Peroxy nitrite, the Breakdown Product of Nitric Oxide, Is Beneficial in Blood Cardioplegia but Injurious in Crystalloid Cardioplegia

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Background—Peroxy nitrite (ONOO−) has been implicated as a primary mediator in the deleterious effects of nitric oxide (NO) in crystalloid solutions, possibly due to a lack of detoxification mechanisms, leading to the formation of ‘·OH. In contrast, ONOO− may exert cardioprotective effects in blood environments secondary to detoxification and the subsequent formation of NO-donating nitrosothiols. This dichotomy in physiological effects of ONOO− may exist between crystalloid and blood cardioplegia (BCP) environments. In the present study, we tested the hypothesis that ONOO− is cardiotoxic in crystalloid cardioplegia but cardioprotective in BCP in ischemically injured hearts.

Methods and Results—In anesthetized dogs on cardiopulmonary bypass, global 37°C ischemia was imposed for 30 minutes, followed by 60 minutes of intermittent 4°C hyperkalemic crystalloid (Plegisol) or BCP with (+) or without (−) 5 μmol/L authentic ONOO−. After 2 hours of reperfusion, left ventricular (LV) function (end-systolic pressure-volume relations, in percent of baseline) was 56±3% in Plegisol−, which was further reduced in Plegisol+ to 40±4%.* In contrast, posts ischemic systolic function was better in BCP+ groups than in BCP− groups (96±2% versus 82±2%, respectively). Differences in functional recovery could not be attributed to differences in hemodynamics. LV end-diastolic stiffness was significantly increased with the addition of ONOO− in both Plegisol (298±26% versus 466±30%*) and BCP (201±22% versus 267±13%*) groups. Consistent with increased LV chamber stiffness, myocardial edema was increased in BCP+ compared with BCP− (78.9±0.3% versus 76.4±0.3%*) and in Plegisol+ compared with Plegisol− (81.1±0.3% versus 79.6±0.4%). Creatine kinase activity was significantly increased in Plegisol+ (48±6) compared with that in Plegisol− (31±6) but was unchanged in BCP− (14±2) relative to BCP+ (18±1). Nitrotyrosine (ng/mg protein) accumulation in LV myocardial biopsy samples confirmed myocardial exposure to ONOO− or its metabolites (Plegisol− 1.2±0.1, Plegisol+ 3.3±0.3*, BCP− 1.4±0.2, BCP+ 2.9±0.2*).

Conclusions—We conclude that (1) the postcardioplegic cardiodynamic effects of ONOO− depend on its environment and (2) ONOO− in crystalloid solution impairs postcardioplegic systolic and diastolic functional recovery, whereas (3) ONOO− in BCP increases functional recovery. This environment-dependent dichotomy in the effect of ONOO− may affect the benefits of NO-related adjuncts to crystalloid or BCP solutions (*P<0.05 versus group without ONOO−).

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Key Words: peroxy nitrite ■ cardioplegia ■ cardiopulmonary bypass ■ nitric oxide ■ superoxide

Nitric oxide (NO) produced endogenously or added exogenously to the myocardium in the form of NO donors has been shown to attenuate myocardial reperfusion injury.1–5 NO inhibits neutrophil adherence to the endothelium,5 diminishes platelet aggregation,6 induces coronary vasodilation,3 and attenuates free radical generation by activated neutrophils.5 NO therapy has been advocated as an addition to the surgical myocardial protection armamentarium. However, the beneficial effects of NO are not universally accepted; several studies have demonstrated injury after NO administration during ischemia/reperfusion rather than cardioprotection. A possible mechanism for the deleterious actions of NO observed in some studies is the near-diffusion limited reaction of NO with superoxide anion producing the potentially cytotoxic anion peroxy nitrite (ONOO−).5–8 The toxicity by ONOO− results from the conversion of ONOO− to peroxynitrous acid (ONOOH) with subsequent cleavage and release of an intermediate with hydroxyl radical (‘OH)-like activity.8 However, the generation of ONOO− from adjacent NO, and the resultant cytotoxic effects, would limit the benefits of this therapeutic approach.
The physiological effects of ONOO\textsuperscript{\textminus} are inconsistent. Studies that involved nonblood environments (ie, culture media, crystalloid perfusates) have demonstrated toxic properties of ONOO\textsuperscript{\textminus} manifested as increased myocardial stunning, increased infarct size,\textsuperscript{6,7,9} and greater endothelial injury. On the other hand, in vivo models or blood-perfused ex vivo models have demonstrated a reduction in myocardial infarct size, inhibition of neutrophil adherence, and improved endothelial function by authentic ONOO\textsuperscript{\textminus}. Opposing effects of ONOO\textsuperscript{\textminus} in postischemic hearts related to blood versus crystalloid environments have been recently reported by Lopez et al.\textsuperscript{10}

