

The Effects of Na Movement on Surgical Myocardial Protection: The Role of the Na⁺-H⁺ Exchange System and Na-Channel in the Development of Ischemia and Reperfusion Injury

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Objectives: We investigated whether the Na⁺-H⁺ exchange inhibitor, HOE642 (Hoe), and/or the Na channel blocker, mexiletine (Mex), enhance a cardioprotective effect on St. Thomas' Hospital cardioplegic solution (STS) to clarify the mechanism by which intracellular Na⁺ is accumulated after cardioplegic arrest.

Materials and Methods: Isolated working rat hearts were perfused with Krebs-Henseleit bicarbonate buffer (KHBB). The hearts were then arrested with STS and subjected to normothermic global ischemia (30 min). This was followed by Langendorff reperfusion (15 min) and then a working reperfusion (20 min). In study A, we added Hoe (5, 10, and 20 μM), Mex (70 μM), or a combination of Hoe (20 μM) and Mex (70 μM), to STS. In study B, we added Hoe (20 μM), Mex (70 μM), or a combination of Hoe (20 μM) and Mex (70 μM) to KHBB during the first 3 min of Langendorff reperfusion.

Results: In study A, the addition of Hoe (10 and 20 μM) to STS showed a significantly greater postischemic recovery of cardiac output compared to the control group [63.1±5.7% (10 μM), 62.7±4.7% (20 μM), and 55.5±4.6% (control), respectively]. The postischemic recovery of cardiac output was significantly greater in the group of the combined addition (Hoe and Mex) to STS than that in the control, 20 μM Hoe, 70 μM Mex groups [70.3±3.7% (Hoe and Mex), 55.5±4.6% (control), 62.7±4.7% (Hoe 20 μM), and 60.2±4.7% (Mex 70 μM), respectively]. The myocardial water content in the postischemic period was 565.1±29.1, 525.8±2.9, 509.4±19.6, and 532.2±20.1; it was 497.3±9.1 mL/100 g dry weight in the control; and 10 μM Hoe, 20 μM Hoe, and 70 μM Mex in the combined use groups. In study B, there was no significant difference in the postischemic recovery of cardiac output in all experimental groups.

Conclusion: The combined use of the Na⁺-H⁺ exchange inhibitor and Na⁺ channel blocker during cardioplegia may achieve a superior cardioprotective effect on myocardial damage because of ischemia and reperfusion. (*Ann Thorac Cardiovasc Surg* 2007; 13: 301–307)

Key words: HOE642, mexiletine, ischemia and reperfusion injury, cardioplegia

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Introduction

Preoperative myocardial dysfunction is still one of the major causes of operative morbidity and mortality in patients undergoing cardiovascular operations, despite remarkable improvements in cardioprotective techniques. Therefore further improvements of protective techniques are still required. The mechanisms responsible for myocardial damage resulting from ischemia and reperfusion

have been widely studied in the field of basic and clinical research. Extensive experimental studies provide evidence that suggest that a rise in cytosolic free Ca^{2+} concentration during ischemia and reperfusion contributes to the development of cell damage during ischemia and reperfusion.^{1,2)} A major pathway of increased Ca^{2+} entry is through the Na^+ - Ca^{2+} exchange secondary to a rise in intracellular Na^+ concentration.³⁻⁵⁾ Therefore the prevention of Na^+ accumulation may exert beneficial effects for myocardial protection against ischemia and reperfusion injury in terms of intracellular Ca^{2+} -overload. The mechanism of intracellular Na^+ accumulation during ischemia and reperfusion has not been completely clarified. Several pathways for an increase in intracellular Na^+ have been reported. The Na^+ - H^+ exchanger has been shown to play an important role for the intracellular Na^+ accumulation during ischemia and reperfusion.^{6,7)} Other mechanisms such as the noninactivating Na^+ channels and the increase of permeability of cell membrane during ischemia and reperfusion have also been suggested to contribute to the intracellular Na^+ accumulation.⁸⁾ The purpose of this study was to assess whether an Na^+ - H^+ exchange inhibitor [HOE642 (Hoe); Hoechst, Germany] and an Na^+ channel blockade [mexiletine (Mex); Boehringer Ingelheim, Japan] contribute to enhance the cardioprotective effects of St. Thomas' Hospital cardioplegic solution (STS).

