandam V, Furukawa S, Prendergast TW, Todd BA, Eisen HJ, McClurken JB. Standard criteria for an acceptable donor heart are restricting heart transplantation. Ann Thorac Surg. 1996;62(5):1268-75.

5. Marshall VC. Renal preservation. In: Morris PJ, ed. Kidney transplantation: principles and practice. Philadelphia: WB Saunders; 2001. p.113-34.

6. Koch A, Radovits T, Loganathan S, Sack FU, Karck M, Szabó GB. Myocardial protection with the use of L-arginine and N-alpha-acetyl-histidine. Transplant Proc. 2009;41(6):2592-4.

7. Ning XH, Chen SH, Xu CS, Li L, Yao LY, Qian K, et al. Hypothermic protection of the ischemic heart via alterations in apoptotic pathways as assessed by gene array analysis. J Appl Physiol. 2002;92(5):2200-7.

8. Ning XH, Xu CS, Song YC, Childs KF, Xiao Y, Bolling SF, et al. Temperature threshold and modulation of energy metabolism in the cardioplegic arrested rabbit heart. Cryobiology. 1998;36(1):2-11.

9. Gomes OM, Gomes ES, Carvalho JI, Faraj M. Adaptações técnicas na preparação de Langendorff para estudo de corações isolados de pequenos animais. Coração. 1999;9:36-8.

10. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med. 2007;357(11):1121-35.

11. Cleveland JC Jr, Meldrum DR, Rowland RT, Banerjee A, Harken AH. Optimal myocardial preservation: cooling, cardioplegia, and conditioning. Ann Thorac Surg. 1996;61(2):760-8.

12. Pereda D, Castella M, Pomar JL, Cartaña R, Josa M, Barriuso C, et al. Elective cardiac surgery using Celsior or St. Thomas No. 2 solution: a prospective, single-center, randomized pilot study. Eur J Cardiothorac Surg. 2007;32(3):501-6.

13. Loganathan S, Radovits T, Hirschberg K, Korkmaz S, Koch A, Karck M, et al. Effects of Custodiol-N, a novel organ preservation solution, on ischemia/reperfusion injury. J Thorac Cardiovasc Surg. 2010;139(4):1048-56.

14. Lee S, Huang CS, Kawamura T, Shigemura N, Stolz DB, Billiar TR, et al. Superior myocardial preservation with HTK solution over Celsior in rat hearts with prolonged cold ischemia. Surgery. 2010;148(2):463-73.

15. Fischer JH, Jeschkeit S. Effectivity of freshly prepared or refreshed solutions for heart preservation versus commercial EuroCollins, Bretschneider's HTK or University of Wisconsin solution. Transplantation. 1995;59(9):1259-62.

16. Ebel D, Preckel B, You A, Mullenheim J, Schlack W, Thamer V. Cardioprotection by sevoflurane against reperfusion injury after cardioplegic arrest in the rat is independent of three types of cardioplegia. Br J Anaesth. 2002;88(6):828-35.

17. Poole-Wilson PA, Langer GA. Effects of acidosis on mechanical function and Ca2+ exchange in rabbit myocardium. Am J Physiol. 1979;236(4):H525-33.

18. Sumimoto R, Kamada N. Lactobionate as the most important component in UW solution for liver preservation. Transplant Proc. 1990;22(5):2198-9.

19. Ning XH, Xu CS, Song YC, Xiao Y, Hu YJ, Lupinetti FM, et al. Temperature threshold and preservation of signaling for mitochondrial membrane proteins during ischemia in rabbit heart. Cryobiology. 1998;36(4):321-9.

20. Ning XH, Xu CS, Song YC, Xiao Y, Hu YJ, Lupinetti FM, et al. Hypothermia preserves function and signaling for mitochondrial biogenesis during subsequent ischemia. Am J Physiol. 1998;274(3 Pt 2):H786-93.

21. Ferreira R, Burgos M, Llesuy S, Molteni L, Milei J, Flecha BG, et al. Reduction of reperfusion injury with mannitol cardioplegia. Ann Thorac Surg. 1989;48(1):77-83.

22. Vassallo DV, Lima EQ, Campagnaro P, Faria AN, Mill JG. Mechanisms underlying the genesis of post-extrasystolic potentiation in rat cardiac muscle. Braz J Med Biol Res. 1995;28(3):377-83.

23. Pinheiro BB, Fiorelli AI, Gomes OM. Effects of ischemic postconditioning on left ventricular function of isolated rat hearts. Rev Bras Cir Cardiovasc. 2009;24(1):31-7.