In cardiac surgery, different perfusate environments exist in the use of crystalloid versus blood cardioplegia (BCP). In surgical models of myocardial ischemia/reperfusion injury, opposing biological effects of NO-related therapy have been reported, possibly related to the environmentally dependent fate of ONOO\textsuperscript{\textminus}. Accordingly, in the present study, we used an in vivo canine cardiopulmonary bypass model to test the hypothesis that biological effects of the NO byproduct ONOO\textsuperscript{\textminus} are dependent on a crystalloid or BCP environment. Specifically, we tested the hypothesis that ONOO\textsuperscript{\textminus} in crystalloid cardioplegia would be deleterious, whereas it would be cardioprotective in BCP.

**Methods**

The dogs were handled in compliance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (NIH publication No. 85-23, revised 1985). The study protocol was approved by the Institutional Animal Care and Use Committee of Emory University.

**Surgical Procedure**

Heartworm-free adult mongrel dogs of either sex weighing 25 to 35 kg were anesthetized with sodium thiopental after premedication with morphine sulfate (4 mg/kg). Maintenance anesthesia consisted of an intravenous mixture of fentanyl citrate (0.3 μg·kg\textsuperscript{\textminus}1·min\textsuperscript{\textminus}1) and diazepam (0.03 mg·kg\textsuperscript{\textminus}1·min\textsuperscript{\textminus}1) infused continuously. After median sternotomy and heparinization (300 U/kg), the vena cavae were isolated, and the azygous vein was ligated. The left subclavian artery was isolated and cannulated with a 22F tapered arterial cannula, and the pericardium was opened and tents, forming a pericardial well. Millar MPC-500 temperature-compensating solid-state catheters (Millar Instruments) were placed in the proximal aorta via the right internal mammary artery and through the left ventricular (LV) apex to measure arterial and LV pressures, respectively. The right carotid artery was dissected free via a ventral cervical incision for the placement of a 7F octapolar impedance catheter (Webster) across the aortic valve into the LV to measure instantaneous LV chamber conductance converted to volume as described previously.\textsuperscript{11} The isolated vena cavae were transected and canulated, and the venous return cannulae (28F) were temporarily lodged in the right atrium to prevent obstruction of venous return while the circulation was intact. Preischemic baseline data with intact circulation were obtained in both the steady state and during gradual preload reduction imposed by transient bicalval occlusion. This results in sequentially declining LV pressure-volume loops from which systolic and diastolic parameters are derived. Cardiopulmonary bypass was instituted with the use of a Cobe membrane oxygenator (Cobe Cardiovascular, Inc) primed with 1.5 L hetastarch (Hespan; DuPont Pharmaceutical). The left and right ventricles were vented with the use of direct cannulation and gravity drainage. A double-lumen aortic root cannula (DLP, Inc) was inserted for cardioplegia delivery and simultaneous monitoring of cardioplegia infusion pressure.

