

## Effect of Intravenous Administration of Antioxidants Alone and in Combination on Myocardial Reperfusion Injury in an Experimental Pig Model

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### ABSTRACT

**BACKGROUND:** Several antioxidants have been found to have conflicting results in attenuating myocardial reperfusion injury. These studies were done primarily in experimental protocols that did not approximate clinical situations.

**OBJECTIVE:** The aim of this study was to test the efficacy of 3 different antioxidants (ascorbic acid [AA], desferrioxamine, and *N*-acetylcysteine [NAC]) administered IV alone and in combination in a closed-chest pig model.

**METHODS:** Farm-raised domestic male pigs (aged 3–5 months, weight of 30–35 kg) were assigned to 1 of 5 groups to receive treatment as follows: group A, AA 100 mg/kg; group B, desferrioxamine 60 mg/kg; group C, a loading dose of NAC 100 mg/kg for 20 minutes and a 20-mg/kg maintenance dose; group D, all 3 drugs in combination; and group E, normal saline (control group). The infusion of all drugs was started 15 minutes before and completed 5 minutes after reperfusion, except for the administration of NAC, which was terminated 60 minutes postreperfusion. Myocardial ischemia (45 minutes) and reperfusion (210 minutes) were achieved percutaneously by circumflex artery balloon occlusion. Ejection fraction, left ventricular end-diastolic pressure (LVEDP), flow in the infarcted artery, and all ventricular arrhythmias were recorded. Oxidative stress was estimated by serial measurements of thiobarbituric acid reactive substance (TBARS) concentration in coronary sinus blood. Infarct size was assessed as a percentage of the area at risk (I/R ratio) using the tetrazolium red staining method.

**RESULTS:** The 25 pigs were divided into 5 groups of 5 pigs each. No significant between-group differences were found in I/R ratio or in oxidative stress (as measured by TBARS concentration). Group C developed significantly more ventricular ar-

rhythmias than the control group (80% vs 0%,  $P = 0.02$ ). No other differences among groups were found. LVEDP was significantly elevated in all treatment groups (mean LVEDP difference [SD] for group A, 6.0 [1.6] mm Hg; group B, 17.6 [1.9] mm Hg; group C, 3.6 [1.7] mm Hg; group D, 6.8 [3.2] and group E, 5.4 [3.4] mm Hg). LVEDP elevation was found to be significantly higher in group B compared with all the other groups (all,  $P < 0.001$ ). No significant between-group differences were found in the other parameters measured.

**CONCLUSION:** In this experimental pig model, the antioxidants AA, desferrioxamine, and NAC administered alone or in combination did not reduce the deleterious effects of reperfusion injury and specifically the extent of myocardial necrosis. (*Curr Ther Res Clin Exp.* 2008;69:423–439) © 2008 Excerpta Medica Inc.

**KEY WORDS:** myocardial infarction, reperfusion injury, antioxidants, combination.

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## INTRODUCTION

Myocardial reperfusion injury is a well-documented phenomenon that partially offsets the benefits of successful pharmaceutical or mechanical restoration of coronary blood flow in acute myocardial infarction.<sup>1</sup> This phenomenon causes a spectrum of clinical events, including arrhythmogenesis, temporary or permanent loss of contractile function (myocardial stunning or necrosis), and endothelial or microvascular dysfunction (no-reflow phenomenon).<sup>2,3</sup>

Oxidative stress through production of reactive oxygen species (ROS) is considered an important mediator in the pathophysiology of this phenomenon.<sup>4–7</sup> To attenuate myocardial reperfusion injury, researchers have tested numerous antioxidants either alone or in combination. Although many of these antioxidants have been found to be effective in experimental models, they have not been found to be effective in clinical trials.<sup>8</sup> One reason for this contradiction is that it is difficult to approximate clinical myocardial ischemia/reperfusion conditions in experimental protocols.<sup>9,10</sup> Another reason is the complexity of this paradoxical phenomenon. Reperfusion injury involves many different mechanisms that cannot be suspended by a single antioxidant; a combination of antioxidants is needed.<sup>11</sup> Therefore, researchers have proposed studying a combination of antioxidants (antioxidant cocktails) in experimental models that resemble the clinical setting of myocardial ischemia/reperfusion.<sup>8,12–14</sup>

We hypothesized that administration of a combination of antioxidants that act at different sites in the pathophysiologic cascade of reperfusion injury may be more effective than administering these agents alone. We selected 3 well-known antioxidants—ascorbic acid (AA), desferrioxamine, and *N*-acetylcysteine (NAC)—that act at 3 different sites in the reperfusion cascade.