Materials and Methods

Animals

Male Sprague-Dawley rats (280 to 330 g body weight) were used in all studies. The animals received human care in compliance with the *Principles of Laboratory Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 85-23, revised 1985).

Experimental model

An isolated working rat heart preparation was used for this study. The rat was anesthetized with ether. It was rapidly excised after heparinization and placed in a cold Krebs-Henseleit bicarbonate buffer (KHBB) solution. After cannulation of the aorta, the heart was perfused with KHBB at a perfusion pressure of 100 cmH₂O in Langendorff mode for a 5-min stabilization period. During this stabilization period, the pulmonary artery was cut, and through the pulmonary vein the left atrium was cannulat-

ed. The heart was then converted to working mode perfusion, and preischemic cardiac function (aortic flow, coronary flow, and heart rate) was measured during four 5-min periods of working mode perfusion. It was then arrested with a 3-min infusion of STS at a perfusion pressure of 60 cmH₂O and subjected to 30 min of normothermic (37°C) global ischemia. This was followed by a 15-min Langendorff reperfusion and then a 20-min working reperfusion. The postischemic cardiac function was measured as a preischemic method. Throughout the experiment, Langendorff reservoir, lung, elastic chamber, heart chamber, and atrial chamber were maintained at 37°C by temperature-regulated pumps (Fig. 1).

Perfusion medium

The perfusion medium was KHBB containing (mM) NaCl 118.5, NaHCO_3 25.0, KCl 4.7, MgSO_4 1.2, KH_2PO_4 1.2, CaCl_2 2.5, and glucose 10.0 at pH of 7.4. The buffer was filtered (5 μm pore size) before use and was continuously gassed with 95% oxygen and 5% carbon dioxide. The cardioplegic solution was STS containing (mM) NaCl 110.0, KCl 16.0, MgCl_2 16.0, CaCl_2 1.2, and NaHCO_3 10.0 at pH 7.8, which has osmolarity of 324 mOsm/L. The cardioplegic solution was filtered (5 μm pore size) before use.

Experimental protocol

In study A, the animals were divided into six groups ($n = 6$ rats/group). In study B, the animals were divided into four groups ($n = 6$ rats/group). Hoe was used as an Na^+ - H^+ exchange inhibitor. Mex was used as an Na^+ channel blocker. In study A, 5 μM HOE642 (group H5), 10 μM Hoe (group H10), 20 μM Hoe (group H20), 70 μM Mex (group M), or a combination of 20 μM Hoe and 70 μM Mex (group HM) was given to STS (Fig. 2). In study B, 20 μM Hoe (group H-R), 70 μM Mex (group M-R), or a combination of Hoe 20 and Mex 70 μM (group HM-R) was given to reperfusate during the first 3 min of the Langendorff reperfusion period (Fig. 2).

Measurement

Aortic flow was measured with an electromagnetic flowmeter (Nihon Kohden MFV-3200; Japan) installed between the elastic chamber and the top of the lung. Coronary flow was measured from the effluent of the right heart. Cardiac output was derived from the sum of aortic flow and coronary flow. Aortic pressure was measured by connecting a fluid-filled tube from a side arm of the cannula to a pressure transducer. Heart rate was calculat-