**Experimental Protocol**

After 30 minutes of normothermic global ischemia, the animals were randomized (n=7/group) to receive either crystalloid cardioplegia (Plegisol; Abbott) or 8:1 BCP each with (+) or without (−) 5 μmol (final concentration) authentic ONOO\textsuperscript{\textminus} delivered with the MPS cardioplegia delivery system (Quest Medical).\textsuperscript{12} The MPS cardioplegia delivery system is a microprocessor-controlled electromechnical instrument with a bladder-based delivery system to enable programmable precise delivery of cardioplegia components and additives with a high degree of accuracy.\textsuperscript{13} Authentic ONOO\textsuperscript{\textminus} solution was added to the additive pouch of the MPS system after adjustment of the concentration as described previously\textsuperscript{13} and delivered to the aortic root via a separate light-protected tube for a final concentration of 5 μmol/L. A hypothermic (4°C) multidose delivery method was used. The compositions of 8:1 BCP and Plegisol are given in Table 1. Cardioplegia was delivered for 3 minutes at induction (high [K\textsuperscript{\textminus}]) and hypo- thermic terminal delivery of 3 minutes for each infusion (low [K\textsuperscript{\textminus}]) delivered at 50 mm Hg. Systemic rewarming to 37°C was achieved, and the cross-clamp was removed immediately after completion of the terminal cardioplegia infusion.

**Reperfusion**

After removal of the aortic cross-clamp, mean arterial pressure was gradually increased from 50 to 70 mm Hg as electromechanical reanimation was observed. When ventricular fibrillation occurred, DC countershocks of 10 to 20 W·s were applied. The heart was maintained in the total vented bypass state for the initial 30 minutes of reperfusion, after which cardiopulmonary bypass was discontinued and cannulas were withdrawn. Cardiodynamic data were collected every 30 minutes for the next 90 minutes. After the final data collection, the heart was excised and immediately immersed in cold Krebs-Henseleit solution consisting of the following composition (in mmol/L): NaCl 118, KCl 4.7, KH\textsubscript{2}PO\textsubscript{4} 1.2, MgSO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 12.5, and glucose 10.

**Data Acquisition and Analysis**

Analog hemodynamic and cardiodynamic data were recorded on a microcomputer system (IBM-PC) with an analog-to-digital converter (model DT2801A; Data Translation) and sampling at 250 Hz. Data were analyzed with the use of an interactive videographics program previously described.\textsuperscript{11} LV chamber conductance was converted to volume with use of a Leycom Sigma 5 signal conditioner and

<table>
<thead>
<tr>
<th>TABLE 1. Composition of Cardioplegia</th>
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<tr>
<td>Constituent</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Potassium ion (KCl), mEq/L</td>
</tr>
<tr>
<td>Calcium ion (CaPc), mmol/L</td>
</tr>
<tr>
<td>Magnesium ion, mmol/L</td>
</tr>
<tr>
<td>pH (THAM buffer) at 37°C</td>
</tr>
<tr>
<td>Osmolality, mOsm/L</td>
</tr>
<tr>
<td>Hematocrit, %</td>
</tr>
<tr>
<td>Oxygen content</td>
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</table>

CPD indicates citrate-phosphate-dextrose; and THAM, tris(hydroxymethyl)-aminomethane.
TABLE 2. Characteristics of the Cardioplegia Solutions

<table>
<thead>
<tr>
<th></th>
<th>BCP−</th>
<th>BCP+</th>
<th>Pleg−</th>
<th>Pleg+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume, mL</td>
<td>1005±26</td>
<td>1019±37</td>
<td>1033±41</td>
<td>1024±27</td>
</tr>
<tr>
<td>(delivery 1, 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume, mL</td>
<td>634±14</td>
<td>655±18</td>
<td>688±33</td>
<td>666±22</td>
</tr>
<tr>
<td>(delivery 2, 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Potassium, mEq/L (1, 4)</td>
<td>19.6±1.2</td>
<td>20.4±0.9</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Potassium, mEq/L (2, 3)</td>
<td>9.8±0.6</td>
<td>10.2±0.8</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>23.4±2.1</td>
<td>24.0±1.3</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

1, 4 indicates induction and terminal infusions of cardioplegia solution; 2, 3, second and third intermittent infusions of cardioplegia solution; and NA, variable is not acceptable.

