PREVENTION OF CELL SWELLING WITH LOW CHLORIDE ST. THOMAS' HOSPITAL SOLUTION IMPROVES POSTISCHEMIC MYOCARDIAL RECOVERY

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Objective: In isolated myocytes cardioplegia-induced cell swelling can be prevented by lowering the KCl product by replacing Cl⁻ with an impermeant ion. This study tested the hypothesis that Cl⁻ substitution in St. Thomas' Hospital cardioplegic solution would result in superior myocardial protection in the intact, blood-perfused heart. **Methods:** Using a parabiotic, isolated rabbit heart Langendorff model, hearts were exposed to 1 hour of hypothermic (10° to 12° C), global ischemia followed by 30 minutes of reperfusion. Isosmotic cardioplegia was administered as a single 50 ml bolus of either standard St. Thomas' Hospital solution ($[K^+]_o \times [Cl^-]_o = 2566.4 \text{ (mmol/L)}^2$) or low Cl⁻ St. Thomas' Hospital solution ($[K^+]_o \times [Cl^-]_o = 700 \text{ (mmol/L)}^2$). Chloride was replaced by a large, impermeant ion, methanesulfonate. Postreperfusion systolic function and atrioventricular conduction times were measured before ischemia and after reperfusion. **Results:** Hearts receiving low Cl⁻ St. Thomas' Hospital cardioplegia demonstrated significantly better postischemic functional recovery ($74\% \pm 3\%$) compared with those treated with standard high Cl⁻ St. Thomas' Hospital solution ($55\% \pm 4\%$, $p = 0.003$). In addition, atrioventricular conduction times remained normal in the low Cl⁻ group but were significantly prolonged in the St. Thomas' Hospital group. **Conclusions:** Lowering the KCl product of St. Thomas' Hospital solution makes it isotonic with plasma and prevents cellular edema. This ameliorates the detrimental functional and electrophysiologic sequelae of hypothermic, hyperkalemic cardioplegia. (J Thorac Cardiovasc Surg 1998;115:1196-202)

Previous attempts to prevent myocardial edema have focused on the osmolality of the cardioplegic solutions and the use of membrane stabilizing agents.¹⁻⁴ However, a solution may be isosmotic with plasma and still induce cell swelling if it is hypotonic. Although osmolality is an intrinsic property of the

solution, tonicity depends on the properties of both the solution and the cell membrane, and membrane properties are highly sensitive to temperature.⁵ Recent studies on isolated rabbit and human myocytes suggest that edema may be a direct consequence of the tonicity of isosmotic cardioplegic solutions.^{6,7} Lowering the KCl product by partially replacing $[Cl^-]_o$ with a large, impermeant ion made St. Thomas' Hospital solution isotonic and prevented cell swelling during the period of exposure.⁶ However, extrapolation of data from single myocytes to the intact heart must be done cautiously. The single cell model fails to incorporate the myriad interactions between myocytes and neural, vascular, and interstitial elements, and the complex geometry of the intact heart.^{6,7} Therefore this study was designed to test the hypothesis that the prevention of cellular swelling by changing the tonicity of the cardioplegic solution would ameliorate the detrimental contractile and electrophysiologic sequelae of hypothermic, hyperkalemic cardioplegia in the more clinically relevant intact heart.

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Methods

Adult New Zealand White rabbits of either sex, weighing 2.8 to 3.1 kg, were used in this study. All animals received humane care in American Association for the Accreditation of Laboratory Animal Care–approved, United States Department of Agriculture–registered facilities in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985.) Experiments were designed to compare the effect of standard and low Cl^- St. Thomas’ Hospital cardioplegic solution on postischemic function of hearts mounted and perfused on a parabolic Langendorff apparatus.

Experimental preparation

Preparation of the support animal. The support animal was anesthetized by intramuscular administration of acepromazine maleate (INN: acepromazine) (1 mg/kg) and xylazine (17.5 mg/kg) followed by ketamine (62.5 mg/kg). A tracheostomy was performed, and mechanical ventilation was initiated (model 683, Harvard Apparatus, Dover, Mass.). Ventilator settings were adjusted to maintain arterial pH between 7.35 and 7.5, carbon dioxide tension (PCO_2) between 35 and 45 mm Hg, and oxygen tension (PO_2) more than 200 mm Hg.