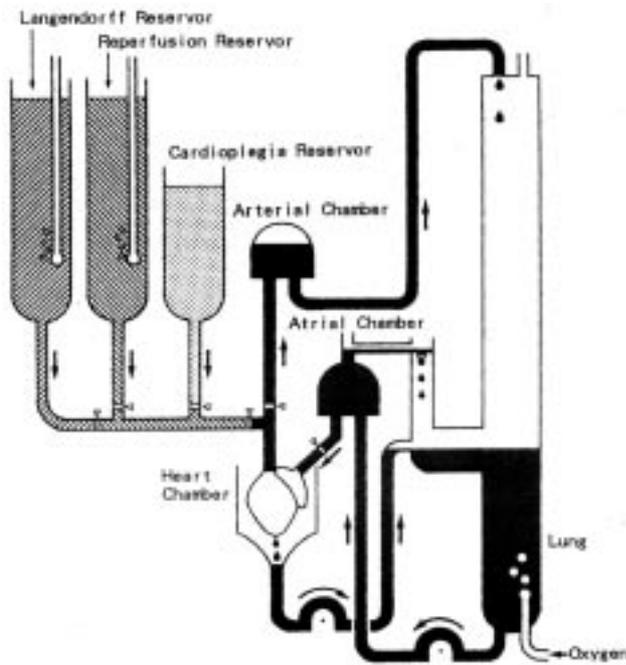


Fig. 1. Experimental model.

The isolated perfused working heart model is a left heart preparation in which oxygenated perfusion medium enter the cannulated left atrium at a pressure equivalent to 18 cm H₂O. The perfusate passes to the left ventricle, from which it is spontaneously ejected through an aortic cannula against a hydrostatic pressure equivalent to 100 cm H₂O. Coronary flow from the right side of the heart can be a sample for enzyme or pooled and recirculated with the aortic flow. In the Langendorff non-working mode, the atrial cannula is clamped and perfusion fluid is allowed to flow into the aorta from a reservoir located 100 cm above the heart. Ischemic cardiac arrest can be induced by clamping the aortic cannula. Short periods of preischemic coronary infusion of cardioplegic solution can be achieved by the use of a reservoir located 60 cm above the heart and attached to a side arm of the aortic cannula.

LM	WM	CI	Ischemia	LM	LM	WM
5'	20'	3'	30' at 37°C	3'	12'	20'

Fig. 2. The experimental protocol for study is represented diagrammatically. LM, Langendorff mode; WM, working mode; CI, cardioplegic infusion.

ed from recorded aortic pressure waves (Fukudadenshi AU-5500N; Japan). At the end of the reperfusion period, the heart was removed from the perfusion apparatus; the atria and great vessels were discarded. The remaining myocardium was weighed immediately before and after 24 h of desiccation at 100°C.

Expression of results

Postischemic cardiac function was assessed and expressed as a percentage of the preischemic value. Myocardial water content was calculated from the difference between wet and dry weights and expressed as mL per 100 g dry weight.

Statistical analysis

Data were expressed as means ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used in each study, and the Bonferroni approach was used for individual comparisons when a significant difference was found. The values of P<0.05 were considered to be significant.

Results

Study A

There were no significant differences between the groups in the baseline values of preischemic cardiac function (Table 1). The postischemic recovery of cardiac function and myocardial water content after reperfusion are shown in Table 2. The postischemic recovery of aortic flow, coronary flow, or cardiac output was significantly greater in the H10 and H20 groups than in the control group. The percent recoveries of aortic output were improved from 55.5±4.6% to 63.1±5.7% and 55.5±4.6% to 62.7±4.7% by Hoe 10 µM and 20 µM, respectively (P<0.05). The postischemic recovery of cardiac output was significantly greater in the group of the combined addition (Hoe and Mex) to STS than in the control, 20 µM Hoe, 70 µM Mex groups [70.3±3.7% (Hoe and Mex), 55.5±4.6% (control), 62.7±4.7% (Hoe 20 µM), and 60.2±4.7% (Mex 70 µM), P<0.05 respectively]. Although the recoveries of cardiac function showed a better tendency in the H5 and M groups than in the control group, no significant differ-

