ZINC SUPPLEMENTATION ENHANCES THE EFFECTIVENESS OF ST. THOMAS' HOSPITAL NO. 2 CARDIOPLEGIC SOLUTION IN AN IN VITRO MODEL OF HYPOTHERMIC CARDIAC ARREST

The present study was done to assess the effectiveness of a zinc-supplemented cardioplegic solution in an in vitro model of hypothermic arrest. Isolated hearts were perfused in the nonworking mode. All hearts were subjected to 2 hours of hypothermic arrest, at 10° C, followed by 60 minutes of recovery. In protocol 1, arrest was initiated with infusion of cardioplegic solution with or without 30 μ mol/L zinc for 5 minutes, which was then reinfused for 5 minutes every 15 minutes during arrest. In protocol 2, arrest was initiated with infusion of cardioplegic solution with or without 40 μ mol/L zinc for 10 minutes. Cardioplegic solution (without zinc) was then reinfused for 5 minutes before the hearts were rewarmed. In protocol 1 hearts, peak postischemic left ventricular developed systolic pressure was 106 ± 5 mm Hg and 80 ± 3 mm Hg in zinc-treated versus control hearts, respectively ($p < 0.05$ by repeated-measures analysis of variance). In protocol 2 hearts, recovery of postischemic left ventricular developed systolic pressure peaked at 74 ± 4 mm Hg and 46 ± 8 mm Hg in zinc-treated and control hearts, respectively ($p < 0.05$, repeated-measures analysis of variance). Similar effects were observed for the left ventricular rate of relaxation ($p < 0.05$, repeated-measures analysis of variance). Except for some minor effects, lactate dehydrogenase release was not affected by zinc supplementation. The present study demonstrates that zinc supplementation further enhances the normally observed preservation of postarrest cardiac function and suggests possible clinical utility for this metal as an additive to standard crystalloid cardioplegic solutions. (J THORAC CARDIOVASC SURG 1995;110:1642-8)

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Since the development of cardiopulmonary bypass (CPB), the use of cold cardioplegic solutions to arrest the heart and decrease myocardial oxygen debt has become commonplace.¹ Yet, despite the use of these solutions, the occurrence of postoperative cardiac complications, such as low-output syndrome, continues to be a major problem after CPB.² Research in this area has generally focused

on the development of more effective chemical approaches to myocardial preservation during CPB. The realization that reactive oxygen intermediates (ROI) play a role in the postischemic myocardial stunning injury^{3,4} began a new era in myocardial preservation. Investigators have reasoned that the postoperative cardiac complications of cardiac operations that require CPB may in fact represent a form of myocardial stunning involving formation of ROIs. This reasoning is strengthened by the findings of several recent studies in human beings that demonstrate the presence of by-products of ROI production, such as increased oxidized glutathione,⁵ malondialdehyde,⁶ and alkoxyl radicals⁷, in post-CPB coronary sinus blood. Thus, in both in vitro and in vivo experimental models, investigators have attempted to augment current cardioplegic solutions with free radical "scavengers" such as superoxide dismutase and catalase.⁸⁻¹⁰ Despite some early promising results, superoxide dismutase has yet to find clinical usefulness.¹¹ The results of one limited

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clinical study indicated that intravenous superoxide dismutase had no effect on ventricular function in patients undergoing thrombolysis because of anterior wall acute myocardial infarction.¹²

One of the problems with the use of ROI scavengers is that they seek to remove the toxic species after they are formed. From a kinetic standpoint, it would seem more advantageous to prevent formation of the more reactive species, such as $\cdot\text{OH}$, in the first place. In two previous studies, we demonstrated the antiarrhythmic¹³ and cardiac protective¹⁴ qualities of zinc when it is added to Krebs–Henseleit buffer in isolated heart models of regional and global ischemia. Zinc, a nonredox-active transition metal, was tested because of its chemical similarities to the redox-active transition metals, iron and copper.¹⁵ In our previous studies on the globally ischemic heart, a model of stunning, we demonstrated that zinc diminished the early reperfusion burst of $\cdot\text{OH}$ through a mechanism that appeared to be related to antagonism of the pro-oxidant activity of copper.¹⁴ Partly on the basis of the success of these past studies, we tested the ability of zinc to preserve postcardioplegic myocardial function in *in vitro* models of hypothermic and intermittent hypothermic cardioplegic arrest when it is added to a commonly used crystalloid cardioplegic solution. In the present study, despite differences in the perfusion media and protocols and in the temperature, we demonstrate that zinc maintains its cardioprotective qualities even in these models of prolonged hypothermic arrest.

Material and methods

Animals. All studies were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee of North Shore University Hospital. Male Sprague–Dawley rats (275 to 450 gm) were obtained from Charles River Laboratory, Inc. (Wilmington, Mass.) or Taconic Farms (Germantown, N.Y.) and were allowed at least 3 days of in-house acclimatization before experimental use. During this time, all animals were allowed ad libitum access to Purina Lab Chow (Ralston Purina Co., St. Louis, Mo.) and water.