Heparin (2500 U) was given through an ear vein. The right femoral artery was cannulated, the cannula connected to a pressure transducer (model P231D, Gould Inc., Cleveland, Ohio), and blood pressure was continuously displayed. Systolic blood pressure was maintained greater than 80 mm Hg by transfusion of either blood collected from the donor animal or electrolyte solution (Plasma-Lyte, Baxter Healthcare Corp., Deerfield, Ill.), and serial hematocrits were measured.

The left internal jugular vein and carotid artery were cannulated to provide extracorporeal circulation, and arterial blood was delivered to perfuse a modified Langendorff apparatus as described previously.⁸ Column effluent was returned to the support animal.

Preparation of the donor animal and isolated heart. The donor animal was also prepared as described above. A rapid cardiectomy was accomplished through a median sternotomy, and blood for transfusion was collected from the thoracic cavity. The aorta was rapidly cannulated, the heart suspended from a modified Langendorff apparatus, and blood perfusion begun.

To monitor contractile function, a fluid-filled latex balloon was placed into the left ventricle and secured with a purse-string suture in the mitral valve annulus. The balloon was connected by way of polyethylene tubing to a pressure transducer (model P231D, Gould, Cleveland, Ohio). The zero pressure reference was set at the level of the aortic valve.

Two needle electrodes were secured in the right atrial appendage and connected to a pacemaker (model 5320, Medtronic, Inc., Minneapolis, Minn.). The heart was paced at a constant rate throughout the study. Two additional electrodes were placed on the left ventricular epicardium to monitor a bipolar electrogram. The electrodes were connected to a preamplifier and amplifier

(model 11-G5407-58 and 13-4615-58, Gould Inc.) and band pass filtered between 0.05 and 1000 Hz. The pressure and electrogram waveforms were displayed continuously and digitized at 1000 Hz with a WINDAQ/200 system (DATAQ Instruments, Akron, Ohio). Coronary flow was measured by an in-line flow probe and continuously monitored with a flowmeter (model T206, Transonic Systems, Inc., Ithaca, N.Y.).

The heart was enclosed in a water-jacketed beaker, and myocardial temperature was monitored with a probe placed in the right ventricle (model BAT 8, Bailey Instruments, Saddle Brook, N.J.). Hearts were warmed and cooled by switching flow between a 37° C water bath (model D1, Haake Co., Berlin, Germany) and a 10° C water bath (model 9010, Fisher Scientific, Pittsburgh, Pa.) connected in parallel to the water-jacketed beaker. Blood perfusate was maintained at 37° C by a separate water bath (model D1, Haake Co., Berlin, Germany). At hourly intervals, heparin (500 U) was administered to the support animal.

Experimental protocol. After instrumentation, hearts were given 30 minutes to equilibrate, and baseline data were acquired. Hearts that did not generate a systolic pressure exceeding 80 mm Hg at an end-diastolic pressure (EDP) of 10 mm Hg were excluded from the study. Intracavitary left ventricular pressure waveforms and left ventricular bipolar electrograms were recorded at seven fixed left-ventricular end-diastolic pressures (LVEDP) (0, 2.5, 5, 10, 15, 20, and 25 mm Hg) attained by adjusting balloon volume. Then balloon volume was reduced to produce a 5 mm Hg LVEDP.

Hearts were randomized to undergo a single 50 ml infusion of one of two cold cardioplegic solutions (10° C) at the onset of 60 minutes of global ischemia (10° to 12° C). Either standard St. Thomas’ Hospital solution ($[\text{K}^+]_o \times [\text{Cl}^-]_o = 2566.4 \text{ [mmol/L]}^2$, Plegisol, Abbott Laboratories, North Chicago, Ill.), or a modified, low Cl^- St. Thomas’ Hospital solution was administered.⁶

Low Cl^- St. Thomas’ Hospital solution ($[\text{Cl}^-]_o = 43.75 \text{ mmol/L}$) was made by substituting an equimolar amount of Na-methanesulfonate for all NaCl and K-methanesulfonate for part of the KCl in St. Thomas’ Hospital solution so that the product $[\text{K}^+]_o \times [\text{Cl}^-]_o$ was 700 (mmol/L)². This low Cl^- cardioplegic solution is isotonic and did not affect the volume of isolated myocytes.⁶ Methanesulfonate was chosen as the substitute for Cl^- because it is a large impermeant anion that does not chelate Ca^{2+} . Furthermore, its pK_a of approximately 1.2 is sufficiently low that negligible amounts of the permeant protonated species are present at physiologic pH.⁶ The osmolarities of the standard and low Cl^- solutions were not significantly different (243 ± 5 and $237 \pm 6 \text{ mOsm}$, respectively).