Calculations

End-Systolic Pressure-Volume Relation
To measure LV systolic performance, the end-systolic point was located at the top left corner of each corrected pressure-volume loop according to the method of Kono et al.1 LV systolic performance was described with the use of end-systolic pressure-volume relation (ESPVR), with slope and volume axis intercept as discriminating variables as described previously.11

End-Diastolic Pressure-Volume Relation
The exponential end-diastolic pressure-volume relation was used to determine the characteristics of LV chamber stiffness (the inverse of compliance) as previously described.12 The exponential β coefficient is the unitless modulus of chamber stiffness used to describe the degree of curvature of the end-diastolic pressure-volume relation.

Preload-Recruitable Stroke Work
LV stroke work (SW) was calculated through point-by-point integration of LV pressure and volume (SW=∫P·dV) over each cardiac cycle, where P is LV instantaneous pressure, and dV is the rate of volume changes. Regression analysis was performed to fit SW for each loop to its corresponding end-diastolic volume (Vsd) with the equation SW=Msw(Vsd-Vsw,0), where Msw is the slope of the linear SW=Vsd relation, and Vsw,0 is the volume intercept where SW=0 mm Hg.

Plasma Creatine Kinase Activity
Blood samples for the measurement of creatine kinase (CK) activity were withdrawn from the femoral artery, and the plasma was separated via centrifugation and analyzed spectrophotometrically as described previously.12 CK activity was expressed in international units per microgram of protein.

Cardiac Myeloperoxidase Activity
Tissue samples weighing ~0.4 g were taken from the anterior wall of the LV for measurement of myeloperoxidase (MPO) activity as an assessment of neutrophil accumulation in the myocardium.13 The activity of MPO was measured spectrophotometrically and expressed as units per 100 mg of tissue. One unit of MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide per minute.

LV Tissue Water Content
Transmural samples of ~0.5 g were taken from the LV free wall after the experiment, quickly blotted of surface moisture and blood, and weighed. The samples were placed in an 80°C oven for 72 hours to desiccate and were reweighed, and the percent water content was calculated as 100*[1−(dry weight/wet weight)].

Determination of Area at Risk and Infarct Size
After excision of the heart, a 37°C solution of 1% triphenyltetrazolium chloride (Sigma Chemical Co) was infused into the aortic root at 100 mm Hg for 10 minutes. The triphenyltetrazolium chloride–stained (non-necrotic) tissue was separated from the pale (necrotic) tissue, and each respective area was weighed.

Tissue Nitrotyrosine
Quantification of tissue nitrotyrosine levels was performed according to a modification of a previously published ELISA procedure13 with a mouse IgG monoclonal anti-nitrotyrosine primary antibody (1:500 in 10% goat serum PBS; Upstate Biotechnology) and a peroxidase-conjugated goat anti-mouse IgG secondary antibody (1:1000, Amersham). The peroxidase reaction product was generated with O-phenylenediamine dihydrochloride (2.2 mmol/L) (Abbott Diagnostics), and absorbance was measured at 460 nm with a spectrophotometer microplate reader (Bio-Tek Instruments, Inc). The amount of nitrotyrosine content in tissue samples was calculated with the use of the standard curve generated from nitrated BSA containing known amounts of nitrotyrosine and expressed as nanograms of nitrotyrosine per milligram of protein.

Statistical Analysis
All data were analyzed with the use of the Sigma Stat program (Jandel Corp). Time-related differences and group/time interactions for longitudinal data (ESPVR, preload-recruitable [PR] SW, end-diastolic parameters, and CK activity) were analyzed with two-way ANOVA for repeated measures adjusted for baseline values. When significance was assigned, Tukey’s post hoc test was used to locate the source of the difference. Single-event, nonrepeated variables were compared between the groups at the same base cardioplegia solution (ie, BCP versus crystalloid) with a standard t test. A value of P<0.05 was considered statistically significant. All values are given as mean±SEM.

Results
In each of the Plegisol plus 5 μmol/L ONOO− (Plegisol+), Plegisol without ONOO− (Plegisol−), and BCP plus 5 μmol/L ONOO− groups (BCP+), 1 animal was excluded because of intraoperative technical complications.

