Comparative Analysis of the Performance of Various Crystalloid Cardioplegic Solutions on Myocardial Protection After Prolonged Cold Ischemia

M.L. Lima, A.I. Fiorelli, O.M. Gomes, B.B. Pinheiro, M.A.F. Da Silva, L.A.B.J. Porto, L. Novaes, N.A.G. Stolf, and D.R. Souza

ABSTRACT

Introduction. The quality and effectiveness of myocardial protection are fundamental problems to expand the use of and consequently good outcomes of donated hearts for transplantation.

Objective. The purpose of this investigation was to compare the cardioprotective effects of Krebs-Henseleit, Bretschneider-HTK, St Thomas, and Celsior solutions using a modified nonrecirculating Langendorff column model of isolated perfused rat heart during prolonged cold storage.

Materials and Methods. After removal 36 rat hearts underwent isolated perfusion into a Langendorff apparatus using Krebs-Henseleit solution for a 15-minute period of recovery; we excluded organs that did not maintain an aortic pressure above 100 m Hg. Subsequently, we equally distributed the hearts into four groups according to the cardioprotection solution; group 1, Krebs-Henseleit (control); group II, Bretschneider-HTK; group III, St Thomas; and group IV, Celsior. Each heart received the specific cardioplegic solution at 10°C for 2-hour storage at 20°C, before a 15 minutes perfusion with Krebs-Henseleit solution for recovery and stabilization. After 60 additional minutes of perfusion, every 5 minutes we determined heart rate (HR), coronary flow (CF), left ventricular systolic pressure (LVSP), and positive and negative peak of the first derivative of left ventricular pressure (+dP/dt and −dP/dt, respectively).

Results. Comparative analysis by Turkey's test showed the following performances among the groups at 60 minutes of reperfusion: HR: II = IV > III > I; CF: II = IV > I = III; LVSP: IV > I = II = III; +dP/dt: IV > I = II = III; and −dP/dt: IV = II > I = II.

Conclusion. Cardioprotective solutions generally used in clinical practice are not able to avoid hemodynamic alterations in hearts exposed to prolonged ischemia. Celsior solution showed better performance than Bretschneider-HTK, St Thomas, and Krebs-Henseleit.

THE EXPANSION OF USE OF MARGINAL DONORS older in heart transplantation over the past years has been accompanied by recipients and donors prolonged ischemic preservation times. This last observation is due to greater as well as remote procurement of cardiac allografts as well as increased recipients on mechanical ventricular support, which makes the native organ extraction significantly more laborious and time-consuming. Increased ischemia times should provide more organs, helping to decrease the list of waiting recipients.

Myocardial protection improves ventricular function after prolonged ischemia; however, the safe cold storage time

From the Laboratory of the Sao Francisco Assis Foundation (M.L.L., A.I.F., O.M.G., B.B.P., L.N.), Belo Horizonte, Brazil; Heart Institute of Sao Paulo University (A.I.F., M.A.F.D.S., L.A.B.J.P., N.A.G.S.), Sao Paulo, Brazil; and Division of the Cardiothoracic Surgery of University Hospital of Örebro (D.R.S.), Örebro, Sweden.

Supported by the Sao Francisco Assis Foundation–Belo Horizonte/Brazil.

Address reprint requests to Alfredo I. Fiorelli, Rua Morgado de Mateus 126/81, Sao Paulo/SP, Brazil CEP: 04015-050. E-mail: fiorelliai@uol.com.br

0041-1345/11/$–see front matter
doi:10.1016/j.transproceed.2010.12.044

for the heart continues to be 4 to 6 hours. Postischemic changes are difficult to interpret in the heart, due to the multiple factors which interact during this process and predispose to misleading conclusions.

The purpose of this investigation was to compare the cardioprotective effects on rat hearts using Krebs-Henseleit, Bretschneider-HTK, St Thomas, and Celsior solutions using an isolated modified, nonrecirculating Langendorff column perfusion.

MATERIALS AND METHODS

All animals used in this study were obtained from our vivarium and received humane care in compliance with the Brazilian College of Animal Experimentation (Brazil) and National Institutes of Health of the United States. The experiment was approved by our Ethics Committee.

Male Wistar-rats weighing 302.3 ± 22.7g (250–300 g) were anesthetized by ether inhalation and intraperitoneal ketamine (50–75 mg/kg) plus xylazine (10–15 mg/kg). Heparin (500 IU) was injected intra-atrially. After an amplified thoracotomy, the hearts were rapidly excised and mounted on a modified nonrecirculating Langendorff column[1] for perfusion with normothermic Krebs-Henseleit solution bicarbonate buffer at a constant pressure of 100 cm H_2O. A saline-filled latex balloon was inserted into the left ventricle through a left atriotomy for isovolumic pressure measurements. Heart rate and balloon pressures were obtained by a direct-writing recorder through a transducer and monitor model-BASE. Coronary flow was measured by timed collection of the coronary venous effluent.

