Deleterious Effect of Hypothermia in Myocardial Protection Against Cold Ischemia: A Comparative Study in Isolated Rat Hearts

M.L. Lima, A.I. Fiorelli, D.V. Vassallo, P.R. Batista, F.V. Simoes, J. Fiorim, I. Stefanon, B.B. Pinheiro, N.A.G. Stolf, and O.M. Gomes

ABSTRACT

Background. There is a growing need to improve heart preservation benefit the performance of cardiac operations, decrease morbidity, and more important, increase the donor pool. Therefore, the objective of this study was to evaluate the cardioprotective effects of Krebs-Henseleit buffer (KHB), Bretschneider-HTK (HTK), St. Thomas No. 1 (STH-1), and Celsior (CEL) solutions infused at 10°C and 20°C.

Methods. Hearts isolated from male albino Wistar rats and prepared according to Langendorff were randomly divided equally into 8 groups according to the temperature of infusion (10°C or 20°C) and cardioprotective solutions (KHB, HTK, STH-1, and CEL). After stabilization with KHB at 37°C, baseline values were collected (control) for heart rate (HR), left ventricle systolic pressure (LVSP), coronary flow (CF), maximum rate of rise of left ventricular pressure during ventricular contraction ($+dP/dt$) and maximum rate of fall of left ventricular pressure during left ventricular relaxation ($-dP/dt$). The hearts were then perfused with cardioprotective solutions for 5 minutes and kept for 2 hours in static ischemia at 20°C. Data evaluation used analysis of variance (ANOVA) in all together randomized 2-way ANOVA and Tukey's test for multiple comparisons. The level of significance chosen was $P < .05$.

Results. We observed that all 4 solutions were able to recover HR, independent of temperature. Interestingly, STH-1 solution at 20°C showed HR above baseline throughout the experiment. An evaluation of the corresponding hemodynamic values (LVSP, $+dP/dt$, and $-dP/dt$) indicated that treatment with CEL solution was superior at both temperatures compared with the other solutions, and had better performance at 20°C. When analyzing performance on CF maintenance, we observed that it was temperature dependent. However, when applying both HTK and CEL, at 10°C and 20°C respectively, indicated better protection against development of tissue edema. Multiple comparisons between treatments and hemodynamic variable outcomes showed that using CEL solution resulted in significant improvement compared with the other solutions at both temperatures.

Conclusion. The solutions investigated were not able to fully suppress the deleterious effects of ischemia and reperfusion of the heart. However, these results allow us to conclude that temperature and the cardioprotective solution are interdependent as far as myocardial protection. Although CEL solution is the best for in myocardial protection,

From the Laboratory of Experimental Cardiovascular Research (M.L.L., A.I.F., B.B.P., O.M.G.), Sao Francisco de Assis Research Foundation, Belo Horizonte, Minas Gerais; the Heart Institute of Sao Paulo University (A.I.F., N.A.G.S.), Sao Paulo; and the Department of Physiological Sciences (D.V.V., P.R.B., F.V.S., J.F., I.S.), Biomedical Center, Federal University of Espirito Santo, Vitoria, Brazil.

Address reprint requests to Melchior Luiz Lima, Rua Benedito Mello Serrano 165, Mata da Praia, Vitoria/ES, Brazil 29065-040. E-mail: mll@ig.com.br

0041-1345/12/$–see front matter
http://dx.doi.org/10.1016/j.transproceed.2012.07.007

more studies are needed to understand the interaction between temperature and perfusion solution used. This will lead to development of better and more efficient cardioprotective methods.

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] In procedures where the ischemia period is short, preservation is not problematic. However, procedures where long ischemic periods are common, myocardial viability may be compromised by the current methods of myocardial preservation.[1] 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]

Because of the shortage of donated hearts, selection criteria are under constant review to increase the number of marginal donors.[2] Nevertheless, studies of myocardial protection have great relevance for the advancement of heart transplantation. Prolonged myocardial ischemia is an independent risk factor for early and late patient survival.