Cardioplegia Characteristics
Table 2 summarizes the cardioplegia characteristics in all groups. There were no significant differences between any of the groups regarding total delivered cardioplegia volume or delivery temperature. With each formulation of cardioplegia (blood or crystalloid), there were no differences in potassium concentrations or (blood cardioplegia) average hematocrit of the cardioplegia solution.

Hemodynamic Parameters
Table 3 shows the insignificant differences in heart rate, mean arterial pressure, and systemic hematocrit among all of the
The crystalloid cardioplegia groups had significantly increased LV end-diastolic pressure compared with the blood groups, with Plegisol+ being significantly greater than Plegisol－ at all reperfusion time points.

ESPVR
All four groups were comparable at the preischemic period in slope (Eₑₑ) of the ESPVR (Figure 1 and Table 4). Generally, recovery of systolic function was better with blood than with crystalloid cardioplegia. At 90 minutes after the discontinuation of cardiopulmonary bypass, postischemic Eₑₑ was significantly diminished to 40.3±4.1% of baseline in the Plegisol－group compared with 56.3±3.3% in the Plegisol+ group (P<0.05). In contrast, in the BCP+ group, 90-minute postischemic Eₑₑ was significantly increased compared with the unsupplemented BCP group (96.0±2.4% versus 81.8±2.4% of baseline, respectively; P<0.05). Significant improvement in Eₑₑ in the ONOO－supplemented BCP was evident only at 2 hours of reperfusion, whereas the dysfunction associated with the ONOO－additive to the crystalloid cardioplegia was statistically significant at all time points.

Chamber Stiffness
Preischemic chamber stiffness was comparable among the four groups (Figure 2). Both crystalloid groups showed a significant increase in chamber stiffness over all of the time points compared with baseline. The Plegisol－group showed the greatest increase in chamber stiffness, with stiffness being significantly greater than that in all other groups at each time point of reperfusion. There were no differences between BCP groups at 30 minutes after cardiopulmonary bypass termination. However, by 90 minutes of beating working reperfusion, LV chamber stiffness in the BCP+ group was significantly greater relative to the BCP－group in contrast to the recovery of systolic function.

Tissue Edema
Postexperimental tissue water content in the Plegisol+ group was greater than that in the Plegisol－group (81.1±0.3% versus 79.6±0.4%, P<0.05) (Figure 3). Similarly, the BCP+ hearts showed significantly greater water content than the BCP－hearts (78.9±0.3% versus 76.4±0.3%, P<0.05). In addition, tissue water content in both crystalloid groups was greater than that in the BCP groups. These group patterns were consistent with the chamber stiffness data (β coefficient).

PRSW
The results of PRSW as an index of integrated (systolic and diastolic) LV performance are given in Figure 4 and Table 4. PRSW was significantly lower in the Plegisol－group than in the Plegisol+ group after 60 and 90 minutes of reperfusion.

### Table 3. Hemodynamic Parameters

|                | Baseline | 5 min | 30 min | 60 min | 90 min |
|----------------|----------|-------|--------|--------|--------|   |
| Heart rate     | Plegisol－| 89.57±3.23 | 92.00±3.82 | 89.29±3.21 | 95.71±2.35 | 90.86±3.47 |
|                | Plegisol+ | 93.86±3.16 | 92.71±3.70 | 93.86±1.81 | 93.29±3.51 | 92.86±3.06 |
|                | BCP－     | 91.86±2.83 | 94.28±4.12 | 91.43±4.02 | 93.43±1.46 | 92.72±3.36 |
|                | BCP+     | 88.00±3.29 | 91.86±4.82 | 91.00±4.70 | 94.71±4.49 | 94.57±5.04 |
| Mean arterial pressure | Plegisol－ | 67.43±2.60 | 66.71±4.46 | 66.86±2.41 | 67.57±3.44 | 66.86±2.91 |
|                | Plegisol+ | 66.14±2.94 | 71.14±3.85 | 67.57±3.02 | 66.29±2.41 | 65.00±2.62 |
|                | BCP－     | 67.57±3.21 | 66.14±2.24 | 70.86±3.46 | 66.86±3.35 | 67.00±4.20 |
|                | BCP+     | 68.43±3.18 | 65.43±3.29 | 65.43±2.69 | 68.29±4.65 | 68.14±3.60 |
| LV end-diastolic pressure | Plegisol－ | 10.57±2.10 | 14.86±1.72* | 14.86±1.90* | 13.86±2.44* | 13.14±1.63 |
|                | Plegisol+ | 10.00±1.56 | 18.71±1.74†  | 19.57±1.82† | 19.00±2.22† | 17.71±1.09† |
|                | BCP－     | 10.14±2.42 | 11.71±1.02  | 10.43±0.92 | 10.57±1.25 | 9.43±1.25 |
|                | BCP+     | 9.00±2.09  | 14.57±1.65† | 15.87±1.14† | 14.14±1.60† | 11.14±1.63 |