To examine whether methanesulfonate itself had a cardioprotective effect, another large impermeant anion, aspartate, was used to lower the $[\text{K}^+][\text{Cl}^-]$ product. Aspartate also has a low pK_a . In this group ($n = 6$), equimolar amounts of Na-aspartate was substituted for all the NaCl and K-aspartate for some of the KCl in St. Thomas’ Hospital solution. This also lowered the $[\text{K}^+][\text{Cl}^-]$ product to 700 (mmol/L)².

After 1 hour of global ischemia, hearts were rewarmed and reperfused for 30 minutes. Intracavitary left ventric-

Table I. Postreperfusion percent recovery of developed pressure and slopes of the LVEDP-diastolic volume relationship under preischemic conditions and after reperfusion

	%DP	m_b	m_r
Standard StT ($n = 8$)	55.1 ± 3.7	156.1 ± 13.8	$175.0 \pm 14.9^\dagger$
Low Cl ⁻ StT ($n = 8$)	$73.7 \pm 2.6^*$	180.9 ± 24.5	$203.4 \pm 22.6^\dagger$

Values are expressed as mean $\pm$ SEM. m_b , Preischemic slope of the LVEDP-V relationship; m_r , postreperfusion slope of the LVEDP-V relationship; St T, St. Thomas' Hospital cardioplegia.

*Statistically different from Standard StT ($p = 0.003$).

† Statistically different from respective m_b (paired t test, $p = 0.044$ and 0.028 for Standard and Low Cl⁻ StT, respectively).

ular pressure waveforms and electrograms were recorded every 5 minutes during the reperfusion period at a fixed balloon volume corresponding to a preischemic LVEDP of 5 mm Hg. After 30 minutes of reperfusion, data were acquired over the identical range of balloon volumes examined before ischemia. At the conclusion of the study, a sample of the left ventricle was excised, blotted, weighed, and then dried until a constant dry weight was reached. The extent of myocardial edema was determined by calculating percent tissue (%H₂O):%H₂O = (Wet weight - Dry weight)/Wet weight.

Data analysis. Digitized pressure waveforms were analyzed, as described previously, using software developed in our laboratory.⁹

End-systolic pressure. End-systolic pressure (ESP) was calculated for each of the seven preischemic and seven postreperfusion balloon volumes. The ESP-volume relationship (ESPVR) was fitted with a least-squares linear regression:

$$ESP = E_{max} \times V + k$$

where E_{max} was the slope of the ESPVR, V was the balloon volume, and k was the y-axis intercept of the ESPVR.

EDP. EDP was calculated for each balloon volume and the EDPVR was fitted with a least-squares linear regression:

$$EDP = m(V - V_o)$$

where m was the slope of the EDPVR and V_o was the balloon volume at which EDP is zero or the x-axis intercept. A linear representation of the EDPVR has been shown to be appropriate over the limited range of volumes examined in this model.¹⁰ The mean linear regression coefficients for the EDPVR were 0.98 ± 0.01 , and 0.99 ± 0.00 for the standard and low Cl⁻ St. Thomas' Hospital solution groups, respectively. The slope of the relationship was used as an estimate of diastolic compliance.

Developed pressure. Developed pressure in the left ventricle (DP) was defined as the difference between ESP and EDP, and 10 consecutive beats were averaged for each balloon volume. The pressure-volume relationship was fitted using the following linear regression:

$$DP = ESP - EDP = (E_{max} \times V + k) - m(V - V_o)$$

Recovery of DP. The recovery of DP, expressed as a percentage, was calculated as the ratio of the postreperfusion DP to the preischemic DP at the same balloon volume. The average percent recovery of DP (%DP) was determined from the following definite integral, approximated using the trapezoidal rule:⁸

$$\%DP = (100) \int_{V_a}^{V_b} \frac{DP \text{ postreperfusion}}{DP \text{ preischemia}} \cdot dV/(V_b - V_a)$$

where V_b was the largest matching postreperfusion balloon volume and V_a was the smallest matching postreperfusion balloon volume.

Statistical analysis. Results are expressed as the mean $\pm$ standard error of the mean. A one-way repeated measures analysis of variance was used for comparisons that involved sequential, time-based measurements. When appropriate, the Kruskal-Wallis analysis of variance on ranks was used as a nonparametric alternative. Individual comparisons between groups were made using a Tukey post test. A t test, or paired t test when appropriate, was used for comparisons between two sets. A χ^2 analysis of contingency tables was used to compare mutually exclusive, categorical data where appropriate.