Crystalloid cardioplegic solutions can be classified as intracellular or extracellular depending on their ion concentration and certain additives. These solutions have different levels of potassium to keep the cell membrane depolarized, free radicals scavengers, impermeants to maintain osmotic pressure and prevent edema, energy substrates, and buffers to prevent acidosis. Myocardial ischemia decreases oxidative metabolism to dwindle in both mitochondrial ATP production and myocardial function.[3]

Studies on myocardial protection have shown improvement in contractile function after long periods of ischemia. Pereda et al., compared the performance of Celsior (CEL) versus St. Thomas-2 (STH-2) solutions, as blood cardioplegia, demonstrating that they were not significantly different.[4] Loganathan et al[5] analyzed the effects of reperfusion up to 24 hours using Bretschneider-HTK (HTK) solution and modified Bretschneider-HTK (Custodiol-N), which improves myocardial and endothelial function during the critical phase of reperfusion after heart transplantation.[5] Lee et al[6] found that Bretschneider-HTK solution exhibited superior protective effects over CEL against prolonged cold ischemia in a syngeneic rat transplantation model.[6]

In addition to the use of cardioplegic solutions for organ preservation, lower temperatures are also used. Hypothermia protects cellular energy metabolism acting to improve 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 combats oxidative stress induced by ischemia and reperfusion.[8] A review by Cleveland et al[9] showed that hypothermia is considered the most important factor for myocardial protection. There is a growing need to investigate and improve heart preservation methods, thus improving performance of cardiac operations, reducing morbidity, increasing the donor pool, and extending its indications and benefits. Therefore, our objective was to compare the efficiency of myocardial protection using 4 widely used cardioplegic solutions infused at 2 different temperatures in isolated heart preparations of rats.

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

We sought to compare the efficiency of myocardial protection using Krebs-Henseleit (KHB), *HTK , STH-1 and CEL solutions* infused at 10°C and 20°C in isolated heart preparations of rats when subjected to ischemia for 2 hours at 20°C.

METHODS

Hearts with an average weight of 1.3 ± 0.0 g were isolated from 64 male albino Wistar rats and immediately subjected to retrograde perfusion according to the method of Langendorff.[10] Rats had a mean weight of 313.1 ± 0.3 g and average size of 23.4 ± 0.0 cm. The research followed the ethical principles for animal experimentation established by the Brazilian College of Animal Experimentation. The Ethics Committee of the St. Francis of Assisi Research Foundation, Belo Horizonte, Brazil, approved all animal care and experimental procedures.

Hearts were randomly divided into 8 groups according to the temperature of infusion (A, 10°C; B, 20°C) and cardioprotective solution used as follows: Groups A1 and B1 were treated with KHB solution (Laboratory of Experimental Cardiovascular Research, St. Francis of Assisi Research Foundation): groups A2 and B2 with HTK solution (Dr. Franz Köhler Chemie GMBH, Germany); groups A3 and B3 with STH-1 solution (Braile Biomédica Industry, Brazil); and groups A4 and B4 with CEL solution (Genzyme Polyclonals S.A.S., France). Table 1 shows the chemical composition of the solutions studied.

After 15 minutes of perfusion at 37°C with KHB solution for stabilization, we collected the values considered baseline (control) for heart rate (HR), left ventricle systolic pressure (LVSP), coronary flow (CF), and the maximum rate of rise of left ventricular pressure during ventricular contraction ($+dP/dt$), and maximum rate of fall of left ventricular pressure during left ventricular relaxation ($-dP/dt$). The hearts were then perfused with their respective cardioprotective solutions for 5 minutes and kept for 2 hours in static ischemia at 20°C. Subsequently, the hearts were reperfused with KHB at 37°C for 60 minutes. Data were collected every 5 minutes. Data evaluation was based on analysis of variance in completely randomized 2-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. The level of significance was set at $P < .05$.