*P<0.05 compared with baseline.
†P<0.05 compared with respective group without ONOO.
In contrast, there was no significant difference noted between the BCP\(^{-}\) and BCP\(^{+}\) groups.

**Plasma CK Activity**
Baseline plasma CK activity levels were comparable among all groups. CK activity in the crystalloid cardioplegia groups with and without ONOO\(^{-}\) were significantly greater than that in either BCP group at all reperfusion time points (Table 5). In the crystalloid cardioplegia groups, the addition of ONOO\(^{-}\) was associated with significantly greater CK activity than that in the Plegisol\(^{-}\) group at 30, 60, and 90 minutes of reperfusion. In the BCP groups, there was a significantly greater CK activity in the BCP\(^{+}\) group compared with the BCP\(^{-}\) group only at 30 and 60 minutes of reperfusion; there was no difference at 90 minutes of reperfusion. At 90 minutes of reperfusion off cardiopulmonary bypass, plasma CK values were significantly greater in both crystalloid groups compared with the BCP groups, and CK activity in the Plegisol\(^{+}\) group was significantly greater than that in all other groups.

**MPO Activity**
The addition of ONOO\(^{-}\) to crystalloid cardioplegia was associated with a significant increase in MPO activity compared with the unsupplemented cardioplegia group, suggesting increased neutrophil accumulation in the myocardium (Figure 5). In contrast, there was significantly lower myocardial MPO activity in the BCP\(^{+}\) group compared with the BCP\(^{-}\) group. Both BCP groups demonstrated significantly lower LV MPO activity than the crystalloid groups.

**Myocardial Infarction**
In this model of global ischemia, there was no infarct identified in any of the hearts. ONOO\(^{-}\) did not induce myocardial necrosis in either the BCP or Plegisol groups.
Nitrotyrosine Data
Figure 6 demonstrates that there was a significant increase in myocardial nitrotyrosine levels in both blood and crystalloid cardioplegia groups supplemented with adjunct ONOO\textsuperscript{−} compared with unsupplemented groups. No difference was found between the BCP\textsuperscript{+} versus Plegisol\textsuperscript{+} groups or between the BCP\textsuperscript{−} versus the Plegisol\textsuperscript{−} groups, although there was a trend for tissue nitrotyrosine levels to be greater in the Plegisol\textsuperscript{+} group.

Discussion
In the presence of superoxide anion, NO is rapidly converted to ONOO\textsuperscript{−} in a biradical reaction that is essentially diffusion limited. Hence, ONOO\textsuperscript{−} is a likely byproduct of NO, especially when NO is elevated by L-arginine or NO-donor compounds. ONOO\textsuperscript{−} production by the ischemic/reperfused heart has been shown to peak in the early seconds of reperfusion at a time coincident with the generation of both NO and superoxide radicals. The subsequent generation of peroxynitrous acid and ultimate conversion to a hydroxyl-like intermediate are likely mediators of biological injury. However, under more biological conditions, certain thiol-containing compounds that are normal constituents of blood, such as glutathione, albumin, and cysteine, may convert ONOO\textsuperscript{−} to nitrosothiols. This detoxification reaction potentially prevents toxic buildup of ONOO\textsuperscript{−} and possibly regenerates NO through these nitrosothiols. However, these detoxifying pathways are absent in crystalloid environments; therefore, ONOO\textsuperscript{−} has the potential of generating either deleterious products or cardioprotective intermediates, depending on the biological environment related to the presence or absence of appropriate endogenous or exogenous detoxifying agents.