AA is a potent water-soluble antioxidant present inside the cells of various body fluids, tissues, and plasma.<sup>15</sup> It acts as a direct scavenger of the ROS produced immediately after myocardial perfusion.<sup>16</sup> The antioxidant properties of desferrioxamine are attributed to its ability to chelate free-ferric ions released from damaged myocardial tissue.<sup>17</sup> In this way, desferrioxamine blocks Fenton reactions and limits the production of potent radicals, thereby reducing the myocardial damage caused indi-

rectly by the conversion of relatively noncytotoxic radicals to highly toxic ones.<sup>18–22</sup> NAC, a thiol group donor, increases endogenous myocardial cell antioxidant defense by restoring intracellular glutathione stores.<sup>7,23,24</sup>

To determine whether the combination of these drugs is effective in preventing myocardial reperfusion injury by reducing oxidative stress, the 3 antioxidants were administered IV alone and in combination in a closed-chest pig experimental preclinical model. Myocardial reperfusion injury was assessed primarily by the extent of induced myocardial necrosis. Secondary end points were the indices of left ventricular function and the incidence of arrhythmias in the reperfusion period. The effect of antioxidants on markers of oxidative stress was also assessed.

## MATERIALS AND METHODS

This study was performed in the Animal and Experimental Laboratory at Ioannina University Hospital, Ioannina, Greece. The study was conducted in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences<sup>25</sup> and approved by the Ethics Committee of Ioannina University Hospital.

### ANIMAL PREPARATION

Twenty-eight young (aged 3–5 months) male, farm-raised, domestic pigs (weight, 30–35 kg) were anesthetized according to the following protocol. All pigs were raised in a modern indoor farm affiliated with the University of Ioannina, specifically designed for small-group pig housing with stable conditions of ventilation, temperature, and feeding. Pigs are raised until the desirable age and weight, specifically for the experiments in our institution. The animals were premedicated with azaperone 4 mg/kg and ketamine 5 mg/kg IM. Anesthesia was induced with thiopental 7 mg/kg IV. After endotracheal intubation, mechanical ventilation was started and anesthesia was maintained with sevoflurane 1% and periodic administration of fentanyl and vecuronium. The animals were ventilated with a mixture of air and oxygen to maintain their arterial blood gas values in the physiologic range. Electrocardiogram (ECG), vital signs, and arterial blood gases were monitored throughout the procedure.

The right internal jugular vein was surgically exposed and a 7 French (Fr) sheath (Glidesheath, Terumo Corp., Tokyo, Japan) was introduced. Under fluoroscopic guidance a 7 Fr multipurpose catheter was placed in the coronary sinus for blood collection; 6 Fr sheaths were positioned in both femoral arteries using the Seldinger technique and heparin 300 IU/kg IV was administered.<sup>26</sup> Under fluoroscopic guidance a 6 Fr pig-tail catheter was placed into the left ventricle to record left ventricular end-diastolic pressure (LVEDP) and to perform left ventriculography to measure ejection fraction (EF) while the left main stem of the left coronary artery was catheterized using a 6 Fr hockey-stick guiding catheter.

### EXPERIMENTAL PROTOCOL

After animal preparation and successful catheterization of the main stem of the left coronary artery, the animals were assigned to 1 of 4 treatment groups (group A, AA 100 mg/kg; group B, desferrioxamine 60 mg/kg; group C, NAC 100 mg/kg for

20 minutes + a 20-mg/kg maintenance dose; group D, AA 100 mg/kg + desferrioxamine 60 mg/kg + NAC 100 mg/kg for 20 minutes + a 20-mg/kg maintenance dose) or to group E (control group), normal saline. The assignment of animals was based on the chronological order of the consecutive experiments (eg, experiment 1, group A; experiment 2, group B; following accordingly from groups A–E in repeat cycles); this process, although incomplete, allowed for randomization of the study experiments. Furthermore, all studied parameters were measured by independent observers blinded to the assigned treatment. Each animal received the allocated intravenous treatment diluted in normal saline to a total volume of 100 mL. The infusion of all substances started 15 minutes before reperfusion and was completed 5 minutes after, except for the administration of NAC which was terminated 60 minutes after reperfusion. The mode of NAC administration was based on previously published studies.<sup>27,28</sup>