Chemicals and reagents. Sodium bicarbonate, sodium chloride, potassium chloride, HEPES, magnesium sulfate, magnesium chloride, d-(+)-glucose, calcium chloride, zinc sulfate, and histidine were obtained from Sigma Chemical Company (St. Louis, Mo.). Sodium heparin and sodium pentobarbital were obtained from the North Shore University Hospital pharmacy. Therapeutic-grade 95% O_2 /5% CO_2 was obtained from General Welding Supply Company (Westbury, N.Y.).

Table I. Composition of St. Thomas’ Hospital No. 2 (Plegisol) cardioplegic solution

Component	Concentration (mmol/L)
Sodium	120.0
Potassium	16.0
Calcium	1.2
Magnesium	16.0
Chloride	160.4
Bicarbonate	10.0

Perfused heart preparation. Rats were injected with sodium heparin (500 units, intraperitoneally) 30 minutes before being anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneally). Hearts were removed rapidly and placed in ice-cold heparinized saline solution. The hearts were then perfused in an orthograde manner through the coronary arteries¹⁶ as previously described¹³ at a constant pressure of 95 cm H_2O .

Perfusate. The perfusate was a modified Krebs–Henseleit buffer consisting of (in millimoles per liter): NaCl 118, KCl 6.1, CaCl_2 2.5, MgSO_4 1.2, NaHCO_3 25, HEPES 1.0, and glucose 11.1. Complete buffer was prepared the day of the experiment by mixing the proper amounts of concentrated stock solutions to which the appropriate quantities of glucose and calcium chloride were added. All concentrated solutions, with the exception of the magnesium sulfate, were treated with iminodiacetic acid chelating resin beads, 50 to 100 mesh (Chelex 100, Bio-Rad, Hercules, Calif., obtained from Sigma Chemical Co.), as previously described.¹⁷ The formula for the cardioplegic solution was that of St. Thomas’ Hospital No. 2 (Plegisol, Abbott Laboratories, Chicago, Ill.) solution¹⁸ as described in Table I and was prepared as a 10-times concentrated solution (without calcium). On the day of the experiment, the concentrated solution was diluted and the proper amount of calcium added. Zinc was added to the cardioplegic solution as the zinc-bis-histidine complex (1 zinc:2 histidines) and was prepared daily.

Experimental protocols

Protocol 1. Protocol 1 consisted of intermittent hypothermic cardioplegia (Fig. 1). Isolated hearts were equilibrated with Krebs–Henseleit buffer at 37° C for 10 minutes. During the 2 hours of hypothermic arrest, cardioplegic solution with or without 30 $\mu\text{mol/L}$ zinc was reperfused for 5 minutes every 15 minutes. This was followed by 60 minutes of reperfusion with Krebs–Henseleit buffer at 37° C.

Protocol 2. Protocol 2 consisted of “no-flow” hypothermic arrest (Fig. 1). Isolated hearts were equilibrated with Krebs–Henseleit buffer at 37° C for 10 minutes. Cardiac arrest was initiated with 10 minutes of perfusion with cardioplegic solution with or without 40 $\mu\text{mol/L}$ zinc at 10° C. During arrest, no cardioplegic solution was infused. Just before reperfusion, all hearts were perfused with hypothermic cardioplegic solution. Hearts were then reperfused for 60 minutes with Krebs–Henseleit buffer at 37° C.

During arrest cardiac temperature was maintained at 10° C by immersion of the heart in cardioplegic solution

Fig. 1. Experimental protocols for cardioplegia studies.

contained in a thermostatically controlled, water-jacketed heart chamber.

Indices of cardiac function. There were six indicators of cardiac function measured in this study. Coronary flow was determined by a timed collection of coronary effluent. Heart rate was calculated from the R-R interval of the electrocardiogram. Left ventricular systolic pressure development and end-diastolic pressure were determined by way of a latex balloon (0.1 ml) that was expanded to exert a physiologic end-diastolic pressure of 5 mm Hg as previously described.¹³ Systolic pressure developed or pulse pressure was calculated as the peak systolic pressure minus the end-diastolic pressure. Contractility was calculated as the maximum rate of rise of the pressure curve or $+dP/dt_{\max}$ and the maximum isovolumetric rate of relaxation was calculated from the rate of fall of the pressure curve or $-dP/dt_{\max}$.

Exclusion criteria. Hearts were excluded from the study if they failed to maintain developed systolic pressure of at least 70 mm Hg or a heart rate of at least 220 beats/min during the 10-minute pretreatment equilibration period. Further, hearts were excluded if a persistent arrhythmia was present during the equilibration period.