Table 1. Chemical Composition of the Solutions Studied

Components (mmol/L)	Krebs-Henseleit Buffer	Bretschneider-HTK	St. Thomas No. 1	Celsior
Lactobionate	—	—	—	80
Manitol	—	30	—	60
Glutamate	—	—	—	20
α-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

RESULTS

To evaluate myocardial protection, we first compared the effect of the treatments (solutions/temperature) on HR in isolated heart preparation of rats. Figure 1 shows the trend in HR of the solutions used in the experiment at 10°C and 20°C, compared with controls, represented by a ratio of 1.0 (basal HR). At 10°C, solutions CEL and KHB provided a more stable HR throughout the length of the experiment. On the other hand, use of HTK and STH-1 solutions at 10°C initially resulted in lower HR (HTK, 0.66; STH-1, 0.55), which increased after 15 and 30 minutes, respectively, and stabilized at a similar HR compared with the other solutions. At 20°C, the KHB, HTK, and CEL solutions resulted in increased recovery of HR in the first 30 minutes followed by stability and reaching similar HRs at 60 minutes (0.88, 0.87, and 0.88, respectively). Interestingly, STH-1 solution at 20°C showed HR above baseline throughout the experiment. These results indicate that all 4 solutions, independent of temperature, are able to recover the HR.

Left ventricular contractility was represented by the corresponding hemodynamic variables LVSP, $+dP/dt$, and $-dP/dt$ (Figs 2–4), and was also evaluated in myocardial protection. These variables show similar trends with the different solutions at either 10°C or 20°C. In CEL solution, all corresponding hemodynamic variables were more stable and with higher rates compared with the other solutions at either temperature, as well as showing a higher recovery at 20°C. With the HTK solution at 10°C, rates increased constantly throughout the 60-minutes period, and were higher compared with the other solutions at this same temperature. At 10°C, the KHB solution resulted in higher rates for all variables compared to STH-1, but was still lower than HTK at LVSP and $-dP/dt$. Despite these differences, KHB reached approximately the same rate of $+dP/dt$ after 40 minutes, compared with HTK. The contractile performance of STH-1 was lower than the other solutions at 10°C. However, at 20°C STH-1 showed similar contractile performance compared to the HTK and KHB solutions. Here, the data show that treatment with CEL is superior to the others at 10°C and 20°C, and shows a better performance at 20°C.

CF was considered as one of the hemodynamics variables to evaluate edema occurrence and extension (a negative factor in the recovery of the heart). All treatments showed a downward trend (Fig 5). However, treatment with HTK solution produced higher flow values compared with the others at 10°C. Moreover, these treatments indicated a decreasing order of efficiency: HTK > CEL > KHB > STH-1. When solutions were tested at 20°C, CEL presented the higher CF values. Again, a decreasing order of efficiency in CF was observed: CEL > HTK > STH-1 > KHB. Together, these results indicate that performance on CF maintenance is temperature-dependent. However, use of

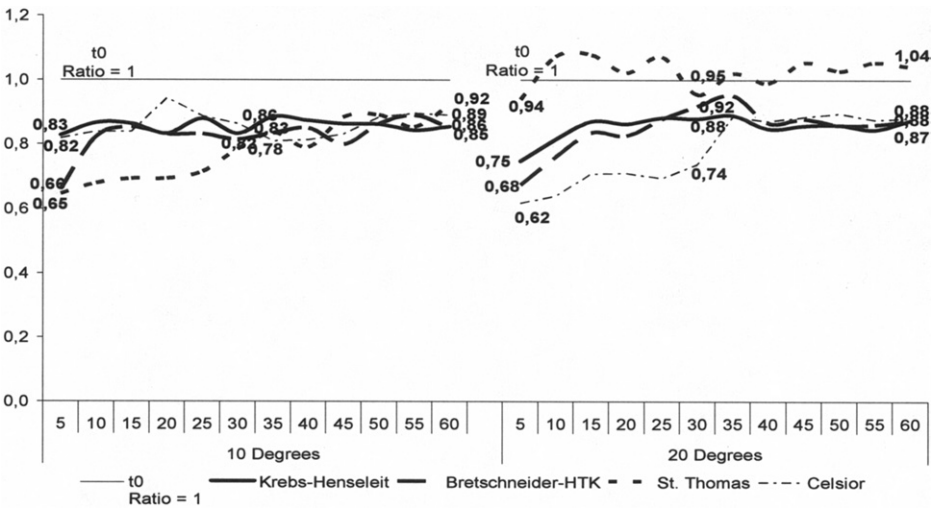

Fig 1. Heart rate (HR) according to the solution and the temperature. Reperfused hearts were monitored for 60 minutes after treatment at either 10°C or 20°C with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas-No. 1, and Celsior. Baseline was calculated after stabilization and before treatment.