The biological fate of ONOO\textsuperscript{−} relative to the presence or absence of detoxification pathways is relevant to cardiac surgery in which blood or crystalloid formulations are used for cardioplegia solutions. In the present study with hearts sensitized by ischemia, authentic ONOO\textsuperscript{−} was added to crystalloid or BCP solutions to simulate the appearance of the breakdown product of pharmacological concentrations of adjunctive NO. The delivery of the labile ONOO\textsuperscript{−} anion to the myocardium was confirmed via the formation of nitrotyrosine in myocardial biopsy samples. We found that in a crystalloid cardioplegia environment, ONOO\textsuperscript{−} was associated with (1) significantly less recovery of postcardioplegia LV function, (2) increased plasma CK activity, and (3) increased neutrophil accumulation in LV myocardium. In contrast, BCP with ONOO\textsuperscript{−} was associated with increased recovery of LV function, lower plasma CK activity, and less MPO activity. However, adjunctive ONOO\textsuperscript{−} in both crystalloid and BCP was associated with increased diastolic chamber stiffness and LV edema. As a whole, both BCP groups showed greater recovery of systolic function, decreased plasma CK activity, and lower myocardial MPO activity compared with the two crystalloid cardioplegia groups.

The greater functional recovery in hearts perfused with blood versus those perfused with crystalloid cardioplegia is consistent with previous reports and may be due to greater oxygen-carrying capacity of blood, the presence of endogenous antioxidants (eg, catalase, glutathione), and more efficient physiological buffers in BCP that are absent in many crystalloid formulations. Hence, in the addition of ONOO\textsuperscript{−} to

<table>
<thead>
<tr>
<th>TABLE 5. CK Activity</th>
<th>Beating Working Reperfusion, min</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>BCP\textsuperscript{−}</td>
<td>1.71±0.23</td>
</tr>
<tr>
<td>BCP\textsuperscript{+}</td>
<td>1.89±0.12</td>
</tr>
<tr>
<td>Plegisol\textsuperscript{−}</td>
<td>2.02±0.63</td>
</tr>
<tr>
<td>Plegisol\textsuperscript{+}</td>
<td>1.70±0.75</td>
</tr>
</tbody>
</table>

* \( \text{P}<0.05 \) compared with respective unsupplemented group at same time point.
cardioplegia solutions, we reproduced the dichotomous physiological effects of both NO and ONOO– in crystalloid and blood-based perfusates reported in the literature. These data imply that when added to crystalloid cardioplegia formulations, NO or NO donors should be used with appropriate detoxifying agents to avoid potential deleterious effects of the breakdown product ONOO–.

The toxicity of ONOO– in either blood or crystalloid environments has been related in part to its concentration in solutions. In studies in which ONOO– demonstrated toxic effects, its concentration was in the micromolar to millimolar range. Under normal in vivo conditions, the concentration of precursor NO ranges between 1 to 20 nmol/L, which can increase 100- to 1000-fold via activation of the inducible isoform of NO synthase. Because of the 1:1 stoichiometry of the reaction between NO and superoxide anion, these values of NO represent the upper limit of ONOO– concentration generated in vivo, with the assumption of an adequate supply of superoxide anion. In the present experiment, the 5 μmol/L concentration used in cardioplegia solutions may have been supraphysiological but is within the range expected when NO-donor agents are used from which ONOO– could be derived. It is not known from the present study whether the use of lower concentrations of ONOO– in the crystalloid cardioplegia solution could have avoided the injury observed in the present study; this would require extended concentration-response studies.