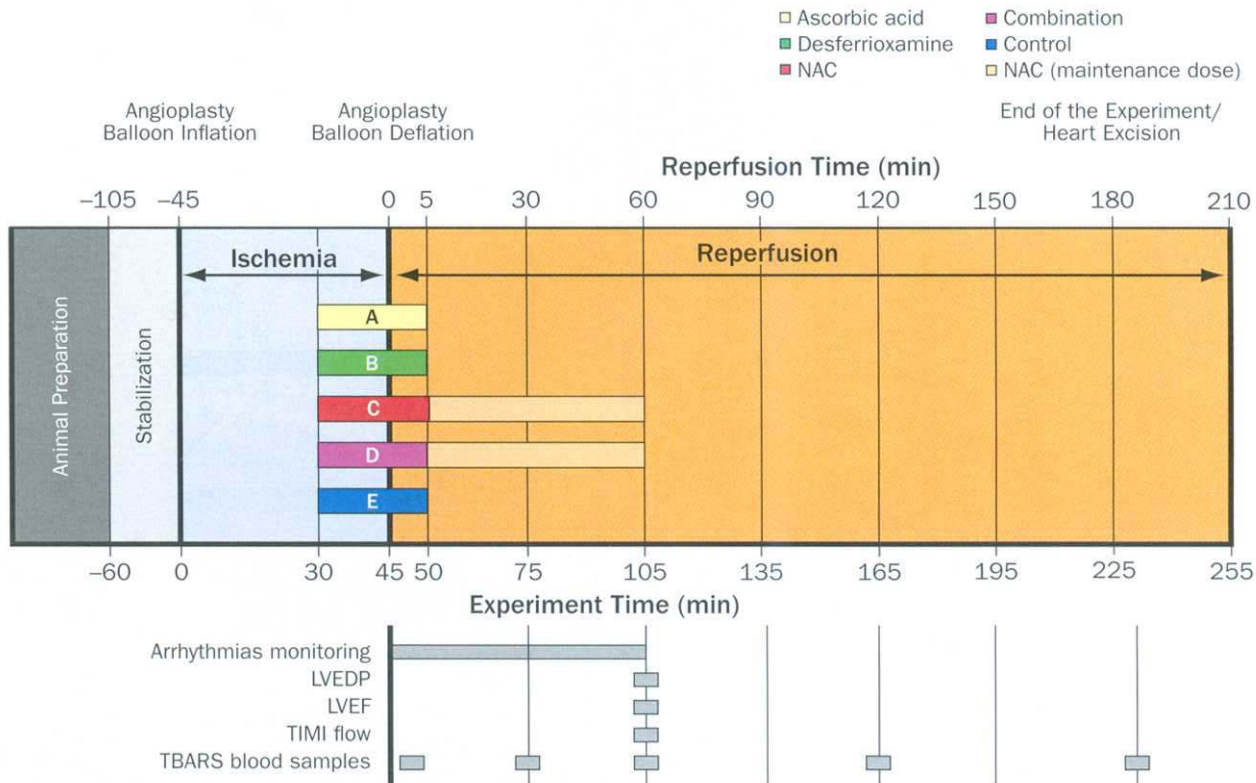
Coronary artery occlusion was accomplished percutaneously by inflating an angioplasty balloon catheter (2.5 × 20 mm) positioned in the middle circumflex artery (LCX) over a 0.014-in guidewire. Vessel occlusion was confirmed by the lack of contrast medium penetration beyond the position of the balloon and by the appearance of ST-segment elevation in the limb ECG leads. The balloon remained inflated for 45 minutes and was subsequently deflated to restore blood flow. Figure 1 shows a schematic representation of the experimental protocol.

#### **MONITORING OF VENTRICULAR ARRHYTHMIA AND ASSESSMENT OF LVEDP, LEFT VENTRICULAR SYSTOLIC FUNCTION, AND CORONARY BLOOD FLOW**

During the first 60 minutes of reperfusion, all animals were monitored for ventricular arrhythmias (ventricular fibrillation, sustained ventricular tachycardia [SVT], nonsustained ventricular tachycardia [NSVT], and idioventricular rhythm). SVT that required cardioversion was considered to be as important as ventricular fibrillation (VF) and was recorded as such. LVEDP measurements were obtained prior to ischemia and 60 minutes after reperfusion. At the same time points, both coronary angiography and left ventriculography at the 30° right anterior oblique projection were performed. Flow in the LCX was graded according to the Thrombolysis In Myocardial Infarction (TIMI) classification as follows<sup>29</sup>: TIMI 0 = total occlusion of the infarct-related artery; TIMI 1 = slow and incomplete opacification of the infarct-related artery after the site of occlusion; TIMI 2 = slow but complete opacification of the infarct-related artery; TIMI 3 = normal opacification. Using left ventriculography, EF was measured offline by an independent investigator who was blinded to the administered treatment (CAAS, Pie Medical, Maastrich, The Netherlands).

#### **ASSESSMENT OF THE ISCHEMIC AREA AT RISK AND THE PROPORTION OF THE INFARCTED MYOCARDIAL ZONE**

Two hundred ten minutes postreperfusion the angioplasty balloon was repositioned in the LCX using the side branches as landmarks and was reinflated to occlude the vessel. Then, 2 mL/kg of Evans blue 2% solution was infused into the beating left ventricle through the pig-tail catheter to delineate the area at risk (ie, the area subjected to ischemia and subsequent reperfusion). Three minutes after the administra-



**Figure 1. Schematic representation of the experimental protocol. NAC = N-acetylcysteine; LVEDP = left ventricular end-diastolic pressure; LVEF = left ventricular ejection fraction; TIMI = thrombolysis in myocardial infarction; TBARS = thiobarbituric acid reactive substance.**

tion of Evans blue solution, the animal was sacrificed by rapid intracardiac injection of a potassium chloride solution.

