Effect of the Na^+/H^+ Exchange Inhibitor Eniporide on Cardiac Performance and Myocardial High Energy Phosphates in Pigs Subjected to Cardioplegic Arrest

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Background. **Pharmacologic Na^+/H^+ exchange inhibition has been suggested to ameliorate cardiac performance depression associated with myocardial ischemia/reperfusion. The purpose of our experimental study was to investigate the impact of the novel Na^+/H^+ exchange inhibitor Eniporide (EMD 96785) on cardiac performance and high energy phosphate content in a clinically relevant pig model of cardioplegic arrest.**

Methods. **We subjected 21 pigs (47 ± 12 [SD] kg) to cardiopulmonary bypass (CPB) and 60 minutes cold (4°C) crystalloid cardioplegic arrest (Bretschneider). The pigs were randomized to receive either systemic infusion of 3 mg/kg Eniporide before cardioplegia with added 2 μmol/L Eniporide (ENI-CP+iv; n = 7); 3 mg/kg Eniporide in cardioplegia only (ENI-CP; n = 7); or no Eniporide (control; n = 7). For cardiac performance determination we measured preload recruitable stroke work and Tau, the time constant of left ventricular (LV) isovolumic relaxation using sonomicrometry and micromanometry before CPB as well as 30, 60, and 120 minutes after weaning off CPB. LV and right ventricular myocardial adenine nucleotides (ATP, ADP, and AMP), glycogen, and water content were determined at the end of the experiments.**

Results. **Neither for standard hemodynamics including vascular pressures and cardiac index nor for cardiac performance factors did we find statistically significant differences between the groups. Similarly, myocardial adenine nucleotides, glycogene, and water content did not differ significantly between the groups.**

Conclusions. **In this acute study we did not find significant effects of the Na^+/H^+ exchange inhibitor Eniporide on cardiac performance and high energy phosphate content in healthy pig hearts subjected to ischemia/reperfusion induced by crystalloid cardioplegic arrest.**

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Since the introduction of cardiopulmonary bypass (CPB) and cardioplegic arrest the surgical techniques and myocardial protection during cardiac surgery have dramatically improved. However CPB and cardioplegic arrest are still associated with global myocardial ischemia reperfusion, which has been reported to induce post-CPB myocardial dysfunction including arrhythmias, contractile dysfunction, and myocyte necrosis [1, 2]. Although most patients do well after cardiac surgery, post-CPB myocardial dysfunction remains a serious risk for the immediate postoperative period and therefore deserves further investigation.

Myocardial dysfunction after ischemia reperfusion has been partially attributed to the activation of the Na^+/H^+ exchange system, which permits entry of extracellular Na^+ into the cardiac myocyte in exchange for intracellular H^+ equivalents and is involved in intracellular pH regulation. One trigger for Na^+/H^+ exchange system activation is intracellular acidosis that usually accompanies myocardial ischemia and reperfusion. Once activated, the Na^+/H^+ exchange system leads to an increase in intracellular Na^+ and a subsequent activation of the Na^+/Ca^{2+} exchanger followed by cytosolic calcium overload, which is thought to play a major role in the development of myocardial stunning and subsequent myocardial cell death [3, 4].

Consequently, several experimental studies have demonstrated that pharmacologic Na^+/H^+ exchange inhibition and subsequent avoidance of calcium overload can protect the myocardium after ischemia reperfusion [5–7]. For example, Scholz and associates [8] demonstrated in a rat model that the Na^+/H^+ exchange inhibitor cariporide (HOE 694; Aventis Pharmaceuticals, Strasbourg, France) prevented ventricular arrhythmias and fibrillation following 15 minutes regional myocardial ischemia. In a canine model, Gumina and colleagues [9] reported significantly better preserved endothelial function after

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ischemia reperfusion in animals pretreated with the Na^+/H^+ exchange inhibitor Eniporide (Merck KgaA, Darmstadt, Germany). Cox and associates [10] found that Na^+/H^+ exchange inhibition using Eniporide decreased myocardial edema immediately after CPB and 2 hours cardioplegic arrest and improved cardiac performance in dogs.

Initial clinical trials with the Na^+/H^+ exchange inhibitor cariporide (HOE 642) also exhibited positive effects on myocardial function after ischemia reperfusion [11]. However, the first enthusiasm vanished when cariporide was evaluated in a large dose finding Phase II/Phase III (Guardian) clinical trial to assess its efficiency in patients with acute coronary syndromes. Overall results failed to demonstrate improved myocardial protection and only subgroup analysis revealed significant risk reductions with the highest cariporide dose especially in high risk patients undergoing coronary artery bypass surgery [12].