The physiological actions of ONOO– in crystalloid cardioplegia may have been due to conversion to deleterious mediators, whereas in BCP, the physiological actions may have been governed by the production of cardioprotective mediators. These mediators are largely labile and not easily measured. ONOO– directly produces nitration of enzymatic proteins, lipid peroxidation, and DNA fragmentation, thereby increasing injury. In addition, ONOO– has been shown to increase vascular permeability, which may have been the cause of the increase in myocardial water content demonstrated in both cardioplegia groups. However, the cardioprotection observed with BCP may be related to the conversion of ONOO– to nitrosothiols, which can then act like NO donors. NO may also be responsible for increasing cell permeability. S-Nitrosothiols derived from ONOO– can activate guanylate cyclase and dilate pulmonary vascular smooth muscle, which is inhibitable by hemoglobin, suggesting an NO-like mediator. In addition, S-nitrosothiols can release authentic NO over prolonged periods of time. The inhibition of neutrophil accumulation in the myocardium by ONOO– has been demonstrated in an in vivo rat preparation to be related to the downregulation of P-selectin, a mechanism similar to the antineutrophil effects of the precursor to ONOO–, NO. The present experiment did not confirm whether cardioprotection by ONOO– in BCP involved the formation of nitrosothiols or authentic NO.

Authentic ONOO– was associated with significantly lower postcardioplegia recovery of systolic function in the crystalloid cardioplegia group but was associated with greater recovery of systolic function in the BCP group. Postcardioplegia functional depression in the crystalloid group may be related to the direct deleterious effects of ONOO– mediated by the intermediate with hydroxyl-like activity. The beneficial effects in the postcardioplegia systolic function in the BCP group may be due to detoxification of ONOO– by plasma or red cell glutathione or by plasma cysteine and albumin, with the resultant production of a nitrosothiol or authentic NO. The postcardioplegic attenuation of neutrophil accumulation in the ONOO–-supplemented BCP group is consistent with the antineutrophil effects of NO. Nossuli et al have shown that ONOO– in vivo attenuates neutrophil adherence to coronary artery endothelium and reduces neutrophil accumulation in postischemic myocardium. Data from Nossuli et al also support a myocardial tissue glutathione-dependent detoxification of ONOO– to a nitrosothiol. In the present study, we did not measure nitrosothiols or NO to substantiate a nitrosothiol-based detoxification mechanism.

In contrast to the group differences in systolic function, diastolic dysfunction was noted in all groups. The diastolic dysfunction in hearts receiving ONOO– may have been secondary to the increased tissue water content in both groups, due at least in part to an ONOO–-mediated increase in vascular permeability. The extent of increases in water content were relatively similar to the increases in chamber stiffness in the ONOO–-treated groups. The diastolic dysfunction seen in the BCP groups receiving ONOO– is likely responsible for masking the effects of preserved systolic function, assessed on the basis of ESPRV, because overall global performance measured with PRSW integrates both systolic and diastolic components.

In summary, the present study demonstrated that the addition of a purported metabolite of NO, ONOO–, to crystalloid or BCP produced a duality in physiological actions regarding postcardioplegic systolic function, CK activity, and neutrophil accumulation. Authentic ONOO– at upper physiological concentrations exerted deleterious effects on postcardioplegia recovery of systolic function in crystalloid cardioplegia solution, whereas it enhanced postcardioplegia systolic function in BCP. A decrease in postcardioplegic integrated LV performance, assessed with the use of PRSW, between untreated and ONOO–-treated groups persisted in the crystalloid cardioplegia group, but an enhanced recovery of overall performance was masked in the BCP group, possibly due to a diastolic filling defect secondary to increased tissue water content. Although ONOO– is not advo-
cated as a cardioprotective additive in any cardioplegic solution, NO is advocated for cardioprotection. However, the benefits of NO-related therapy may depend on the environmentally sensitive generation of ONOO⁻. Indeed, deleterious effects of NO-related strategies in crystalloid cardioplegia have been reported and may be due to the cytotoxic effects of ONOO⁻. The use of thiol agents such as glutathione may detoxify ONOO⁻ in crystalloid cardioplegia solutions and therefore prevent deleterious effects in crystalloid solutions incorporating NO-related therapeutic strategies. However, no such detoxifying agents are required in BCP.

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