Chemical analysis. Lactate dehydrogenase (LDH) activity in pulmonary artery effluent was determined with use of the method described by Bergmeyer, Bernt, and Hess¹⁹ and is expressed in Racker units.

Statistical analysis. Analysis of differences of cardiac functional recovery and LDH release were analyzed with a repeated-measures analysis of variance (ANOVA) in which the within-group factor was time. Differences between two individual groups were analyzed with an independent Student's *t* test. In all cases, results were considered to be significant at the $p < 0.05$ level. All statistical analyses were done with the SPSS/PC+ (SPSS Inc., Chicago, Ill.) statistical analysis package.

Results

Effect on heart rate and coronary flow. The effect of zinc-supplemented cardioplegic solutions on

postischemic heart rate is illustrated in Fig. 2. Treatment of hearts with zinc according to protocol 1 resulted in a significantly ($p < 0.05$, repeated-measures ANOVA) lower heart rate in the postcardioplegia period. By the end of the reperfusion period, heart rate in control versus zinc-treated hearts had returned to 95% and 80% of precardioplegic values, respectively. This was not the case for protocol 2, because there were no significant differences between control and treated hearts with this protocol. Heart rate in both groups had returned to approximately 80% of precardioplegic values by the end of the experiment.

Zinc-supplemented cardioplegic solutions had little effect on coronary flow (data not shown). In protocol 1 hearts, the postarrest coronary flow rate returned to a peak of 82% of prearrest values (control, 17 ± 1 ml/min; zinc-treated, 16 ± 1 ml/min). In protocol 2 hearts, postarrest coronary flow rates returned to a peak of 95% of prearrest values (control, 19 ± 1 ml/min; zinc-treated, 19 ± 2 ml/min).

Effect on left ventricular systolic pressure development. The effect of zinc-supplemented cardioplegic solutions on postischemic systolic pressure development is illustrated in Fig. 3. Treatment of hearts with zinc according to both protocols 1 and 2 resulted in significant ($p < 0.05$, repeated-measures ANOVA, for both protocols) improvement in postcardioplegic left ventricular systolic pressure development. In the protocol 1 groups, maximal recovery for zinc-treated hearts was virtually 100%, whereas that of control hearts was 80% of precardioplegic values. In the more severe protocol 2 treatment

Fig. 2. Effect of zinc-supplemented cardioplegic solution on postischemic heart rate. Isolated hearts were equilibrated with Krebs-Henseleit buffer at 37° C for 10 minutes and then subjected to 2 hours of hypothermic (10° C) global ischemia. This was followed by 60 minutes of reperfusion with Krebs-Henseleit buffer at 37° C. **A**, Intermittent hypothermic cardioplegia. Cardiac arrest was initiated with 5 minutes of perfusion with cardioplegic solution with or without 30 μmol/L zinc at 10° C, which was then reperfused for 5 minutes every 15 minutes during arrest (control, $N = 12$; zinc-treated, $N = 8$). **B**, "No-flow" hypothermic arrest. Cardiac arrest was initiated with 10 minutes of perfusion with cardioplegic solution with or without 40 μmol/L zinc at 10° C. Just before reperfusion, all hearts were perfused with hypothermic cardioplegic solution (control, $N = 9$; zinc-treated, $N = 7$). Statistical analysis is presented in Results section.

groups, maximal recovery was 76% and only 50% in zinc-treated and control hearts, respectively.

Effect on left ventricular contractility and rate of relaxation. As illustrated in Fig. 4, postischemic contractility ($+dP/dt_{max}$) was also improved in the zinc-treated hearts. The difference between control and treated hearts was significant ($p < 0.05$, repeated-measures ANOVA) in the protocol 1 groups. In the protocol 2 groups, because of relatively large variability in the control hearts, the difference, while apparent, was not significant. Similar, but more marked, effects of zinc-supplemented cardioplegic solutions were observed on the rate of left ventric-

Fig. 3. Effect of zinc-supplemented cardioplegic solution on postischemic left ventricular systolic pressure development. Presentation same as in Fig. 2.

ular relaxation ($-dP/dt_{max}$) (Fig. 5). Differences between control and treated hearts were significant ($p < 0.05$, repeated-measures ANOVA) in both protocol groups.

Effect on LDH release. As illustrated in Fig. 6, zinc-treatment had virtually no effect on LDH release, except for some minor differences ($p < 0.05$, t test) observed toward the end of the reperfusion period (protocol 2 only).