The heart was excised rapidly (within 3 minutes), the atria and the right ventricle were discarded, and the left ventricle was cut from the base to the apex in parallel slices 0.5 cm in thickness. Members of an experienced team of the Forensic Department of our institution who were blinded to the treatment of each animal were responsible for preparing and staining the excised heart and for assessing the infarcted area and the area at risk of the myocardium studied. The area at risk of each slice was the area that was not stained blue. In each slice, the borders of the area at risk were demarcated with India ink to maintain their visibility after incubation of the slices in triphenyl tetrazolium chloride (TTC). The slices were then incubated in a 1% TTC solution for 30 minutes at 37°C in a dark room. At the end of this process, 2 areas could be identified within the area at risk (the Evans blue unstained area): (1) the TTC-stained area, with a characteristic brick-red color, was the ischemic but viable myocardium; and (2) the TTC-unstained area represented the nonviable (infarcted) tissue.<sup>30</sup> The slices were photographed with a digital camera. The areas at risk and the infarcted areas were measured using a computer program (UTHSCSA-ImageTool, version 3.00, San Antonio, Texas). These areas were multiplied by the thickness of the slice (0.5 cm), and the volumes of risk and infarction were calculated for each slice. By summing these volumes, the total volume of risk and infarction were estimated for the whole left ventricle. The *I/R ratio* was defined as the proportion of the myocardium at risk (R) that was necrotic (infarcted) (I). The total left ventricle (LV) area and the proportion of the myocardium at risk to the total LV area were also measured.

#### EVALUATION OF OXIDATIVE STRESS

Oxidative stress was assessed using thiobarbituric acid reactive substances (TBARS), an indicator of lipid peroxidation widely used as an indirect method of estimating oxidative stress in clinical and experimental studies.<sup>31–33</sup> Measurement of TBARS concentration was made in blood samples collected from the coronary sinus. The first blood sample (control sample taken at time point 0 minutes) was taken just after the catheter was inserted in the coronary sinus and before ischemia was initiated. After reperfusion, blood samples were collected at 2, 30, 60, 120, and 180 minutes. Blood was carefully aspirated from the coronary sinus into solid glass reagent tubes containing ethylenediaminetetraacetic acid (Greiner Bio-One GmbH, Kremsmünster, Austria). All samples were immediately placed in ice and centrifuged at 5000 rpm at 4°C for 15 minutes to separate serum. The serum was then collected with a small pipette, placed in 1.5-mL Eppendorf tubes, and stored at –80°C until processing. TBARS concentrations were measured using the fluorometric method previously described in detail by Wasowicz et al.<sup>34</sup>

#### STATISTICAL ANALYSIS

All continuous variables are expressed as mean (SD) unless mean (SEM) is indicated. Statistical significance among groups was evaluated using 1-way analysis of

variance (ANOVA) followed by the Bonferroni multiple-comparison test (for post hoc comparisons). Repeated-measures ANOVA of TBARS concentration within the treatment groups was performed using general linear model repeated measures to attempt to reduce repeated sampling error (a bias inherent with this type of comparison).<sup>35</sup> AUC was used to evaluate data about the time course of the release of TBARS after reperfusion. Categorical variables of separate groups were compared using the Fisher exact test.  $P \leq 0.05$  was considered statistically significant. Statistical analysis was performed using SPSS version 13.0 (SPSS Inc., Chicago, Illinois). The number of animals studied in each group was based on similar complex experimental studies in the literature<sup>10,36</sup> and on the expectation of a large difference to be achieved with the pharmacological intervention (eg, the administration of antioxidants compared with the control infusion would reduce the I/R ratio by >50% or would abolish arrhythmias).

## RESULTS

Of the 28 pigs included in the study, 3 died prior to group assignment and initiation of ischemia (2 due to fatal bradycardia at the time of intubation and initiation of anesthesia and 1 due to left main artery dissection during catheter engagement). The surviving 25 animals were assigned to the 5 study groups ( $n = 5$  per group). Heart rate and mean aortic pressure were similar in all groups throughout the experiment.