After a 15-minute recovery period, we excluded the hearts that did not maintain an aortic pressure above 100 m Hg. Subsequently, the hearts were arrested by a 50 mL hypothermic (4°C) coronary artery infusion with one of the four solutions and then immersed in the same solution for 2 hours at 20°C. Thirty-six hearts were distributed in four study groups (eight each) according to the cardioprotection solution, namely: group I, Krebs-Henseleit (control); group II, Bretschneider-HTK; group III, St Thomas; and group IV, Celsior. On completion of the storage interval, the hearts were transferred back to the Langendorff column for a 15-minute reperfusion at 37°C with Krebs-Henseleit solution for ischemic recovery and stabilization. This perfusion was maintained for an additional 60 minutes, every 5 minutes during which we determined the following parameters: heart rate (HR), coronary flow (CF), left ventricular systolic pressure (LVSP), positive and negative peak of the first derivative of left ventricular pressure (+dP/dt and −dP/dt, respectively). At baseline, these variables were tested at the balloon volume required to produce an end-diastolic pressure of approximately 10 mm Hg. The balloon was deflated during the ischemic period and reinflated after the reperfusion was started at the same volume as during the preischemic period.

Statistical Analysis

The results were expressed in tabular form: namely, before ischemia as well as 5, 30, and 60 minutes after reperfusion. For time before ischemia (control), we considered the average of all values for each parameter. The bootstrap method was utilized to enlarge the confidence of results by increasing the number of samples using a resampling probabilistic process.[2] Data were analyzed by one-way analysis of variance and Tukey multiple comparison test. A probability level of $P < .05$ was considered statistically significant.

RESULTS

Initially, we analyzed the variability of all investigated parameters by variation coefficients that measure relative dispersion around the mean. The sample was considered to be stable (variation coefficient <15%). Table 1 shows the hemodynamic parameters analyzed in each study group.

In all groups, the HR and LVSP stayed significantly below the average values that hearts showed before the ischemia and did not recuperate within 60 minutes of reperfusion. The Celsior group showed the best and earliest recovery. The Krebs-Henseleit group showed the worst performance for HR, and paradoxically the St Thomas group, for LVSP. With respect to +dP/dt, −dP/dt and CF, only the Celsior group stayed above or near the minimum values. The St Thomas group showed the worst results. Finally, Table 2 compares the groups by Tukey test.

DISCUSSION

Currently the major part of cardiac surgery is performed after cardiac arrest induced by cardioplegic solutions. None of the cardioprotective methods is ideal. Crystalloid cardioplegic solutions can be divided into intracellular (Bretschneider-HTK) and extracellular (Celsior and St Thomas) types, depending on their sodium context. Cardioplegic solutions are essentially constituted of high potassium levels to maintain membrane depolarization; scavengers of reactive oxygen species; impermeants to maintain osmotic pressure and prevent allograft edema; energy substrates and buffers to prevent acidosis. In 1996, Cleveland et al performed a bibliographic review on myocardial protection, observing that hypothermia was the most important factor.[3] Short ischemic periods did not pose major problems; however, challenges began with periods beyond 4 hours. Thus satisfactory organ preservation methods are mandatory for a successful heart transplantation after a prolonged ischemic time; they may result in expansion of the donor pool.

Clinical investigations on the comparative performance of cardioplegic solutions offer the greatest difficulties in interpretation of results. The Langendorff system was chosen because it has been standardized for evaluation of myocardial protection in our laboratories. It is possible to analyze direct effects on the heart with exclusion systemic interferences.[1]

Myocardial ischemia decreases mitochondrial oxidative metabolism with decreased adenosine triphosphate production and myocardial function. Our results demonstrated that deleterious effects of prolonged myocardial ischemia occurred with all cardioprotective solutions and that none of the solutions integrally conserved rat heart function. Myocardial contractility and relaxation, as studied by LVSP, +dP/dt, and −dP/dt showed persist ever of the ischemic injury over 60-min reperfusion. In 2010, Loganathan et al.[4] showed that myocardial and endothelial function may improve gradually until 24 hours of the reperfusion.

Hemodynamic data have been analyzed by natural frequency of recovery of the sinoatrial node after