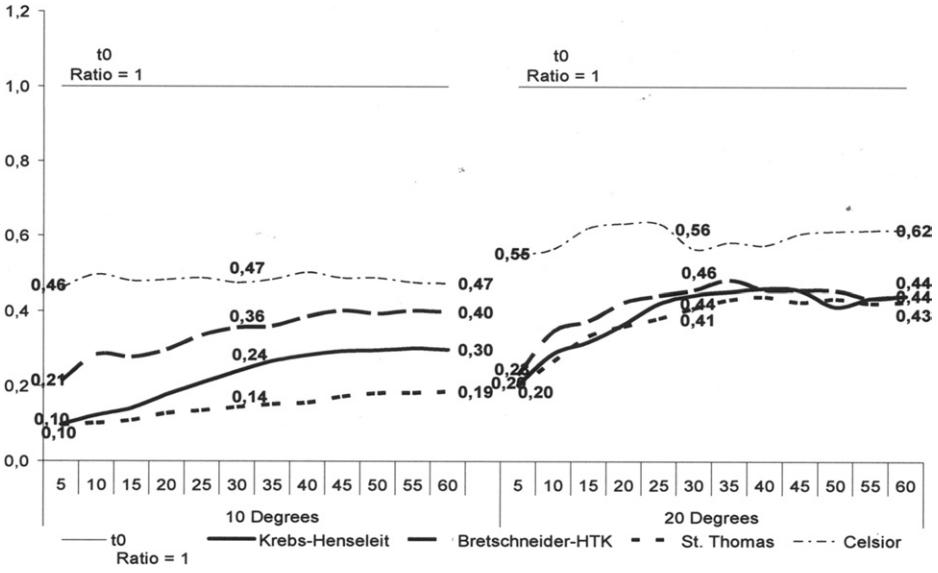

Fig 2. Left ventricle systolic pressure (LVSP) according to the solution and the temperature. Reperfused hearts were monitored for 60 minutes after treatment at either 10°C or 20°C with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas-No. 1, and Celsior. Baseline was calculated after stabilization and before treatment.

HTK at 10°C and CEL at 20°C suggests better protection against development of tissue edema.

To better evaluate the efficiency of myocardial protection a study was undertaken of multiple comparisons among treatments (Table 2). For HR, only CEL versus HTK at 10°C and STH-1 versus HTK at 20°C were not significantly different. For LVSP and CF, all comparisons were significantly different. In $+dP/dt$ and $-dP/dt$, only HTK versus KHB at 20°C was not significantly different. Overall, use of CEL solution resulted in significant improvement in hemodynamic variable outcome compared to the other solutions at both temperatures.

DISCUSSION

We compared the efficiency of myocardial protection using 4 commonly used cardioplegic solutions. These solutions were tested at either 10°C or 20°C in isolated heart preparations of rats. Together, our results show improved myocardial protection at 20°C when CEL solution was used.

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. Thus, heart rate is ultimately a variable capable of providing indirect information on the severity of injury caused by ischemia and reperfusion.[11] All solutions provided preservation of the HR, but the results were below the baseline value for this variable at both temperatures. It was observed that, after 30 minutes of reperfusion, all solutions were stable, except for the STH-1 solution at 20°C, which had a HR greater than baseline throughout reperfusion. We suggest that, at this