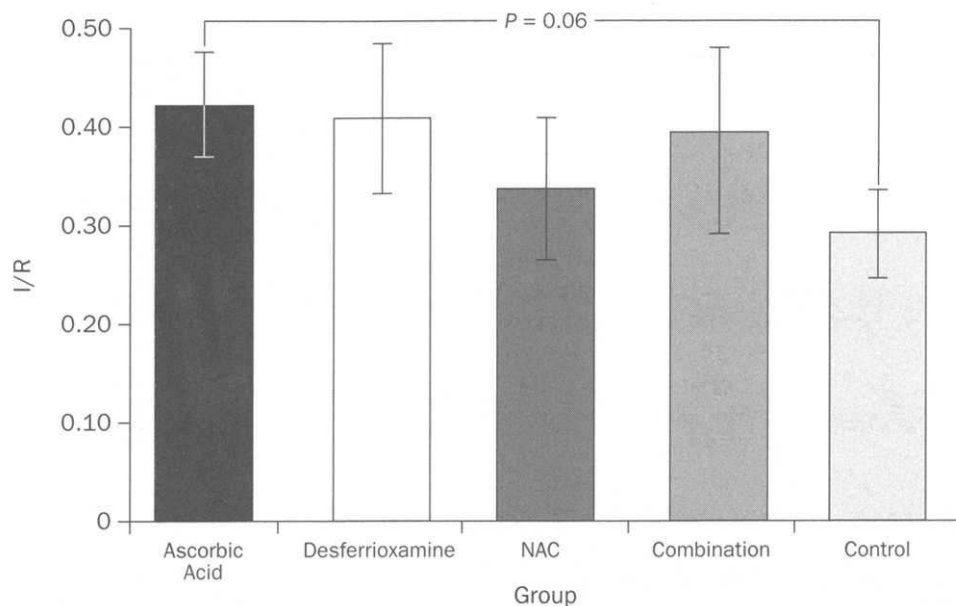
### I/R RATIO

At the end of the procedure, the I/R ratio was as follows: group A, 0.42 (0.05); group B, 0.40 (0.07); group C, 0.33 (0.07); group D, 0.39 (0.08); and group E, 0.29 (0.04) (Figure 2). Comparison using ANOVA found a significant difference in I/R ratio among the groups ( $P = 0.03$ ). Post hoc analysis revealed a trend towards a larger I/R ratio in the group that received ascorbic acid (group A) in comparison to the control group ( $P = 0.06$ ). No significant statistical differences were found between the other treatment groups. The proportion of the area of myocardial risk to the total LV area did not differ among the treatment groups.

### ARRHYTHMIAS

In group E no animal developed VF/SVT, while in groups A, B, C, and D, 1, 2, 4, and 1 animals, respectively, developed VF/SVT after reperfusion. There was a significant difference in the incidence of VF/SVT between groups E and C ( $P = 0.02$ ). Two animals had multiple episodes of VF/SVT: 1 in group A had 5 consecutive episodes of VF/SVT and 1 in group D had 2 episodes of VF. In all of these episodes the animals were successfully defibrillated. Most of the VF/SVT episodes (6 [86%]) occurred during the first 10 minutes after reperfusion.

The mean (SD) numbers of NSVT and idioventricular rhythm episodes were as follows: group A, 11.4 (8.9); group B, 14.8 (5.8); group C, 11.0 (3.8); group D, 14.4 (9.9); and group E, 9.8 (6.9). There were no significant between-group differences.



**Figure 2. Mean (SEM) proportion of the at-risk myocardium that is infarcted (I/R ratio) by treatment group. NAC = N-acetylcysteine.**

#### LEFT VENTRICULAR FUNCTION

LVEDP was significantly elevated 60 minutes after reperfusion in all treatment groups compared with baseline (all,  $P < 0.01$ ) (Figure 3). The mean (SD) differences were as follows: group A, 6.0 (1.6) mm Hg; group B, 17.6 (1.9) mm Hg; group C, 3.6 (1.7) mm Hg; group D, 6.8 (3.2) mm Hg; and group E, 5.4 (3.4) mm Hg. Using ANOVA, LVEDP elevation was found to be significantly higher in group B compared to all the other groups (all,  $P < 0.001$ ). No significant differences were found between the other groups.

One hour after reperfusion, EF was found to be significantly reduced compared with baseline in all of the groups (all,  $P < 0.001$ ) (Figure 4). No significant between-group differences were found in changes in EF after reperfusion.

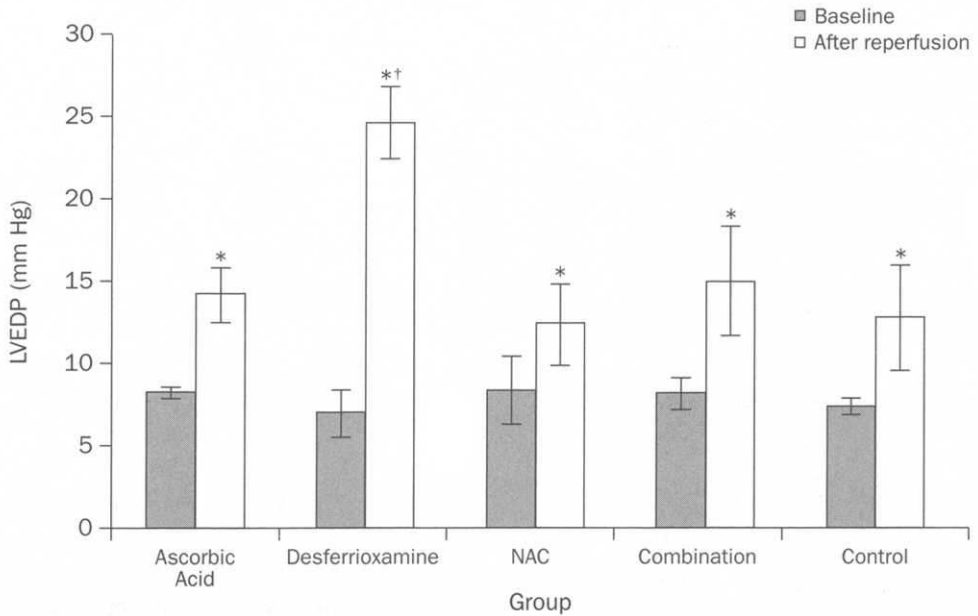
#### CORONARY FLOW

Optimal coronary blood flow (TIMI score, 3) after reperfusion was found in 3 animals in group C; 1 each in groups A, D, and E; and none in group B. No significant difference was found among group comparison.

