Original Paper

Pharmacology

Pharmacology 2004;72:106–112
DOI: 10.1159/000079139

Received: February 5, 2004
Accepted: March 1, 2004

S-Nitroso Human Serum Albumin Improves Oxygen Metabolism during Reperfusion after Severe Myocardial Ischemia

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Key Words
Heart preservation · Ischemia/reperfusion · Controlled reperfusion · Myocardial blood flow · Myocardial oxygen metabolism · Nitric oxide · Postischemic contractile function

Abstract
Nitric oxide (NO) supplementation may modify myocardial oxygen consumption and vascular function after ischemia. We investigated the effects of the NO donor, S-nitroso human serum albumin (S-NO-HSA), on cardiac oxygen metabolism during controlled reperfusion on normothermic cardiopulmonary bypass after severe myocardial ischemia. Pigs randomly received either S-NO-HSA or human serum albumin prior to and throughout global myocardial ischemia. Myocardial oxygen utilization is impaired at the onset of reperfusion, which is not amenable to S-NO-HSA. However, NO supplementation during ongoing supply dependency of oxygen consumption eventually leads to greater myocardial oxygen delivery and consumption. In conjunction with a better washout of lactate, this indicates an improved capillary perfusion in the S-NO-HSA group during reperfusion, which results in a better contractile function post bypass.

Part of the results were presented at the Annual Meeting of the International Anesthesia Research Society, San Diego 2002, and printed in abstract form in a supplement of *Anesthesia & Analgesia*.

Introduction

After initial hyperemia, progressive warm ischemia increases coronary artery resistance. Elevated coronary artery resistance, however, correlates with decreased left ventricular developed pressure at reperfusion and a lower energetic index. Nitric oxide (NO) repletion during myocardial ischemia enhances vascular function again [1]. NO determined in microvessels shows a rapid increase after initiation of ischemia that peaks after 15–20 min but drops below baseline levels during prolonged ischemia. Likewise, during early reperfusion, NO levels decline exponentially below 1 nmol/l in association with an increase of superoxide radicals (O_2^-) production [2]. NO depletion causes disarrangement of endothelial nitric oxide synthase (eNOS), which consequently starts to produce both NO and O_2^-.

Exogenous NO can exert its cardioprotective effect through various different mechanisms. When given be-

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fore ischemia, the two structurally unrelated NO donors, diethylenetriamine/NO and S-nitroso-*N*-acetypenicillamine, have been shown to mimic late preconditioning [3, 4]. During reperfusion, NO inhibits platelet aggregation, attenuates leukocyte-endothelium interactions and decreases endothelin levels [5, 6]. Furthermore, it diminishes calcium uptake of the mitochondria by reversible inhibition of the respiratory chain [7].

Recently, we could also demonstrate that inhibition of the derangement of eNOS by S-NO-HSA, a novel NO donor for intravenous application, is an important mechanism, which mitigates ischemia-reperfusion injury in skeletal muscle [8]. In this compound, NO is bound to human serum albumin as an S-nitroso moiety. The half-life of S-NO-HSA in blood is approximately 15 min [8]. NO supplementation by S-NO-HSA confers endothelial protection, prevents eNOS decoupling and normalizes eNOS function, which results in sufficient endogenous NO formation and limited O_2^- production during reperfusion. Therefore, NO supplementation may affect both systemic oxygen delivery (DO_2) by regulating microvascular blood flow via normalization of eNOS function and oxygen consumption (VO_2) by altering cellular respiration. Both mechanisms could potentially influence the refilling of high-energy stores and consequently organ function.

The role of NO supplementation during severe myocardial ischemia/reperfusion with supply dependency of VO_2 on myocardial oxygen metabolism during controlled reperfusion has not yet been elucidated. We hypothesized that, under these conditions, the long-lasting NO release by S-NO-HSA would have a beneficial effect on microvascular perfusion and would consequently enhance myocardial oxygen delivery (mDO_2), which in turn may contribute to an improved myocardial pump function after severe global myocardial ischemia.