These data indicate that the clinical relevance of Na^+/H^+ exchange inhibition remains unclear, and thus deserves further investigation. Therefore, the purpose of our experimental study was to investigate the impact of the novel Na^+/H^+ exchange inhibitor Eniporide (EMD 96785; Merck KGaA) on cardiac performance and high energy phospate content in a clinically relevant pig model of CPB and cardioplegic arrest. We chose a pig model because the pig heart is more comparable to the human heart than other animal species.

Material and Methods

Animal Preparation and CPB

All animal procedures were approved by the Animal Welfare Representative of the University of Cologne and were consistent with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication 85 to 23, revised 1985).

Twenty-one pigs (47 ± 12 kg [standard deviation]) of either sex were premedicated with 6 to 8 mg/kg Azaperon (Stresnil; Janssen-Cilag, Helsinki, Finland) and 0.02 to 0.04 mg/kg atropin (Atropinsulfat Braun, 0.5 mg, Melsungen, Germany) and underwent tracheal intubation and mechanical ventilation using a volume-cycled respirator (Engström 300; Engström Medical AB Solna, Sweden). Anesthesia was induced with 0.5 mg/kg propofol (Disoprivan 2%; AstraZeneca, Wilmington, DE) and 10 mg/kg ketamin (Ketavet; Pharmacia, Piscataway, NJ), and was maintained by continious intravenous administration of 1 mg/kg per minute Ketamin (Ketavet).

Fluid-filled catheters were placed into the left common carotid artery and the left internal jugular vein and connected to pressure transducers for arterial and central venous pressure monitoring, arterial and venous blood sampling, and fluid administration, respectively.

Following median sternotomy and pericardiotomy, another catheter was placed into the pulmonary artery for pulmonary artery pressure monitoring. All pigs received 300 IU heparin/kg for systemic anticoagulation and 250 to 500 mg methylprednisolon (Urbason; Hoechst Marion Roussel, Aventis Pharamaceuticals) to prevent any cross reaction with foreign pig blood used for priming the extracorporeal circuit. Additional doses of 100 IU/kg heparin were administered every 60 minutes throughout the experiment. An umbilical tape was placed around the inferior vena cava for cardiac preload manipulation. A micromanometer-tipped pressure transducer (Millar Instruments, Inc, Houston, TX) was introduced into the left ventricular cavity through the apex. Two sonomicrometry crystals (10 MHz; Sonometrics Corporation, London, Ontario, Canada) were placed in the left venticular subendocardium across the septum/free-wall axis of the left ventricle. The crystals were then connected to a sonomicrometer (Sonometrics Corporation) for signal processing.

The extracorporeal circuit (Stöckert, Germany) and the membrane oxygenator (Cobe Cardiovascular Inc., Arvada, CO) were primed with heparinized pig blood. Extracorporeal circulation was prepared by cannulating the ascending aorta (20F arterial perfusion cannula) and the right atrium-inferior vena cava using a two-stage venous cannula (34/38F). Additionally, the left ventricle was vented with a 12F catheter inserted through the left atrium. Subsequently, CPB was started and pigs were cooled to 28°C. Ten minutes later the aorta was cross-clamped, and cardioplegic arrest was initiated by antegrade administration of cold (4°C) crystalloid cardioplegic solution *Bretschneider*-HTK, Custodiol (10 mL/kg^{-1}; perfusion pressure 70 to 80 mm Hg). External myocardial cooling was accomplished by intermittent instillation of iced (4°C) saline solution into the pericardium. Additional 3 mL/kg cardioplegia were given at 20 and 40 minutes of cardioplegic arrest or if electrical or mechanical activity of the heart occurred. The amount of cardioplegia administered was similar in all three groups. Whole-body hypothermia at 28°C was maintained for 45 minutes of aortic cross clamping followed by rewarming to 37°C. After 60 minutes cardioplegic arrest the cross-clamp was removed and the heart was reperfused on normothermic CPB for 30 minutes. The pigs were then weaned off CPB and all cannulas were removed. At 120 minutes post-CPB pigs were euthanized with anesthesia overdose and intravenous potassium.