#### OXIDATIVE STRESS

TBARS concentrations were assessed at different time points throughout the reperfusion period (Figure 5). No significant between-group differences were found in baseline TBARS concentrations. Compared with baseline (0 minutes), TBARS concentrations were significantly elevated at 30 minutes in group E and at 120 minutes in





**Figure 3. Mean (SEM) left ventricular end diastolic pressure (LVEDP) at baseline and 60 minutes after reperfusion. \* $P < 0.05$  baseline versus 60 minutes after reperfusion; † $P < 0.001$  desferrioxamine group versus all other groups. NAC = *N*-acetylcysteine.**

group C (both,  $P = 0.02$ ). In group B, TBARS concentrations decreased significantly at 2 minutes and 120 minutes (both,  $P = 0.002$ ) after reperfusion. No significant change in TBARS concentrations was noted in group D.

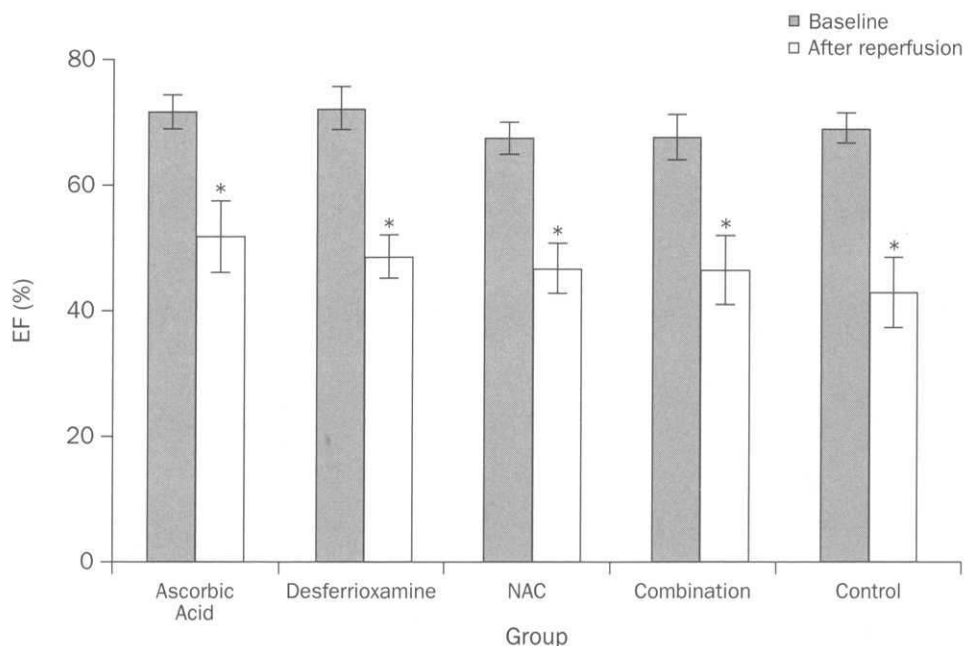
ANOVA of AUC for all groups and for the total reperfusion period (180 minutes) revealed no statistically significant between-group differences. Multiple comparisons between TBARS concentrations at each time point found significantly higher concentrations in group A compared with group B at 120 minutes after reperfusion ( $P = 0.05$ ) (Figure 5).

## DISCUSSION

This study examined the effects of AA, desferrioxamine, and NAC administered intravenously alone and in combination on reperfusion injury using an experimental closed-chest pig model of a 45-minute ischemia period followed by 210 minutes of reperfusion. No significant effect on reperfusion injury was found with any of the treatments.

### ASCORBIC ACID

Experimental studies of the antioxidant effects of AA after reperfusion have reported contradictory findings. Some studies reported a protective effect when AA was administered alone or in combination with other agents in isolated cardiomyocytes