Table 1. Preischemic cardiac function in hearts arrested with St. Thomas' Hospital cardioplegic solution containing various concentrations of HOE642 and/or mexiletine

Group	C	H5	H10	H20	M	HM
AF (mL/min)	78.3±3.6	77.8±3.2	79.3±4.1	77.8±6.7	77.3±3.6	78.0±1.6
CF (mL/min)	18.9±0.8	19.0±1.7	18.4±1.2	18.4±0.4	19.7±1.5	18.9±1.2
CO (mL/min)	97.1±4.1	96.8±4.2	97.6±5.2	96.3±7.1	96.7±4.5	96.5±3.3
HR (beats/min)	329.4±24.8	327.0±32.2	326.9±32.2	322.9±25.7	321.6±29.5	308.6±19.3

Values are expressed as means ± SD. C, control; H5, 5 μM HOE642; H10, 10 μM HOE642; H20, 20 μM HOE642; M, 70 μM mexiletine; HM, combined use of 20 μM HOE642 and 70 μM mexiletine. AF, aortic flow; CF, coronary flow; CO, cardiac output; HR, heart rate. *n* = 6/group.

Table 2. Postischemic recoveries of cardiac function in hearts arrested with St. Thomas' Hospital cardioplegic solution containing various concentrations of HOE642 and/or mexiletine

Group	C	H5	H10	H20	M	HM
% AF	49.9±5.4	53.2±3.9	58.0±5.6*	57.2±5.0*	54.4±5.2	64.9±3.6**‡
% CF	78.9±1.5	82.8±4.1	85.2±5.6*	85.7±4.9*	83.4±3.8*	92.6±5.5**‡
% CO	55.5±4.6	58.8±3.4	63.1±5.7*	62.7±4.7*	60.2±4.7	70.3±3.7**
% HR	99.9±3.9	97.4±2.3	100.9±1.3	102.8±11.3	98.8±2.5	97.3±4.2
WC (mL/100 g dry wt)	565.1±29.1	545.5±27.5	525.8±2.9*	509.4±19.6*	532.2±20.1*	497.3±9.1**‡

Values are expressed as mean±SD. *, *P*<0.05 vs. C; †, *P*<0.05 vs. group H20; ‡, *P*<0.05 vs. group M. C, control; H5, 5 μM HOE642; H10, 10 μM HOE642; H20, 20 μM HOE642; M, 70 μM mexiletine; HM, combined use of 20 μM HOE642 and 70 μM mexiletine. % AF, percent recovery of aortic flow; % CF, percent recovery of coronary flow; % CO, percent recovery of cardiac output; % HR, percent recovery of heart rate. WC, myocardial water content (mL/100 g dry wt). *n* = 6/group.

ences were detected. There was a tendency of lower myocardial water content in group H5 than in group C. Myocardial water content in groups H10, H20, M, and HM was significantly lower than in group C. Group HM showed the lowest myocardial water content, which was significantly lower than in group M. Although there was no significant difference in terms of myocardial water content between groups HM and H20, a tendency of less myocardial content was seen in the HM group.

Study B

There were no significant differences between the groups at baseline values in the preischemic cardiac function (Table 3). There was no significant difference between the groups in terms of the postischemic recovery of aortic flow, coronary flow, cardiac output, or heart rate (Table 4). There was no significant difference between the groups in terms of myocardial water content (Table 4).