Results

There were no significant differences in pH, PO₂, PCO₂, serum sodium, potassium, calcium, or hematocrit in the support animal between baseline data acquisition and after 30 minutes of reperfusion. The mean hematocrit was 31 ± 1 at baseline versus 30 ± 2 after reperfusion ($p = 0.53$). There were no significant differences in times to electrical and mechanical arrest between low Cl⁻ St. Thomas' Hospital (28.2 ± 2.8 seconds and 27.6 ± 2.9 seconds, respectively) and standard St. Thomas' Hospital solutions (31.4 ± 3.2 seconds and 27.1 ± 1.2 seconds, respectively).

Postischemic diastolic compliance. Postreperfusion changes in diastolic compliance were quantified by determining the slope of the LVEDP-V (Table I). Both groups demonstrated significantly decreased compliance after reperfusion compared with before ischemia. However, there was no difference between standard and low Cl⁻ solutions.

Postischemic systolic function. Low Cl⁻ St. Thomas' Hospital cardioplegia resulted in significantly improved recovery compared with standard St. Thomas' Hospital cardioplegia as assessed by %DP (Table I). The systolic dysfunction presented early in the reperfusion period and remained constant over the 30-minute reperfusion interval (Fig. 1).

Fig. 1. Developed pressure (*DP*) under preischemic conditions (time = 0 minutes) and effect of standard and low Cl^- St. Thomas' Hospital solution on *DP* during the reperfusion period (time = 60 to 90 minutes). Values are expressed as mean $\pm$ standard error of the mean. *Significant difference versus St. Thomas' Hospital solution, with *p* values shown in parentheses.

Coronary flow. Both groups exhibited a significant increase in coronary flow immediately on reperfusion (Fig. 2). Reperfusion hyperemia lasted longer in the low Cl^- St. Thomas' Hospital group. At 5 minutes, the flow in the standard cardioplegia group had returned to preischemic levels, whereas coronary blood flow was still significantly elevated in the low Cl^- group.

Myocardial tissue water. Mean percent tissue water for standard and low Cl^- St. Thomas' Hospital cardioplegia was $78.0\% \pm 0.4\%$ and $78.6\% \pm 0.2\%$, respectively. There were no statistically significant differences between the two groups.

Atrioventricular conduction times. The P-R interval was significantly prolonged throughout the reperfusion period compared with baseline in the standard St. Thomas' Hospital group (Fig. 3). In contrast, no conduction delay was observed in the low Cl^- St. Thomas' Hospital group.

Alternative anion. An alternative anion, aspartate, was examined to investigate whether there was any protective effect of methanesulfonate itself. Both of the low Cl^- solutions had identical $[\text{K}^+][\text{Cl}^-]$ products. The low Cl^- St. Thomas' Hospital solution

formulated with aspartate had an identical protective effect when compared with the methanesulfonate group. There was a $69\% \pm 2\%$ recovery of *DP* in this group. This was significantly better than standard St. Thomas' Hospital solution ($p < 0.05$) and not significantly different than the methanesulfonate group. Similar to the methanesulfonate group, there also was no significant prolongation of the P-R interval in the aspartate hearts, 119 ± 5 msec before versus 110 ± 9 msec after ischemia ($p = 0.62$).

Discussion

Under hypothermic conditions, the activity of the transporters and pumps that normally regulate cell volume are markedly depressed, which leaves only passive fluxes of ions to modulate cell volume.^{6, 7, 11} Because of the high membrane permeabilities to K^+ and Cl^- , the KCl product inside the cell must equal the KCl product outside the cell under these conditions.⁵ This would predict that cell swelling would occur with exposure to St. Thomas' Hospital solution because St. Thomas' Hospital solution has a much greater KCl product ($2566.4 \text{ [mmol/L]}^2$) than either blood plasma (350 to 550 $[\text{mmol/L}]^2$) or

Fig. 2. Coronary blood flow (CBF) under preischemic conditions (time = 0 minutes) and effect of standard and low Cl^- St. Thomas' Hospital solution on CBF during the reperfusion period (time = 60 to 90 minutes). Values are expressed as mean $\pm$ standard error of the mean. * $p < 0.05$ versus respective preischemic level.

physiologic crystalloid (e.g., Tyrode's solution, 700 $[\text{mmol/L}]^2$). By lowering the KCl product of cardioplegic solution while maintaining osmolality constant by partially replacing $[\text{Cl}^-]_o$ with a large, impermeant ion, cell swelling was prevented in isolated myocytes.⁶ This study was designed to examine whether lowering the KCl product would improve myocardial protection in an intact, blood-perfused heart.

