Attenuation of postcardioplegia injury with inhibitors of the sodium-hydrogen exchanger

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There is increasing evidence that the myocardial necrosis that occurs during coronary artery bypass grafting (CABG) surgery is due to, in part, inadequate myocardial protection and that one of the underlying mechanisms is related to activation of the sodium-hydrogen exchanger (NHE). The NHE is one of 2 intracellular alkalinizing exchangers in mammalian cells; the second mechanism is the Na-HCO₃ exchanger. To date, 7 different isoforms of the NHE have been identified and are termed NHE-1 through NHE-7. NHE-1 is the primary isoform in mammalian myocardium. It is a 110-kd glycosylated protein localized in the sarcolemmal membrane of the cardiomyocyte. Its activity is regulated, in part, by a proton sensor on the cytosolic surface of the exchanger that is sensitive to intracellular H⁺ concentration ([H⁺]). In the resting state the NHE-1 is relatively quiescent, but during ischemia, increased intracellular accumulation of H⁺ stimulates the proton sensor, and activity of the exchanger is, in turn, increased by allosteric modifications of the molecule. The NHE-1 is also stimulated by other exogenous stimuli, such as cytokines, endothelin 1 (ET-1), thrombin, α-adrenergic agents, and byproducts of ischemic metabolism, such as hydrogen peroxide and lysophosphatidylcholine.

NHE-1 activity has been implicated as a mechanism underlying both myocardial and endothelial cell dysfunction caused by ischemia-reperfusion injury. During ischemia, the associated increase in [H⁺] caused by anaerobic metabolism stimulates the NHE-1, which leads to a concomitant increase in intracellular Na⁺ concentration ([Na⁺]). Extrusion of Na⁺ ions is limited, however, by the inhibition of the Na⁺/K⁺-adenosine triphosphatase pump that occurs as a result of ischemia-induced depletion of adenosine triphosphate. The increased [Na⁺], slows or reverses the direction of the Na⁺/Ca²⁺ exchanger, which normally extrudes Ca²⁺ in exchange for Na⁺ when operating in the forward direction. This reversal results in a deleterious increase in intracellular Ca²⁺, which is known as intracellular calcium overload. This process is thought to be one of the key mechanisms underlying ischemia-reperfusion injury in the heart, manifested as contractile dysfunction, endothelial injury, necrosis, and apoptosis. It is important to note that it is not the NHE-1 activation per se that directly results in myocyte injury and cell death but rather its indirect effect associated with inhibition of Na⁺/K⁺-adenosine triphosphatase and activity normally designed to prevent intracellular Na⁺ overload.

As a consequence of the deleterious physiologic effects of Ca²⁺ overload indirectly triggered by NHE-1 activity, a number of compounds have been developed to inhibit its activity, such as HOE 642 or cariporide. The long-term objective is to use these compounds to treat patients at risk of ischemia-reperfusion injury (ie, patients who undergo CABG surgery). Most experimental studies have demonstrated that NHE-1 inhibition is associated with a reduction in postsischemic arrhythmias, necrosis, contractile dysfunction, and apoptosis. The preponderance of data suggest that these beneficial effects are exerted when the agent is administered before ischemia rather than afterward (ie, during reperfusion). There is evidence suggesting that prolonged ischemia might reduce the activity of the exchanger, and the exchanger could be stimulated at the time of reperfusion. However, results have been variable when NHE-1 inhibitors have been administered at reperfusion. Although cariporide was administered perioperatively in the GUARDIAN clinical...
trial, there are preclinical studies in which addition of NHE-1 inhibitors to cardioplegia solutions was cardioprotective.\(^4\) Castellá and colleagues\(^5\) reported that a single 5 mg/kg intravenous pretreatment infusion of cariporide reversed postcardioplegia contractile dysfunction and reduced markers of myocardial oxidant injury. They have also shown that administration of cariporide as an adjunct to the terminal reperfusion flush of amino acid–enhanced cardioplegia at a dose of 40 mg/L (approximately 16 mg) was cardioprotective.