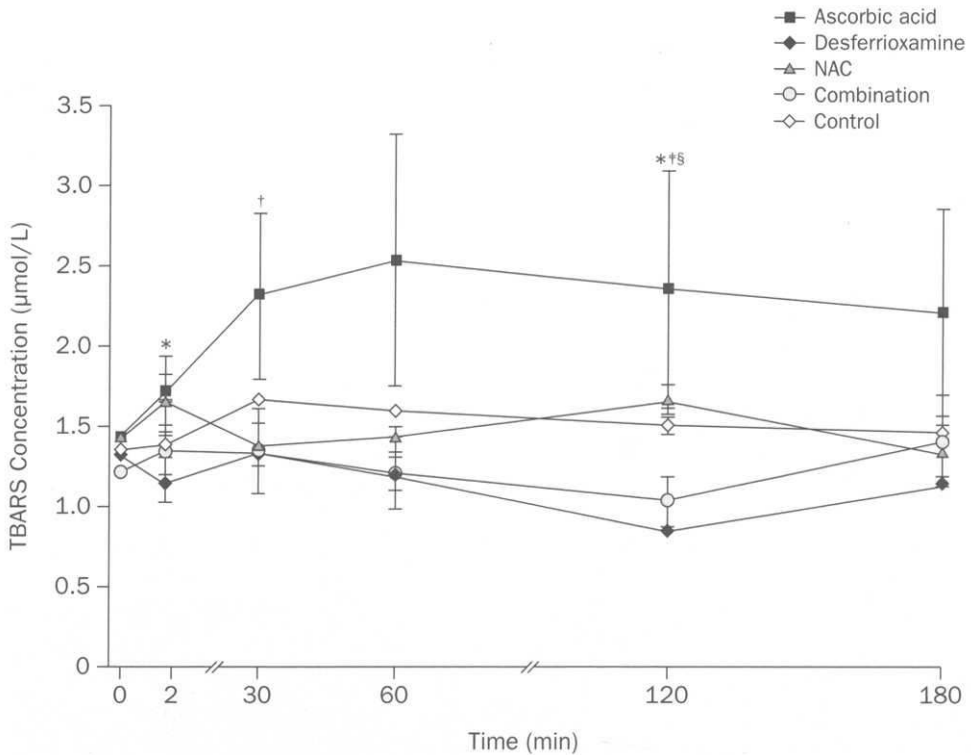
**Figure 4. Mean (SEM) ejection fraction (EF) measured off-line at baseline and 60 minutes postreperfusion. No significant between-group differences were found. NAC = N-acetylcysteine. \*All,  $P < 0.001$  versus baseline.**

and in vivo experimental models,<sup>37,38</sup> while others found no such protective effect from reperfusion injury, even when the drug was administered prior to coronary occlusion.<sup>13,39</sup>

In our study, the infarct size increased numerically, though not significantly, in animals receiving AA alone compared with the control group. In the same animals, an increase was also observed in oxidative stress measured by TBARS concentration. These findings are in accordance with the results of previous in vitro studies that reported possible prooxidative activity of AA that was attributed either to ascorbate auto-oxidation or to a significant reduction of intracellular reducing agents, such as glutathione.<sup>40,41</sup> Ascorbate auto-oxidation is a well-described phenomenon occurring especially in the presence of iron or copper ions released in myocardial tissue as free catalytic metals.<sup>21,40</sup> The significant depletion of endogenous antioxidants, particularly those of glutathione, prohibits the intracellular reduction of dehydroascorbate (the major transport form of AA) to AA, thereby ameliorating its protective effects or even demonstrating a prooxidative action.<sup>41</sup>

#### **DESFERRIOXAMINE**

Desferrioxamine has been widely studied as a free-radical scavenger. Previous studies have reported beneficial effects, mostly when desferrioxamine was administered before the onset of ischemia.<sup>19,42</sup> Subsequent clinical trials found desferrioxamine had a protec-



**Figure 5.** Mean (SEM) thiobarbituric acid reactive substance (TBARS) concentrations at different time points throughout the reperfusion period. \* $P = 0.002$  desferrioxamine group versus baseline; † $P = 0.02$  control group versus baseline; \* $P = 0.02$  NAC group versus baseline;  $^{\S}P = 0.05$  ascorbic acid group versus desferrioxamine group. NAC = *N*-acetylcysteine.

tive role, particularly in patients undergoing coronary artery bypass graft (CABG) surgery.<sup>32,43</sup> However, desferrioxamine can only be administered early in cases of elective reperfusion, such as CABG surgery, but not in acute myocardial infarction.

Our model was designed to resemble clinical scenarios of myocardial infarction; therefore, the drugs had to be administered after the initiation of ischemia and prior to reperfusion. Desferrioxamine was not found to provide sufficient protection against reperfusion under these circumstances. In addition, a significant increase in LVEDP was found 1 hour after reperfusion, indicating significant left ventricular dysfunction in these animals, possibly associated with a toxic effect of desferrioxamine on the myocardium. This phenomenon has been mainly attributed to an oxidative action of desferrioxamine.<sup>44</sup> Borg and Schaich proposed a dose-dependent biphasic antioxidant/prooxidant behavior of desferrioxamine: at low doses it responds as a chelating agent, while at high doses it paradoxically amplifies oxidative damage.<sup>44</sup> In our study, however, oxidative stress (measured by TBARS concentration) was not found to increase in animals receiving desferrioxamine; on the contrary, a significant decrease in TBARS

concentrations was found, particularly 120 minutes after reperfusion. This observation provides evidence that oxidative toxicity may not be the reason for desferrioxamine failure; other mechanisms may be implicated in the worsening LV function observed in the current study. Because of its relatively low lipophilic characteristics, desferrioxamine might not sufficiently penetrate cellular and mitochondrial membranes. Therefore, it cannot protect sensitive intracellular homeostasis mechanisms, leading to cardiac myocyte dysfunction and subsequent impaired LV function.<sup>45</sup>

### **N-ACETYLCYSTEINE**

Administration of NAC early in the ischemic period can attenuate both postreperfusion myocardial necrosis and stunning.<sup>24,46</sup> However, if it is administered late (after ischemia initiation or on reperfusion), NAC was not found to be protective<sup>27,47</sup>; our results confirm this finding.