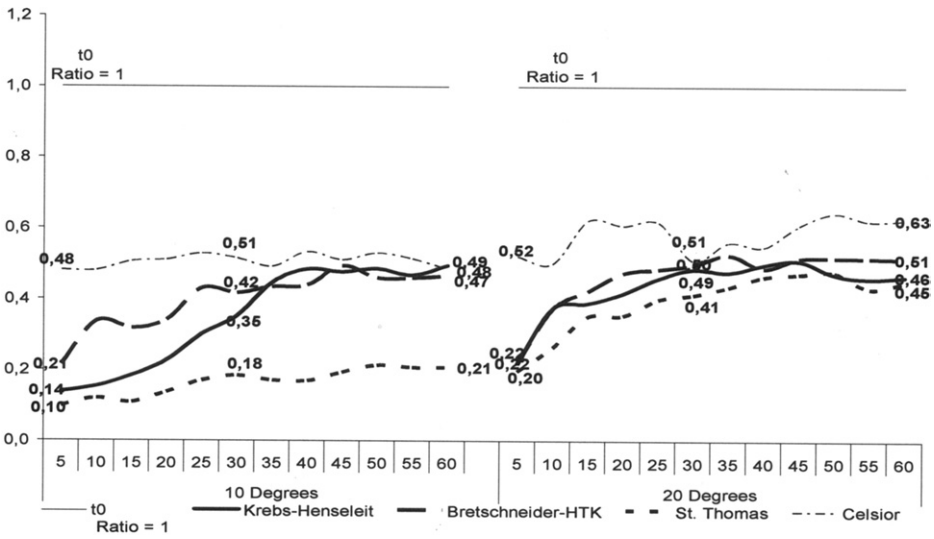

Fig 3. Maximum rate of rise of left ventricular pressure during ventricular contraction ($+dP/dt$), according to the solution and the temperature. Reperfused hearts were monitored for 60 minutes after treatment at either 10°C or 20°C with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas-No. 1, and Celsior. Baseline was calculated after stabilization and before treatment.

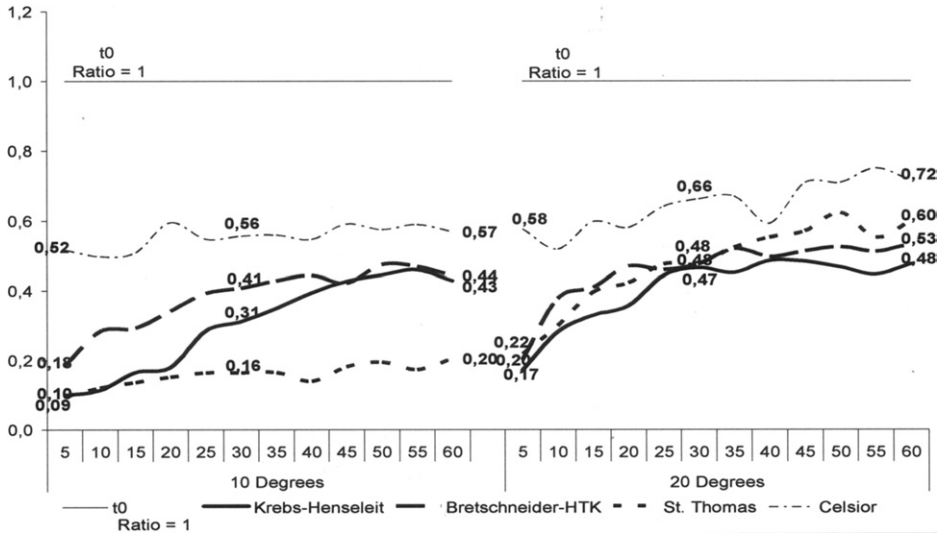

Fig 4. Maximum rate of fall of left ventricular pressure during left ventricular relaxation ($-dP/dt$), according to the solution and the temperature. Reperfused hearts were monitored for 60 minutes after treatment at either 10°C or 20°C with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas-No. 1, and Celsior. Baseline was calculated after stabilization and before treatment.

temperature, there was less inhibition of ion exchangers in the cell membranes allowing for increased HR, probably owing to intracellular calcium overload.[12] It is worth noting that the chemical composition of STH-1 solution has the highest concentration of Ca^{2+} and K^{+} among the solutions tested. At the lower temperature, the membrane ion exchangers potentially become less functional, thereby leading to decreased release of intracellular calcium.[12] This could explain the marked difference in HR between 10°C and 20°C when STH-1 was used.