Material and Methods

This prospective, randomized, blinded study was approved by the local animal investigation committee. All animals received care in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health [NIH Publ. 85-23, rev. 1985]. Pigs (Österreichische Landrasse) weighing 30 ± 4 kg were sedated with 20 mg/kg ketamine i.m., anesthetized with 15 mg/kg pentobarbital, intubated and ventilated with a mixture of oxygen in air. Respirator settings were adjusted to achieve normoventilation, normoxia, and keep peak airway pressures <20 cm H_2O. End-tidal pCO_2 and pulse oximetry were employed to control respirator variables. Anesthesia was maintained with 8 mg/kg/h propofol, 0.7 mg/kg/h piritramide, a synthetic opioid, and 0.1 mg/kg/h pancuronium. Ringer's solution was infused at a rate of 10 ml/kg/h and fluid-filled catheters were placed in both internal jugular veins and the right carotid artery. A Swan-Ganz pulmonary artery catheter was then introduced via the right external jugular vein, thereby we determined cardiac output (CO), central venous pressure (CVP), pulmonary artery pressure (PAP), and blood temperature. Throughout the study, temperature was kept at 37°C with the help of prewarmed infusions and a heating blanket. After thoracotomy, a tip catheter (Millar Tip Catheter, Calif., USA) was introduced in the left ventricle via the apex to monitor left ventricular pressure. Both venae cavae and the ascending aorta were cannulated for cardiopulmonary bypass (CPB). A cotton band was placed around the venae cavae to occlude venous return during CPB. In addition, a small balloon-tipped catheter was inserted in the coronary sinus for collection of coronary venous blood. CPB priming consisted of a balanced electrolyte solution with hetastarch. Systemic anticoagulation was induced with a bolus injection of 600 U/kg BW heparin and maintained by additional heparin doses such as to achieve an activated clotting time (ACT) of >400 s. Management of normothermic CPB included the α-stat method, the use of membrane oxygenators with arterial line filters and a pump flow set at 100 ml/kg/min.

After baseline measurements, the pigs were randomly assigned to either the S-NO-HSA group (n = 6) or the control group (n = 8). The former received an infusion containing S-NO-HSA at a rate of 0.1 μmol/kg/h over 1 h. The preparation of S-NO-HSA has recently been described in detail [14]. The control group received human serum albumin over 60 min at the same rate. Fifteen minutes after the start of the infusion, the pigs were subjected to 15 min of warm global cardiac ischemia by venous inflow obstruction and aortic cross-clamping that eventually resulted in asystole. After opening the cross-clamp again, hearts were paced at a rate of 120 beats/min by DDD pacing and defibrillated when necessary. CPB flow was kept at 100 ml/kg/min and the heart, still unloaded and not ejecting, was reperfused for 30 min on CPB. Mean arterial blood pressure (MAP) was maintained between 50 and 60 mm Hg with the help of intermittent injections of neosynephrine if necessary. Continuing throughout ischemia and the reperfusion period, treatment solution or vehicle was infused at the same rate until it was stopped after 30 min of controlled reperfusion with termination of CPB. Blood samples for lactate, hemoglobin (OSM 2 Hemoxymeter; Radiometer, Copenhagen, Denmark), and blood gas analyses (Blood Gas Analyzer 995; AVL, Graz, Austria) were drawn from the arterial line and the coronary sinus at baseline and at 2, 5, 10, 15, 20, and 30 min of controlled reperfusion. Coronary blood flow (CBF) was measured with an ultrasonic flow probe (3-mm Flowprobe, Transonic Flowprobe; Transonic Systems Inc., Ithaka, N.Y., USA) placed around the proximal part of the left anterior descending artery. Flow was normalized to the body weight of each pig to account for small differences in body weight. Arterial and coronary venous oxygen content (CaO_2, CvO_2), DO_2, and mDO_2, myocardial oxygen consumption (mVO_2), myocardial oxygen extraction (mEO_2), and the myocardial lactate washout (mLact) were calculated using the following formulas:

$$CaO_2\,[\text{ml } O_2/\text{dl}] = 1.36 \times Hb \times SaO_2 + 0.003 \times paO_2$$
$$CvO_2\,[\text{ml } O_2/\text{dl}] = 1.36 \times Hb \times SvO_2 + 0.003 \times pvO_2$$
$$DO_2\,[\text{ml } O_2/\text{min}] = 100\ \text{ml/kg BW/min (CPB flow)} \times CaO_2 \times 10$$
$$mDO_2\,[\text{ml } O_2/\text{min/kg BW}] = CBF/\text{kg BW} \times CaO_2/100$$
$$mVO_2\,[\text{ml } O_2/\text{min/kg BW}] = CBF/\text{kg BW} \times (CaO_2 - CvO_2)/100$$
$$mEO_2\,[\%] = (CaO_2 - CvO_2)/CaO_2 \times 100$$
$$\text{mLact}\,[\text{min} \times 10^{-7}] = CBF/\text{kg BW} \times (\text{lactate a} - \text{lactate v})$$

where Hb = arterial hemoglobin content, BW = body weight, paO_2, pvO_2, SaO_2, SvO_2, lactate a, lactate v = oxygen tension, oxygen saturation and lactate concentration in arterial and coronary sinus blood, respectively.