Animals treated with NAC had the same incidence of NSVT and idioventricular rhythm as the other groups, but they had a relatively higher incidence of VF/SVT (proarrhythmic effect), indicating that late intravenous administration of NAC during ischemia did not protect against reperfusion ventricular arrhythmias. Previous studies found a protective effect of NAC on reperfusion arrhythmias; however, this was achieved only when NAC was infused from the beginning of ischemia until late in the reperfusion period.<sup>9,24,48</sup> Although the exact reason for the proarrhythmic effect of NAC in our pig model cannot be fully clarified, the increased oxidative stress observed as increased TBARS concentration in the NAC group could be a possible cause. This speculation is supported by the finding that thiol-containing compounds, such as NAC, are capable of forming species with potentially toxic effects (eg, thiol, peroxy sulphenyl radicals) that can limit the expected benefits of the antioxidant properties of NAC.<sup>49</sup>

### **COMBINATION OF DRUGS**

In the presence of conflicting data regarding the protective role of antioxidants, researchers have suggested that antioxidant combinations might be more effective than monotherapy in preventing the deleterious effects of reperfusion injury, possibly through a synergic effect.<sup>8,12,13</sup> Previous experiments investigating the action of different drug combinations had contradictory results.<sup>13,50</sup> Our study found that the combination of AA, desferrioxamine, and NAC administered via IV was not effective in protecting against reperfusion injury.

The elevated TBARS concentration in the control group confirmed oxidative stress as one of the important mediators in the pathophysiology of reperfusion injury. However, although the different antioxidant agents decreased the amount of oxidative stress, they did not have a protective effect on reperfusion injury. The route of administration may be an important factor in our findings. Previous studies in pigs found a protective effect of different agents only with retrograde administration (ie, via cardiac veins) and not when they were infused via IV.<sup>10,36</sup> However, IV administration remains the most common route for infusing various drugs during pharmaceutical (thrombolysis) or mechanical (primary coronary angioplasty) reperfusion. Intracoronary

or retrograde administration of these drugs can be done in a setting of primary coronary angioplasty but not in the case of pharmaceutical IV thrombolysis. In addition, such administration requires special complex catheterization techniques that will increase the time to perfusion, thereby ameliorating the potential benefit.

Other important factors in this study were the timing and the period of administration of the antioxidant agents. All of these drugs were found to have positive results in studies in which they were administered before the initiation of ischemia.<sup>46,51,52</sup> However, in clinical practice it is not feasible to administer these drugs before the onset of symptoms of myocardial infarction. In our experimental protocol, all drugs were administered just before reperfusion, as would have been done in the case of a patient presenting with acute myocardial infarction requiring thrombolysis or primary percutaneous intervention. In the present study, we approximated the drug doses that have been found to be beneficial in experimental and clinical trials.<sup>24,28,52-57</sup> It remains unknown whether the administration of the combination of these drugs in different doses, for different time periods, and via different routes would have resulted in more or less favorable outcomes.

#### **LIMITATIONS**

The most important limitation of the present study was the small number of animals in each group. Only treatment effects of great magnitude would be expected to be revealed with such a small number of animals, and these were not observed in our study.

Although the present experimental study was designed to resemble the actual clinical conditions of myocardial ischemia/reperfusion, all such experimental models have important differences compared with the setting of myocardial infarction in humans.

TBARS concentration in coronary sinus blood samples, which has been widely used in clinical and experimental studies as an indirect marker of oxidative stress, was used for that purpose in this study. However, direct measurement of ROS using electron spin resonance spectroscopy or study of oxidative stress markers in myocardial tissue might result in different findings.

Finally, all animals were euthanized by intracardiac injection of potassium, a widely acceptable method of euthanasia in fully anesthetized animals that causes immediate asystole.<sup>58</sup> Therefore, an impact of potassium on the ischemic or necrotic area of the myocardium was not anticipated and has not been studied in detail. Furthermore, because this procedure was used in all 5 groups, it should not have affected the between-group comparisons.

As reperfusion injury remains an unresolved clinical problem, studies of new drugs that achieve high concentrations in the myocardial zone and studies of alternate routes of administration are needed.

#### **CONCLUSION**

We found that in this experimental closed-chest pig model mimicking clinical scenarios of myocardial infarction and reperfusion that AA, desferrioxamine, and NAC alone or in combination did not provide significant protection from reperfusion oxidative damage.

## ACKNOWLEDGMENTS

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