Functional consequences of low Cl^- hyperkalemic cardioplegia. Lowering the KCl product significantly improved postischemic recovery of DP, ameliorating myocardial stunning. This is consistent with the fact that intracellular edema has been shown to result in ultrastructural disturbances such as mitochondrial swelling and vacuolization of the sarcoplasm.¹² Moreover, derangements in intracellular volume may adversely influence the regulation of cellular metabolism, hormone, and transmitter release.¹²⁻¹⁴ Further studies will be needed to address the specific mechanisms involved in this injury.

An alternate explanation for our results is that the Cl^- ion possesses some inherent toxicity. However, this ubiquitous ion has not been shown in any

previous study to be toxic within its physiologic range of concentrations. Another explanation for our findings would be that methanesulfonate itself possessed inherent cardioprotective effects. However, we obtained identical results with an alternate impermeant anion, aspartate. These data strongly suggest that it is the correction of the hypotonicity of the cardioplegic solution that is critical, not the specific impermeant anion used to achieve this result.

Low Cl^- St. Thomas' Hospital solution did not offer better preservation of LV diastolic compliance (Table I). This is not surprising. In isolated myocyte preparations, cell swelling returned to baseline within 20 minutes of reperfusion.⁶ In this study, percent tissue water in the standard St. Thomas' Hospital group was not significantly different from that of the low Cl^- St. Thomas' Hospital group after 30 minutes of reperfusion. Thus one would not have expected a change in ventricular compliance at that point.

Coronary blood flow. Myocardial edema has been associated with decreased coronary perfusion.^{15, 16} In the standard St. Thomas' Hospital

Fig. 3. Effect of standard and low Cl^- St. Thomas' Hospital solution on atrioventricular conduction times after reperfusion. Values are expressed as mean $\pm$ standard error of the mean. *LOW Cl-*, Low Cl^- St. Thomas' Hospital solution; *STANDARD*, Standard St. Thomas' Hospital solution. * $p < 0.05$ versus preischemic P-R interval.

group an initial reperfusion hyperemia dropped to preischemic values almost immediately. In the low Cl^- cardioplegia group reperfusion flow remained elevated for 5 minutes (Fig. 2). One explanation for this is that myocardial, or perhaps vascular, edema early in the reperfusion period blunted the hyperemic response in the standard St. Thomas' Hospital group compared with the low Cl^- group. As edema resolved over the reperfusion period, coronary flow returned to normal levels. Alternatively, whole-cell patch-clamp studies of isolated rabbit coronary artery smooth muscle cells have characterized a chloride current that contributes to coronary vasoconstriction.¹⁷ It is possible that coronary smooth muscle activation was transiently affected by the low Cl^- milieu.

Atrioventricular conduction. Our laboratory and others have shown that cell swelling and edema are associated with slowed ventricular conduction.^{9, 18} In this study, the conduction delay observed after standard cardioplegia (Fig. 3) was not seen in the low Cl^- cardioplegia group. In previous work, we have hypothesized that the conduction delays associated with hyperkalemic solutions were due to an increase in cell volume, which decreased the extracellular space, resulting in a higher resistance to current flow.^{18, 19} This hypothesis is supported by our observations in isolated myocytes⁶ and by this

study. Because slowed conduction velocity plays a central role in arrhythmogenesis,^{20, 21} the postoperative arrhythmias common after cardiac procedures may be prevented by reformulating standard cardioplegic solutions.

Advantages and disadvantages of the blood-perfused isolated heart Langendorff model. The advantages and drawbacks of this model have been previously described.⁸ The more physiologic nature of this model is a distinct advantage over nonparabolic and crystalloid-perfused models. However, although this model offers a closer approximation to the clinical scenario, care should be taken in extrapolating results from in vitro studies to the clinical setting.

Conclusion

These results demonstrate a clear correlation between the prevention of cell swelling and the amelioration of the detrimental contractile and electrophysiologic sequelae of hypothermic, hyperkalemic cardioplegia. Blood-perfused isolated hearts treated with low Cl^- , St. Thomas' Hospital cardioplegic solution displayed significantly improved postischemic functional recovery and shorter atrioventricular conduction times when compared with standard high Cl^- St. Thomas' Hospital solution. New strategies aimed at correcting the hypotonicity

of cardioplegic solutions and preventing cell swelling may improve myocardial protection with hyperkalemic cardioplegia.

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