Na^+/H^+ Exchange Inhibition

Eniporide is a specific Na^+/H^+ exchange inhibitor that was administered in this trial as follows after randomizing the pigs into three groups: one group (n = 7) received a systemic bolus of 3 mg/kg Eniporide 15 minutes before cardioplegia plus 2 μmol/L Eniporide in cardioplegia (ENI-CP+iv); the second group (n = 7) received 3 mg/kg Eniporide in cardioplegia only (ENI-CP); and the control group (n = 7) received a similar volume of saline vehicle. The dose for the ENI-CP+iv group was chosen according to in vitro and in vivo experiments evaluating Eniporide's binding specificity and efficiency as well as experiments with CPB circuits to ensure that no significant binding of Eniporide to the oxygenator occurred [13]. The dose for the ENI-CP group was chosen to investigate if high

myocardial Eniporide concentration by cardioplegia administration in the absence of systemical pretreatment resulted in improved myocardial performance.

Hemodynamics, Preload Recruitable Stroke Work, and Tau

Hemodynamic data were simultaneously logged into a Toshiba Satellite Notebook (Tustin, CA) using an analog-to-digital data acquisition device. Cardiac output was measured using a Transit Time Flowmeter Module (TTFM; Transonic Systems Inc., Ithaca, NY) placed around the ascending aorta.

Using the left venticular (LV) sonomicrometry crystals and LV pressure signals, we measured LV pressure loops. These data were recorded at a frequency of 200 Hz during 12 seconds of inferior vena cava occlusion and disconnection from the respirator (Sonolab/Sonoview; Sonometrics Corporation). All measurements were recorded in triplicate. The preload recruitable stroke work (PRSW), a load insensitive LV contractility index, was calculated as the slope of the relation between left ventricular end-diastolic volume and left ventricular stroke work as previously described [14–17]. Tau, the time constant of the energy consuming LV isovolumetric relaxation, was calculated using a monoexponential model with a zero asymptote [18]. All calculations for PRSW and Tau values were processed with the Sofware SonoSOFT (Sonometrics Corporation).

All data were measured at following time points: before CPB initiation (baseline) as well as at 30, 60, and 120 minutes post CPB.

Measurement of High Energy Phosphates and Nucleotides

At the end of the experiments, transmural tissue samples (1 g) from the right and left ventricle were taken and fixed by freeze-clamping in liquid nitrogen. The samples were freeze dried in vacuo at −30°C, homogenized and deproteinized in 10 mL of 1/3 mol/L perchloric acid per gram wet tissue weight at 0 to 4°C using the Ultraturrax tissue homogenizer (Janke & Kunkel, Staufen, Germany) followed by centrifugation and neutralization of the supernatant with 2N potassium hydroxide and final centrifugation and filtration to remove the potassium perchlorate precipitate. The extracts were injected into a chromatograph in 40-μL volumes using an AS2000 Autosampler (Merck/Hitachi, Darmstadt, Germany) [19].

The chromatographic system consisted of an L-6200 HPLC pump (Merck/Hitachi), a model L-4500A Diode Array Detector (Merck/Hitachi), and a 250×4 mm stainless steel column including a guard column, each packed with LiChrospher 100 RP-18 (5 μm; Merck). The eluent consisted of degassed 25 mmol/L potassium phosphate buffer with 2 mmol/l 11-aminoundecanoic acid (Merck-Schuchard, Hohenbrunn, Germany) in methanol-water (92:8 v/v) at a column temperature of approximately 20°C (ambient temperature). The eluent flow rate of 1.0 ml/min resulted in a pressure of approximately 150 bar. In a recirculating isocratic system with 1 L eluent volume contaminated eluent was separated using an Eco-saver[19].

Left ventricular and right ventricular (RV) myocardial adenine nucleotides (ATP, ADP, AMP) were measured chromatographically from the peak areas with spectral peak identification using HSM D-7000 HPLC-software (Merck/Hitachi). Glucose and lactate were measured by enzymatic tests. After destruction of the existent glucose in the tissue homogenate in 28.5% NaOH, the precipitated glycogen was hydrolyzed in HCl and measured after neutralization in a glucose test.

Myocardial Water Content

At the end of the experiments transmyocardial specimens were taken from a fat-free area of the LV anterior wall. Specimens were weighted than freeze dried after lyophilization. The individual dry weights were corrected for residual water content determined in heat-drying experiments [20]. Myocardial water content (MWC) was calculated as (wet weight − dry weight) divided by wet weight and expressed as a percentage. All measurements were performed in triplicate.