Discussion

The results of this study demonstrate that zinc supplementation of a standard cardioplegic solution (St. Thomas' Hospital No. 2) further enhances the normally observed preservation of postarrest cardiac function. One of the most novel aspects of this study was that prolonged perfusion with zinc-containing solutions was not necessary to obtain significant cardiac protection. This is in contrast to findings in our previous kinetic studies in the normothermic globally ischemic heart.¹⁴ In that study we showed that to obtain optimal protection, zinc must be present both before and after ischemia. The critical period was clearly the preischemic period, because treatment at the start of reperfusion only actually decreased postischemic recovery.¹⁴ Al-

Fig. 4. The effect of zinc-supplemented cardioplegic solution on postischemic left ventricular contractility ($+dP/dt_{\max}$). Presentation same as in Fig. 2.

though zinc is unlike other agents that have been tested, such as superoxide dismutase, in that myocardial cells do absorb zinc,¹⁴ there was some question as to whether sufficient amounts of the metal would be available because of the low temperatures at which the cardioplegic solution was administered. Cellular uptake of zinc is known to be a facilitated process that has been demonstrated to be significantly impaired at hypothermic temperatures.²⁰ Yet, despite the need for a critical loading period and the possibility of decreased absorption at low temperatures, it was thought that a possible clinical application of zinc might be myocardial preservation during CPB through inclusion in cardioplegic solutions.

In the present study, two protocols for administration of the cardioplegic solution were examined. Protocol 1 represents an *in vitro* model of intermittent hypothermic arrest with the total time of perfusion with zinc-supplemented cardioplegic solution equaling 35 minutes. Protocol 2 represents a model of straight hypothermic arrest, with only 10 minutes of perfusion with a higher concentration of zinc at the initiation of arrest only. This latter treatment interval was chosen because results of previous kinetic studies in the global ischemia model suggested that this period was critical.¹⁴ In both of these

Fig. 5. Effect of zinc-supplemented cardioplegic solution on postischemic left ventricular rate of relaxation ($-dP/dt_{\max}$). Presentation same as in Fig. 2.

models, supplementation of the cardioplegic solution with zinc resulted in clear improvement of cardiac function in the postarrest period, particularly with respect to left ventricular systolic pressure development and rate of relaxation. The lower postarrest heart rate, observed in protocol 1 hearts, is consistent with the negative chronotropic effect of zinc that we have previously reported.^{13,14} The mechanism of the negative chronotropic effect is unclear, but may be related to effects of zinc on movement of divalent cations, such as potassium and calcium.²¹ Effects on heart rate were not observed in protocol 2 hearts because overall less zinc was administered. On the basis of average coronary flow rates, it was estimated that approximately 20 μmol of zinc was administered to protocol 1 hearts, whereas only 6 μmol was administered to protocol 2 hearts. Effects on heart rate do not appear to be related to the protective effect (unpublished observations).

The role of zinc in the heart is not known; however, several studies have suggested an increased requirement by the postischemic heart. In human beings, it has been demonstrated that plasma zinc levels decrease after acute myocardial infarction.²²⁻²⁵ In experimentally infarcted dog heart, increased uptake of zinc into the mitochondria and

Fig. 6. Effect of zinc-supplemented cardioplegic solution on postischemic release of LDH. Presentation same as in Fig. 2.

microsomal fractions, both possible sites of ROI production,^{26, 27} has been observed.²⁸ These results suggest an increased need for zinc by the ischemic myocardium. Whether the same is true for the hypothermic arrested heart, which has decreased metabolic oxygen debt, is unclear. Nonetheless, we believe that the protection of the heart subjected to hypothermic arrest was consistent with that observed in normothermic globally ischemic hearts¹⁴ and, further, is consistent with the overall hypothesis that zinc is an endogenous antioxidant.²⁹ We^{13, 14, 30} and others³¹⁻³⁴ have proposed that zinc inhibits site-specific formation of radicals catalyzed by redox-active transition metals such as iron and copper. In the crystalloid-perfused isolated heart model, the source of the metals appears to be the heart itself^{14, 35} and possibly Krebs-Henseleit buffer under certain conditions.¹⁷ In extracorporeally circulated blood, during CPB, recent evidence suggests that red blood cells may be a potential source of both chelatable iron and copper.³⁶ Zinc, by virtue of its similarities in coordination chemistry,¹⁵ can displace site-specifically bound copper or iron, or both, though recent evidence suggests that copper is the more important of the two.^{14, 32} Because zinc is nonredox active, site-specific formation of radicals is thus diminished. Assuming that postoperative

myocardial dysfunction is indeed a form of iatrogenic myocardial stunning injury and that ROIs are involved,⁵⁻⁷ it would be reasonable to suggest that a similar mechanism is involved in the current studies.

In summary, these studies demonstrate that zinc, when added to a cardioplegic solution, can exert a protective effect on the heart in a state of hypothermic arrest, at least in this in vitro crystalloid-perfused isolated heart model. Whether zinc will find clinical usefulness as an additive to cardioplegic solutions remains to be seen. Clearly, the results of this study support the continuation of ongoing in vivo large animal studies to confirm the effectiveness and safety of zinc as a cardioprotective agent before clinical testing in human beings.

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