The myocardial contractility was assessed using LVSP, $+dP/dt$, and $-dP/dt$. Analyzing them in an integrated manner, we observed that the effects of ischemia and reperfusion on the myocardium are extremely deleterious, producing a marked reduction in ventricular performance. At 60 minutes of reperfusion, lower rates of contractile depression were observed for CEL solution: 53% at 10°C and 38% at 20°C in LVSP, 52% at 10°C and 37% at 20°C of $+dP/dt$, and 43% 10°C and 28% at 20°C of $-dP/dt$. We believe that concentrations of K^{+} (15 mmol/L) and Ca^{2+} (0.25 mmol/L) of this solution contribute to a better performance by promoting the depolarizing arrest without contributing to an overload of intracellular calcium during the ischemic period.[13]

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 at 10°C and CEL solution at 20°C were those that produced the highest flow values. The other solutions used at the 2 different

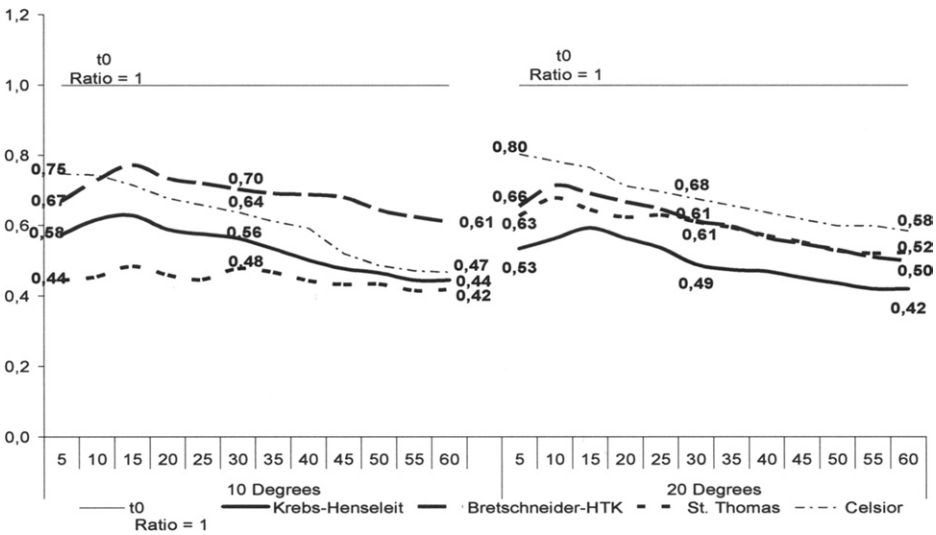

Fig 5. Coronary flow (CF) according to the solution and the temperature. Reperfused hearts were monitored for 60 minutes after treatment at either 10°C or 20°C with the following solutions: Krebs-Henseleit Buffer, Bretschneider-HTK, St. Thomas-No. 1, and Celsior. Baseline was calculated after stabilization and before treatment.

Table 2. Comparison Between Solutions at 10°C and 20°C of the Corresponding Hemodynamic Variables

Temperature	Average Difference	HR (bpm)	LVSP (mmHg)	+d*P*/d*t* (mmHg/s)	−d*P*/d*t* (mmHg/s)	CF (mL/min)
10°C	Krebs-Henseleit - Bretschneider-HTK	−18.8*	−18.1*	−179.1*	−150.1*	−2.7*
	Krebs-Henseleit - St. Thomas-No. 1	9.9*	7.9*	252.9*	186.7*	1.9*
	Krebs-Henseleit - Celsior	−20.2*	−43.8*	−518.2*	−684.6*	−0.6*
	Bretschneider-HTK - St. Thomas-No.1	28.7*	26.1*	431.9*	336.7*	4.6*
	Bretschneider-HTK - Celsior	−1.4	−25.7*	−339.1*	−534.5*	2.1*
	St. Thomas-No.1 - Celsior	−30.1*	−51.8*	−771.1*	−871.2*	−2.5*
20°C	Krebs-Henseleit - Bretschneider - HTK	21.6*	−4.4*	15.1	−14.5	−2.6*
	Krebs-Henseleit - St. Thomas-No. 1	20.0*	2.6*	198.7*	162.7*	−1.6*
	Krebs-Henseleit - Celsior	32.2*	−33.5*	−292.7*	−458.9*	−4.4*
	Bretschneider-HTK - St. Thomas-No.1	−1.6	6.9*	183.6*	177.1*	1.0*
	Bretschneider-HTK - Celsior	10.6*	−29.2*	−307.8*	−444.5*	−1.8*
	St. Thomas-No. 1 - Celsior	12.2*	−36.1*	−491.5*	−621.6*	−2.8*