Discussion

The mechanisms of myocardial injury during ischemia and reperfusion have been extensively studied. Since intracellular massive accumulation of Ca²⁺ was observed

during reperfusion, it has been proposed that intracellular Ca²⁺ overload may be a major cause of myocardial cellular damage.^{1,2)} However, the mechanism of the massive Ca²⁺ influx into the cytosol has not been completely clear. The hypothesis that increased intracellular Na⁺ accumulation during ischemia and reperfusion may contribute to excessive Ca²⁺ uptake by the Na⁺-Ca²⁺ exchanger has been widely accepted.³⁻⁵⁾ Several studies have demonstrated that the inhibition of Na⁺ influx pathways reduced Na⁺ accumulation and contributed to better functional recovery after reperfusion.⁷⁻¹⁰⁾ It has been considered that Na⁺ accumulation during ischemia is one of the major determinants of ischemia and reperfusion injury. Some studies suggested that the inhibition of intracellular Na⁺ accumulation has become a key point to reduce myocardial ischemia and reperfusion injury.^{5,7,9)} The precise mechanism responsible for intracellular Na⁺ accumulation during ischemia is not yet clear, but it is likely that both Na⁺-H⁺ exchange and noninactivating Na⁺ channels contribute to the Na⁺ accumulation.^{8,11,12)} Therefore the present study was designed to investigate whether the Na⁺-H⁺ exchange inhibitor, Hoe, and/or Na⁺ channel blockade, Mex, enhance a cardioprotective effect of the STS and to clarify how intracellular Na⁺ accumulation con-

Table 3. Preischemic cardiac function in hearts reperfused with KHBB solution containing HOE642 and/or mexiletine

Group	C	H-R	M-R	HM-R
AF (mL/min)	78.3±3.6	79.8±5.6	79.2±1.3	79.2±3.1
CF (mL/min)	18.8±0.8	19.0±1.6	19.1±1.1	19.1±1.5
CO (mL/min)	97.1±4.1	97.3±7.9	98.3±1.3	98.1±4.8
HR (mL/min)	329.4±24.8	317.6±41.1	323.8±15.7	314.8±10.7

Values are expressed as means±SD. C, control; H-R, 20 µM HOE642; M-R, 70 µM mexiletine; HM-R, combined use of 20 µM HOE642 and 70 µM mexiletine. AF, aortic flow; CF, coronary flow; CO, cardiac output; HR, heart rate. *n* = 6/group.

Table 4. Postischemic recoveries of cardiac function and myocardial water content in hearts reperfused with KHBB solution containing HOE642 and/or mexiletine

Group	C	H-R	M-R	HM-R
% AF	49.9±5.4	52.2±5.4	50.7±3.9	55.3±5.8
% CF	78.9±1.5	75.4±13.2	79.8±2.2	81.7±3.9
% CO	55.5±4.6	56.7±6.3	56.4±3.5	60.4±5.5
% HR	99.9±3.9	93.2±8.7	98.0±1.5	98.5±4.3
WC (mL/100 g dry wt)	565.1±29.1	566.6±24.9	551.0±14.1	540.3±12.2

Values are expressed as means±SD. C, control; H-R, 20 µM HOE642; M-R, 70 µM mexiletine; HM-R, combined use of 20 µM HOE642 and 70 µM mexiletine. % AF, percent recovery of aortic flow; % CF, percent recovery of coronary flow; % CO, percent recovery of cardiac output; % HR, percent recovery of heart rate; WC, myocardial water content (mL/100 g dry wt). *n* = 6/group.

tributes to the development of ischemia and reperfusion injury after cardioplegic arrest.

Hoe is a highly selective and specific inhibitor of type 1 Na⁺-H⁺ exchanger. Many studies have revealed that it can improve myocardial injury because of ischemia and reperfusion insult by reducing intracellular Na⁺ accumulation.^{13,14} However, the beneficial effect of Hoe added to STS has not been well investigated, and the optimal concentration of it during cardioplegia is still unclear. In our studies with isolated working rat heart preparation, the addition of 10–20 µM Hoe to STS improved postischemic recovery of cardiac function and resulted in less myocardial water content. The role of the Na⁺-H⁺ exchanger in Na⁺ loading during ischemia or reperfusion remains controversial. Some researchers have hypothesized that Na⁺ accumulation occurs mainly during ischemia, and Na⁺ influx through the Na⁺-H⁺ exchange system during reperfusion may be markedly smaller than Na⁺ efflux through Na⁺ efflux pathways.^{15,16} Other studies, however, have shown that Na⁺ accumulation results mainly from reperfusion because low extracellular pH would inhibit the Na⁺-H⁺ exchange system during ischemia.¹⁷ In the present study, the Na⁺-H⁺ exchange inhibitor administered during cardioplegia enhanced a cardioprotective effect,

but it showed no beneficial effect when administered during reperfusion. Therefore our data suggest that intracellular Na⁺ accumulation occurs mainly through the Na⁺-H⁺ exchange system during ischemia, but not during reperfusion.