In this month’s issue of the Journal, the article by Castellá and colleagues\(^6\) provides new insight into this conundrum of optimal timing of NHE-1 inhibition and suggests that pretreatment with an NHE-1 inhibitor might be more important in facilitating recovery from ischemia-reperfusion injury than pH management of the cardioplegic reperfusion advocated previously by this group. In this month’s report the authors tested the hypothesis that pretreatment with the NHE-1 inhibitor cariporide is more effective in attenuating postcardioplegic myocardial injury independent of cardioplegia pH management strategy than buffering blood cardioplegia to pH 7.7 with tris hydroxymethyl aminomethane (THAM) or maintaining an acidic (pH 7.2) environment by using this same blood cardioplegia without THAM. The experiments were performed in a porcine model of 30 minutes of antecedent normothermic ischemia, followed by 30 minutes of cardiopulmonary arrest to create vulnerability to postcardioplegia injury. Cariporide was administered as a pretreatment before global ischemia in the 2 groups receiving either acidic or alkalotic cardioplegia; in 2 other groups acidic or alkalotic cardioplegia was delivered without pretreatment cariporide. Global left ventricular function was assessed by means of pressure-volume analysis, and myocardial damage was determined by measuring creatine kinase-MB. Other blood biochemical measurements included conjugated dienes as a measure of oxidant generation and nitric oxide and ET-1 generation as an indicator of endothelial function. The authors report that that the combination of pretreatment cariporide and acidic or alkalotic cardioplegia was more cardioprotective than either an extracellular acidic or an alkalotic blood cardioplegia without cariporide pretreatment. They conclude that pretreatment with an NHE-1 inhibitor can prevent myocardial injury and that this protection is independent of pH management of the cardioplegia solution. These are important findings and suggest that NHE-1 inhibition represents a novel approach to protecting the heart during surgical intervention. The study adds to the growing body of evidence that NHE-1 inhibitors are cardioprotective in both normal and seriously injured myocardium when administered perenterally or as an adjunct to cardioplegia. The findings and the experimental design of this study, however, raise several important questions and interesting issues.

First, does exogenous pH management with buffers such as THAM actually influence intracellular pH under the experimental conditions imposed? THAM is known to buffer intracellular pH. However, the acidic properties of the nonbuffered cardioplegia in the absence of THAM could have been conferred by the citrate-phosphate-dextrose (CPD) solution used to chelate calcium, and whether this was intracellular acidosis was not determined. The use of CPD to chelate calcium is not specifically mentioned in the article but can be inferred from historical formulations from this group and from the extent of hypocalcemia (0.2 mEq/L) of the solution. In addition, it is unclear whether the CPD-induced hypocalcemia could have influenced the activity of the Na\(^+/\)Ca\(^{2+}\) exchanger and mitigated intracellular Ca\(^{2+}\) accumulation independent of alterations in the pH of the cardioplegia solutions.

Second, how important is the timing of intracellular pH management in the absence or presence of cariporide pretreatment? According to the study design, cariporide was administered before the onset of normothermic global ischemia, whereas the buffering interventions were exerted during the added 30 minutes of “protected” ischemia with intermittent delivery of cardioplegia. Therefore pretreatment with an NHE-1 inhibitor presumably influenced the intracellular milieu before any intracellular changes that might have occurred as a result of modulation of pH in the cardioplegic solution. Thus it is unclear whether the salutary effects of cariporide were due to NHE-1 inhibition during normothermic ischemia or to better myocardial protection during cardioplegia. To answer these questions, intracellular pH, Na\(^{+}\), and Ca\(^{2+}\) will need to be measured during normothermic ischemia, cardiopulmonary arrest, and subsequent reperfusion. In addition, it is possible that cariporide had an effect during reperfusion because of its relatively long half-life.