Table 1. Analyzed Hemodynamic Parameters into Each Study Group

Group	Before Ischemia	Reperfusion Time (min)			*P*
		5	30	60	
Heart rate (beat per min)					
I, Krebs-Henseleit	238.9 ± 21.9	184.0 ± 14.5	191.1 ± 13.3	197.3 ± 9.2	<.001
II, Bretschneider-HTK		198.2 ± 12.1	199.3 ± 17.0	221.3 ± 20.0	<.001
III, St Thomas		156.3 ± 10.5	217.9 ± 4.8	226.7 ± 6.3	<.001
IV, Celsior		214.9 ± 12.7	225.6 ± 17.7	247.8 ± 5.6	<.001
Left ventricular systolic pressure (mm Hg)					
I, Krebs-Henseleit	127.1 ± 113.3	11.4 ± 0.6	24.5 ± 2.9	32.0 ± 4.4	<.001
II, Bretschneider-HTK		27.1 ± 5.9	37.0 ± 4.9	41.9 ± 3.8	<.001
III, St Thomas		12.7 ± 2.1	17.0 ± 1.7	38.0 ± 21.7	<.001
IV, Celsior		76.4 ± 2.4	76.8 ± 8.0	78.2 ± 5.6	<.001
+dP/dt (mm Hg/min)					
I, Krebs-Henseleit	1768 ± 246	228 ± 13	537 ± 52	815 ± 60	<.001
II, Bretschneider-HTK		426 ± 126	1085 ± 634	1460 ± 662	<.001
III, St Thomas		184 ± 30	344 ± 42	352 ± 34	<.001
IV, Celsior		1179 ± 183	1233 ± 143	1134 ± 143	<.001
−dP/dt (mm Hg/min)					
I, Krebs-Henseleit	1697 ± 262	1343 ± 10	452 ± 62	637 ± 63	<.001
II, Bretschneider-HTK		340 ± 74	602 ± 107	615 ± 86	<.001
III, St Thomas		156 ± 6	272 ± 22	475 ± 285	<.001
IV, Celsior		1282 ± 198	1406 ± 169	1422 ± 113	<.001
Coronary flow (mL/min)					
I, Krebs-Henseleit	20.3 ± 1.3	12.8 ± 1.4	11.4 ± 1.0	8.8 ± 0.8	<.001
II, Bretschneider-HTK		15.5 ± 1.1	14.7 ± 1.0	12.7 ± 0.9	<.001
III, St Thomas		10.9 ± 1.1	10.4 ± 0.8	8.7 ± 0.8	<.001
IV, Celsior		14.6 ± 0.6	12.7 ± 0.4	9.7 ± 0.3	<.001

+dP/dt, positive peak of the first derivative of left ventricular pressure; −dP/dt, negative peak of the first derivative of left ventricular pressure; HTK histidine, tryptophan and ketogluterate.

ischemia-reperfusion without interference by an external placemaker. Celsior solution showed the best performance; but the frequencies in all groups remained below the mean preischemia values, probably reflecting the degree of edema. Coronary flow reduction observed after reperfusion period was also related to the edema, which offered greater resistance to blood flow through the heart.

In 2007, Pereda et al,[5] employed a randomized design to compare cardioplegic agents—Celsior versus St Thomas No. 2 solution—in elective cardiac surgery. They did not observe a significant difference in performance between them. In our study, the St Thomas solution showed the worst performance in relation to Celsior or Bretschneider-HTK. Perhaps the addition of blood to the solutions, as proposed by those authors, equalized their performance, whereas it did not occur in our experiment because the solutions were utilized in their original composition.

In this investigation using reperfusion the Langendorff system, Celsior solution exhibited cardioprotective effects against cold ischemia of rat hearts that were superior to those of Bretschneider-HTK or St Thomas. However, in 2010 Lee et al[6] observed the Bretschneider-HTK solution to exhibit superior protective effects over Celsior against prolonged cold ischemia in a syngeneic rat transplantation model. These apparently conflicting results may be linked to the experimental model, because those authors employed allografts stored the in cold for 6 to 18 hours followed by in vivo reperfusion via heterotopic transplantation. In contrast, the Langendorff model excludes systemic effects on cardiac allografts as occur during heart transplantation.

Table 2. Comparative Analysis Among Groups by Turkey Test ($P < .05$)

Variable	Reperfusion Time (min)		
	5	30	60
Heart rate (bpm)	I = IV > II = III	II = IV > I = III	II = IV > III > I
LVSP (mm Hg)	I = II = III = IV	IV > I = II = III	IV > I = II = III
+dP/dt (mm Hg/min)	IV > I = II = III	II = IV > I = III	IV > I = II = III
−dP/dt (mm Hg/min)	IV > I = II = III	IV = II > I = III	IV = II > I = II
Coronary flow (mL/min)	II = IV > I = III	II = IV > I = III	II = IV > I = III

bpm, beats per minute; LVSP, left ventricular systolic pressure; +dP/dt, positive peak of the first derivative of left ventricular pressure; −dP/dt, negative peak of the first derivative of left ventricular pressure.

We recognize the limitations of transfer of results of an experimental investigation to clinical application. At this stage, we choose only to compare the behavior of cardioplegic solutions on myocardial protection after prolonged cold ischemia with respect to hemodynamic variables during isolated perfusion. In future studies, we are interested to investigate the maximum safety period and metabolic-energetic alterations induced by each cardioplegic solution, particularly with respect to preservation of endothelial cell function. Researchers concerned about this theme have direct impact in the expansion of donated hearts to decrease the transplantation waiting list.

In conclusion, cardioprotection solutions used in clinical practical are not capable of avoiding hemodynamic alterations to the heart during prolonged ischemia. We observed Celsior solution showed a better performance than Bretschneider-HTK, St Thomas, or Krebs-Henseleit in respectively decreasing protection.

REFERENCES

1. Pinheiro BB, Fiorelli AI, Gomes OM, et al: Cardiac effects of postconditioning depend critically on the duration of reperfusion and reocclusion episodes. Heart Surg Forum 13:E52, 2010

2. Cevenini G, Barbini P: A bootstrap approach for assessing the uncertainty of outcome probabilities when using a scoring system. BMC Med Inform Decis Mak 10:45, 2010

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

4. 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

5. 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

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