Membrane-stabilization may be another effective way to prevent intracellular Na⁺ accumulation during ischemia and reperfusion. Many studies provide evidence that demonstrates the beneficial effects of several class 1b antiarrhythmic agents on myocardial ischemia and reperfusion injury.^{18–20} The underlying mechanism of these beneficial effects was postulated to be attributable to the prevention of Na⁺ overload by not only blocking the Na⁺ channel, but also membrane stabilization. Mex, an Na⁺ channel blockade classified as a class 1b antiarrhythmic agent, is known to exert antiarrhythmic effects in experimental animal models and in patients suffering from arrhythmia. Mex has also been shown to inhibit intracellular Na⁺ accumulation by the blocking of Na⁺ current and keeping membrane stabilization, and its addition to a preischemic perfusate at a dose of 10–100 µM significantly improves cardiac function after reperfusion.^{18,20} According to these reports, we have employed 70 µM as an effective dose of mexiletine in the present study. When

added to STS, Mex significantly reduced myocardial edema and improved the postischemic recovery of coronary flow, though it did not improve the postischemic recovery of aortic flow or cardiac output. This result means that mexiletine, when added to STS, could exert a cardioprotective effect even under the condition that the Na⁺ channel is inactivated by membrane depolarization with a high potassium (16 mM) cardioplegic solution, suggesting that the mexiletine effect may be exerted by a mechanism other than the Na⁺ channel blocking (e.g., membrane stabilization). When administered during reperfusion, Mex showed no significant cardioprotective effect in either cardiac function or myocardial water content, suggesting that mexiletine may not play an important role in the development of myocardial injury during reperfusion.

It has not been investigated whether the combined use of an Na⁺-H⁺ exchange inhibitor and an Na⁺ channel blockade efficiently enhance the cardioprotective effect of STS. In the present study, the combined use of Hoe (20 μM) and Mex (70 μM) showed beneficial effects on the postischemic recovery of cardiac function and myocardial water content when added to STS, but not on reperfusion medium. Also when added to STS, the combined use of HOE 642 (20 μM) and Mex (70 μM) achieved superior cardioprotective effects compared to the use of each drug alone. The mechanism of beneficial effects of the combined use cannot be fully explained solely in terms of intracellular Na⁺ accumulation during ischemia. Hoe, characterized by a highly selective and specific inhibitor of type 1 Na⁺-H⁺ exchanger, is known to locate on the plasma membrane, working to maintain the intracellular ionic homeostasis. Mex has been characterized by not only a blocking of Na⁺ current, but also by keeping membrane stabilization. Therefore a possible explanation to understand the mechanism responsible for the beneficial effects of a combined use of Hoe and Mex is that Mex may act as a strong membrane stabilizer in addition to the effect of Hoe during ischemia.

Conclusion

The addition of 10 to 20 μM Hoe to STS enhances its cardioprotective effect on myocardial injury because of normothermic ischemia and reperfusion. A combined use of the Na⁺-H⁺ exchange inhibitor (20 μM) and Na⁺ channel blockade (70 μM) during the cardioplegia may achieve superior cardioprotective effects compared to the use of each drug alone. When used during the reperfusion peri-

od, neither Na⁺-H⁺ exchange inhibitor nor Na⁺ channel blockade may have any cardioprotective effect on myocardial injury as a result of ischemia and reperfusion.

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