Third, independent of the timing of action of cariporide or pH modulation, what role if any did cariporide play in directly inhibiting neutrophils or attenuating the triggers of neutrophil activation and recruitment in postcardioplegic myocardium in this preparation? These are relevant questions because ischemia, reperfusion, and cardiopulmonary bypass stimulate the release of proinflammatory cytokines that activate neutrophils, and NHE-1 inhibition blunts neutrophil activation through a pH-dependent mechanism. The NHE-1 isoform is expressed in neutrophils,\(^7\) and its activation maintains an appropriately alkalotic state for optimal reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and generation of superoxide anions. Accordingly, inhibition of NHE-1 blocks respiratory burst activity in activated neutrophils\(^4\) by maintaining the intracellular H\(^+\) load created by the respiratory burst, thereby inhibiting pH-sensitive NADPH oxidase activity.\(^8\) Some studies have shown, however, that higher concentra-
tions of cariporide or other NHE inhibitors might be required to attenuate neutrophil respiratory burst activity than that required to protect myocytes. In the absence of a direct inhibitory effect on neutrophils, cariporide could have attenuated the proinflammatory mediators that recruit neutrophils to activated endothelium, which is a critical step in the initiation of the inflammatory cascade. Indeed, it is known that the function of the coronary vascular endothelium is blunted after nonsurgical and surgical ischemia-reperfusion. The intracellular accumulation of Ca$^{2+}$ induced by NHE activity mediates, in part, the increased expression of the proinflammatory adhesion molecule ICAM-1 on coronary vascular endothelium. This is consistent with reports by other investigators that postischemic and postcardioplectic endothelial dysfunction is attenuated by NHE-1 inhibitors. However, it is not clear whether this is a direct inhibition of neutrophil activation and neutrophil-mediated injury or whether it is an attenuation of proinflammatory mediators that trigger neutrophil activation.

The finding that ET-1 levels are lower after cariporide treatment is an important one. ET-1 can be synthesized by myocytes, as well as by vascular endothelium. Activation of the ET$_4$ receptor by ET-1 released after ischemia and reperfusion causes myocyte injury, as well as vasoconstriction, and ET-1 directly promotes calcium overload in myocytes through activation of the ET$_4$ receptor subtype. This leads to contractile dysfunction. ET-1 also causes endothelial dysfunction by inhibiting the effects of nitric oxide. Finally, increased levels of ET-1 stimulate activity of the NHE-1. The observation by Castellá and colleagues in this month’s issue of the Journal that cariporide, but not buffered cardioplegia, reduced coronary sinus ET-1 levels not only highlights a limitation of cardioplegia buffering strategies but also suggests that a withdrawal of a longer-term secondary trigger of NHE-1 activity might be important in favorably altering the pathogenesis of late reperfusion injury and delayed deleterious postoperative events. This is particularly relevant because there is increasing evidence in patients that inadequate myocardial protection during cardiac surgery for ischemic heart disease is associated with decreased medium and long-term survival.

Finally, the majority of preclinical studies on the benefits of NHE-1 inhibition have been performed in in vitro models (cardiomyocytes and isolated perfused hearts) or in vivo preparations (regional or global acute ischemia) that do not entirely simulate the complex conditions associated with cardiac surgery by using cardiopulmonary bypass, intermittent ischemia, and intraoperative cardioplegia delivery. Also, many patients undergoing CABG surgery have sustained an antecedent ischemic event. After initiating cardiopulmonary bypass, the heart is typically infused intermittently with a cold cardioplegic solution or infused continuously with tepid or warm cardioplegia. This delivery of cardioplegia is a form of reperfusion, with potential benefits and liabilities. On completion of the revascularization operation, the crossclamp is removed, and the heart is perfused globally through the grafts and the native diseased vessels. It is at this time that the human heart is at additional risk of myocardial injury caused by oxidant generation, neutrophil-mediated or neutrophil-independent inflammatory responses, or calcium overload. This complex clinical scenario differs considerably from the relatively straightforward coronary occlusion-reperfusion protocols in which the preconditioning of studies has been conducted. If we are to accept the challenge of extrapolating the cardioprotection conferred by NHE-1 inhibitors reported in preclinical studies to the human situation, it is vital that we use animal preparations that more closely mimic the human condition, such as the model used by Castellá and colleagues. Only in this manner will we be able to more reliably use our preclinical studies to evaluate new and more effective methods of myocardial protection.

References

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