Abbreviations: HR, heart rate; LVSP, Left ventricular systolic pressure; +d*P*/d*t*, maximum rate of rise of left ventricular pressure during ventricular contraction; −d*P*/d*t*, maximum rate of fall of left ventricular pressure during ventricular contraction; CF, coronary flow.
*$P < .05$.

temperatures showed a descending order of efficiency in maintaining coronary flow, as follows: CEL > KHB > STH-1 at 10°C, and HTK > STH-1 > KHB at 20°C. 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 the endothelium, modifying its permeability.[12] Moreover, another antagonistic factor to edema development could be related to the osmotic properties of each solution used.[13] 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, indicating that temperature also interfered with the edema process. 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. Similar to temperature, the comparative performances between them do not obey this order, indicating that Na^+ is not solely responsible for the participation of edema.

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 nonhuman isolated hearts with solutions without blood produces disturbances in heart 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.[14]

The present results allow us to conclude that the solutions studied were not able to fully suppress the deleterious effects of ischemia and reperfusion on the heart, 20°C promoted better protection in isolated rat heart compared with 10°C independent of the solution used, and CEL solution gave the best result of myocardial protection at 10°C and 20°C. Although CEL solution had the best outcome in myocardial protection, further research is still needed that includes other solutions and cardioprotective methods.

ACKNOWLEDGMENTS

The authors thank the Brazilian Association for Organ Transplant for their support and efforts in providing scientific writing workshops.

REFERENCES

1. Hertz MI, Aurora P, Christie JD, et al: Scientific Registry of the International Society for Heart and Lung Transplantation: introduction to the 2010 annual reports. J Heart Lung Transplant 29:1083, 2010

2. Fiorelli AI, Stolf NA, Pego-Fernandes PM, et al: Recommendations for use of marginal donors in heart transplantation: Brazilian Association of Organs Transplantation guideline. Transplant Proc 43:211, 2011

3. Steenbergen C, Murphy E, Watts JA, et al: Correlation between cytosolic free calcium, contracture, ATP, and irreversible ischemic injury in perfused rat heart. Circ Res 66:135, 1990

4. Pereda D, Castella M, Pomar JL, et al: Elective cardiac surgery using Celsior or St. Thomas No. 2 solution: a prospective, single-center, randomized pilot study. Eur J Cardiothorac Surg 32:501, 2007

5. Loganathan S, Radovits T, Hirschberg K, et al: Effects of Custodiol-N, a novel organ preservation solution, on ischemia/reperfusion injury. J Thorac Cardiovasc Surg 139:1048, 2010

6. Lee S, Huang CS, Kawamura T, et al: Superior myocardial preservation with HTK solution over Celsior in rat hearts with prolonged cold ischemia. Surgery 148:463, 2010

7. Ning XH, Chen SH, Xu CS, et al: Hypothermic protection of the ischemic heart via alterations in apoptotic pathways as assessed by gene array analysis. Appl Physiol 92:2200, 2002

8. Ning XH, Xu CS, Song YC, et al: Temperature threshold and modulation of energy metabolism in the cardioplegic arrested rabbit heart. Cryobiology 36:2, 1998

9. Cleveland JC Jr, Meldrum DR, Rowland RT, et al: Optimal myocardial preservation: cooling, cardioplegia, and conditioning. Ann Thorac Surg 61:760, 1996

10. Gomes OM, Gomes ES, Carvalho JI, et al: Adaptações técnicas na preparação de Langendorff para estudo de corações isolados de pequenos animais. Coração 9:36, 1999

11. Ebel D, Preckel B, You A, et al: Cardioprotection by sevoflurane against reperfusion injury after cardioplegic arrest in the rat is independent of three types of cardioplegia. Br J Anaesth 88:828, 2002

12. Ning XH, Xu CS, Song YC, et al: Temperature threshold and preservation of signaling for mitochondrial membrane proteins during ischemia in rabbit heart. Cryobiology 36:321, 1998

13. Sumimoto R, Jamieson NV, Kamada N: Examination of the role of the impermeants lactobionate and raffinose in a modified UW solution. Transplantation 50:573, 1990

14. Lima ML, Fiorelli AI, Gomes OM, et al: Comparative analysis of the performance of various crystalloid cardioplegic solutions on myocardial protection after prolonged cold ischemia. Transplant Proc 43:80, 2011