Statistical Analysis

All data presented are mean ± standard deviation (SD). Data were analyzed for changes between groups and changes over time using multivariate analysis of variance and F-test with repeated measures design and posthoc comparisons using Newman-Keuls test as implemented in the software package Statistica for Windows (StatSoft, Inc., Tulsa, OK). A value of p less than 0.05 was considered significant.

Results

Standard hemodynamic variables for the three groups are summarized in Table 1. There was no significant difference between the groups. Looking for the change over time, there was a significant increase in pulmonary artery pressure for all groups ($p < 0.0001$). Cardiac performance data including PRSW, Tau, cardiac index, and stroke volume index are presented in Figure 1. There were no significant differences between the groups. However, looking for the effect of "time" we found a significant decrease in PRSW ($p = 0.007$), cardiac index ($p = 0.0002$), and stroke volume index ($p < 0.0001$) in all groups. Tau, the time constant of isovolumetric relaxation, did not characterize significant changes over time.

In Figure 2 myocardial ATP, ADP, ATP, and glycogen contents as well as myocardial water content are displayed. Neither adenine nucleotides nor glycogen content nor myocardial water content differed significantly between the groups.

Comment

In this study we did not find significant effects of the Na^+/H^+ exchange inhibitor Eniporide on cardiac performance and high energy phospate content in healthy pig hearts subjected to ischemia-reperfusion induced by crystalloid cardioplegic arrest.

Myocardial dysfunction after ischemia reperfusion has

Table 1. Standard Hemodynamics

Variable	Baseline	30 Minutes Post-CPB	60 Minutes Post-CPB	120 Minutes Post-CPB	Change Between Groups (MANOVA) (*p* Value)	Change Over Time (MANOVA) (*p* Value)
HR (1/min)						
ENI-CP+iv	96.6 ± 28.6	102.1 ± 14.9	111.6 ± 17.0	99.0 ± 18.2	0.51	0.70
ENI-CP	95.0 ± 22.8	95.9 ± 15.8	98.4 ± 16.0	96.7 ± 18.1		
Control	105.7 ± 27.0	110.0 ± 11.5	100.1 ± 9.7	104.1 ± 14.7		
MAP (mm Hg)						
ENI-CP+iv	74.4 ± 8.6	64.4 ± 16.9	63.6 ± 8.9	56.7 ± 14.6	0.84	0.086
ENI-CP	71.4 ± 6.3	68.3 ± 15.4	65.6 ± 16.7	64.7 ± 9.0		
Control	62.1 ± 16.5	62.4 ± 14.0	68.3 ± 12.1	64.6 ± 15.7		
PAP (mm Hg)						
ENI-CP+iv	21.0 ± 3.5	25.9 ± 6.8	27.6 ± 8.3	26.7 ± 7.5	0.97	<0.0001
ENI-CP	20.9 ± 4.5	28.6 ± 4.5	28.7 ± 5.1	25.9 ± 2.9		
Control	22.3 ± 5.3	25.4 ± 7.7	27.0 ± 8.8	28.6 ± 11.1		
CVP (mm Hg)						
ENI-CP+iv	5.4 ± 3.7	6.9 ± 2.9	6.4 ± 3.9	6.4 ± 3.1	0.18	0.37
ENI-CP	9.7 ± 3.1	9.6 ± 2.6	9.0 ± 2.8	9.3 ± 3.6		
Control	6.7 ± 3.6	7.7 ± 3.4	8.0 ± 3.8	8.9 ± 3.7		

Data are mean ± standard deviation.

CP = cardioplegia; CPB = cardiopulmonary bypass; CVP = central venous pressure; ENI = Eniporide; HR = heart rate; MAP = mean arterial pressure; PAP = pulmonary artery pressure.

been attributed to the activation of the Na^+/H^+ exchange system, which permits the entry of extracellular Na^+ into the cell in exchange for intracellular H^+ equivalents, and thus, is involved in intracellular pH regulation and other cell functions. The accumulation of protons during ischemia reperfusion stimulates the Na^+/H^+ exchange system and leads to an increase in intracellular Na^+. Subsequent Na^+/Ca^{2+} exchanger activation may then result in cytosolic calcium overload, which is thought to play a major role in the development of myocardial stunning and myocardial cell death [3, 4]. Another effect of the intracellular Na^+ increase is a concomitant increase in intracellular water leading to myocardial edema, which has been thought to be one of the reasons for myocardial dysfunction after ischemia-reperfusion [10] and which has been shown to impair myocardial function even in the absence of ischemia [14, 21].

Considering this background, pharmacological inhibition of the Na^+/H^+ exchange system appears to be a promising therapeutic approach to preserve myocardial function after ischemia-reperfusion. In fact, Na^+/H^+ exchange inhibitors have been reported to limit infarct size

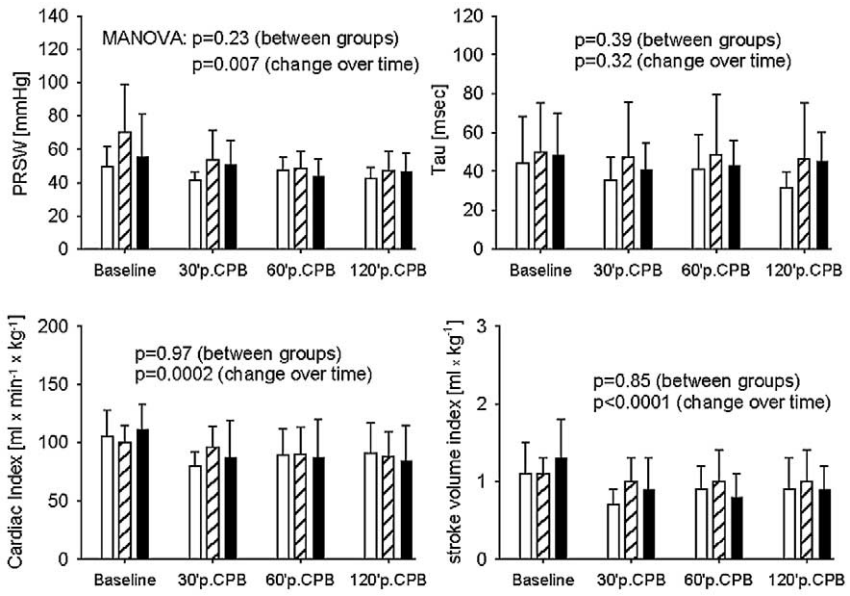

Fig 1. Preload recruitable stroke work (PRSW), a load insensitive left ventricular contractility index, cardiac index, Tau, the time constant for left ventricular isovolumetric relaxation, and stroke volume index did not differ significantly between the groups. □ = control; ▨= ENI-CP; ■ = ENI-CP+iv. (CPB = cardiopulmonary bypass; ENI-CP = Eniporide in cardioplegia only; ENI-CP+iv = Eniporide in cardioplegia plus intravenous bolus.)

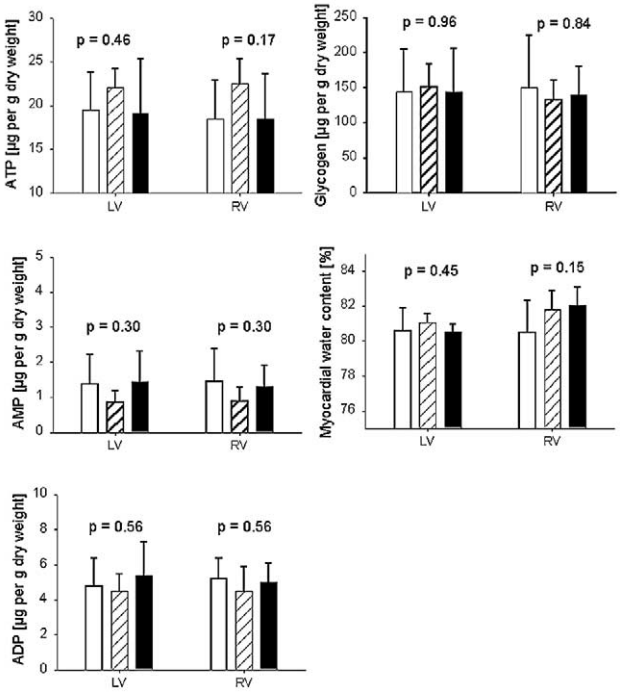

*Fig 2. Left and right ventricular myocardial adenine nucleotides (*ATP, ADP, *and* AMP*), glycogen content, and myocardial water content did not differ significantly at 120 minutes post-cardiopulmonary bypass.* □ *= control;* ▨*= ENI-CP;* ■ *= ENI-CP+iv. (ENI-CP = Eniporide in cardioplegia only; ENI-CP+iv = Eniporide in cardioplegia plus intravenous bolus;* LV *= left ventricle;* RV *= right ventricle.)*

in several animal species [6, 9, 22]. The greatest effect of Na^+/H^+ exchange inhibitors was seen when administered before ischemia, and there was still a marked benefit when given before reperfusion [23, 24].

In contrast to these beneficial experimental effects, clinical application of the Na^+/H^+ exchange inhibitor cariporide in humans failed to demonstrate improved myocardial protection (GUARDIAN trial [12]), which was suggested to be the result of insufficient dosages, at least in part. Additionally, the new Na^+/H^+ exchange inhibitor Eniporide was currently evaluated in a Phase II clinical trial (ESCAMI) in patients with acute myocardial infarction (MI) and also failed to demonstrate cardioprotective effects [25]. These data indicate inconsistent results regarding experimental and clinical Na^+/H^+ exchange inhibition that could potentially be due to species differences.

In a recent experimental investigation Cox and associates [10] found that Na^+/H^+ exchange inhibition with Eniporide decreased myocardial edema immediately after 2 hours cardioplegic arrest and improved preload recruitable stroke work in dogs. Their central hypothesis was that better post-CPB recovery was enhanced by myocardial edema reduction. However, these results were not reproducible in a follow-up study with similar protocol [13]. In fact, despite better preserved preload recruitable stroke work in dogs pretreated with Eniporide versus untreated controls, MWC was increased in both groups but to an even greater extent in the Eniporide group [13]. Explaining these inconsistant results they pointed to the fact that no differentiation between intracellular and extracellular myocardial water content could be made [13]. In our present study we also did not find statistically significant differences in MWC between the three study groups, which suggests that Eniporide does not substantially impact MCW in pigs subjected to CPB and cardioplegic arrest.

In contrast to experimental studies in other species, we did not find significant effects of Eniporide on post-CPB cardiac performance in pig hearts. There are several potential explanations for these results. First, beneficial effects might be dependent on sufficient Na^+/H^+ exchange inhibitor dosages. To account for this we chose two different dosages: either 3 mg per kg body weight as intravenous bolus followed by 2 μmol/L concentration in cardioplegia (ENI-CP+iv) or 3 mg per kg body weight into cardioplegia only (ENI-CP). Thus, we investigated a group with high myocardial Eniporide concentration (ENI-CP) versus a group with systemic plus low myocardial concentration (ENI-CP+iv) versus a control group with no Eniporide exposition. Second, the time of Eniporide administration might influence its effects on cardiac performance. As other studies have shown that Na^+/H^+ exchange inhibition exhibits the strongest effects when given before the ischemic insult [23, 24], we accounted for this by either giving Eniporide as a bolus before cardioplegia plus into cardioplegia (ENI-CP+iv) or into cardioplegia alone (ENI-CP). Third, species differences may influence the effects of Na^+/H^+ exchange inhibition. This study investigated Na^+/H^+ exchange inhibition in a clinical relevant model of CPB and cardioplegic arrest in the pig heart. Since the pig heart is probably more comparable to the human heart than that of other species, we believe our results support the clinical ESCAMI and GUARDIAN trials, which failed to demonstrate clinical relevance of Na^+/H^+ exchange inhibition in both cardiological and cardiac surgery patients.

Limitations of the Study

One limitation of our study results from the small sample size of our groups, which carries the risk for type II errors. However, with seven pigs in each group any difference between groups would be small and would require a very large number of studies to demonstrate. This has to be considered when interpreting the data of the present study. Another limitation is the fact that contractility impairment induced by crystalloid cardioplegia in the control group was only about 20%, and thus, potential beneficial effects attributable to Eniporide are difficult to detect. In a model using more impaired contractility such as in hearts subjected to ischemia before cardioplegia, Eniporide may have revealed other results than those found in our model.

In conclusion, this study failed to demonstrate significant effects attributable to the novel Na^+/H^+ exchange inhibitor Eniporide on cardiac performance, high energy phospate content, and myocardial edema formation in pigs subjected to cardioplegic arrest on CPB. Future

studies are required to determine if Na^+/H^+ exchange inhibition may exhibit beneficial effects in severely compromised hearts, particularly those exposed to acute myocardial ischemia or infarction.

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