After 30 min of reperfusion, the pigs were weaned from bypass. As the catheter in the coronary sinus needs to be removed before termination of CPB, no coronary sinus blood could be analyzed anymore. The blood from the heart lung machine was slowly retransfused to obtain the desired preload and an epinephrine infusion was begun at a rate of 0.28 μg/kg/min 5 min prior to weaning. This dosage had been determined in preliminary studies. Hypotension, i.e. MAP < 50 mm Hg, was treated with bolus injections of neosynephrine to obtain a mean blood pressure in the range from 50 to 60 mm Hg. Heparin was reversed with 300 U/kg protamine and further volume loading was guided by left ventricular end-diastolic pressure, which was aimed to be at 2 mm Hg above or below baseline level. Respirator settings were adjusted according to SaO_2 and $etCO_2$ to achieve normoxia and normocapnia. At the end of the experiment, the pigs were sacrificed with a bolus injection of KCl.

Statistical Analysis

Repeated measure ANOVA with Fisher's PLSD and unpaired t-test were employed. Friedman, Wilcoxon and Mann-Whitney U tests were used when data showed a skewed data distribution. A p value of <0.05 was considered to be significant. Values are reported as means ± SEM.

Results

All 14 pigs completed the experimental protocol and none had to be excluded from statistical analysis. No harmful side effects were seen with the application of S-NO-HSA. In particular, at a dose of 0.1 μmol/kg/h, we did not observe alterations from hemodynamic variables determined at baseline in the course of S-NO-HSA treatment. During reperfusion on CPB, all measurements were made at a constant pump flow of 100 ml/kg/min, a systemic blood pressure ranging between 50 and 60 mm Hg, a heart rate of 120/min with the heart unloaded and thus not ejecting. There was no difference between groups regarding the applied dosages of neosynephrine to stabilize blood pressure. Furthermore, hemoglobin levels on and after termination of CPB were not significantly different between groups.

Due to hemodilution on CPB, arterial hemoglobin dropped from 8 to 6 g/dl and global DO_2 decreased by half but remained at this level throughout reperfusion without a significant difference between groups (data not shown). MDO_2 increased to twice its baseline level immediately after declamping in parallel with a rising CBF (fig. 1, 2). CBF in the S-NO-HSA group appeared to be above those recorded in controls during the first 5 min of reperfusion and mDO_2 was significantly higher at 5 min of reperfusion in the S-NO-HSA group ($p \leq 0.05$). MDO_2 decreased thereafter to reach its lowest level at 15 min ($p \leq 0.05$). Towards the end of reperfusion on CPB it climbed again and was higher in the S-NO-HSA group ($p \leq 0.05$). mVO_2 was neither affected by hemodilution nor by the great oxygen debt at declamping. Furthermore, it did not change in the S-NO-HSA group at 5 min of reperfusion despite a higher mDO_2. However, at 20 and 30 min of controlled reperfusion, we observed higher values for mVO_2 in the S-NO-HSA group ($p \leq 0.05$) and also a significant difference between groups in mDO_2 at 30 min after ischemia ($p \leq 0.05$) (fig. 1).

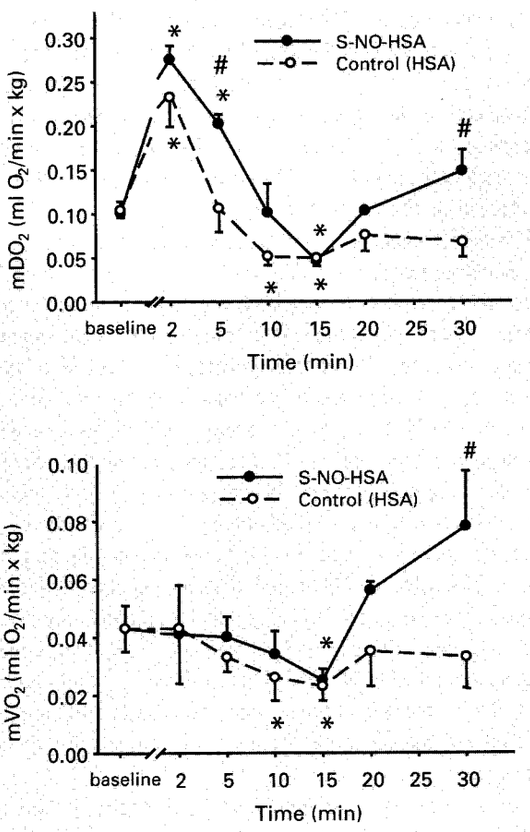

Fig. 1. Myocardial oxygen delivery (mDO_2) and myocardial oxygen consumption (mVO_2) at baseline and during controlled reperfusion at 2, 5, 10, 15, 20, and 30 min on cardiopulmonary bypass at a constant flow rate, blood pressure and heart rate with the hearts unloaded. S-NO-HSA = S-NO-HSA group; control = control group receiving human serum albumin (HSA). * $p < 0.05$ (vs. baseline), # $p < 0.05$ (S-NO-HSA vs. control group).

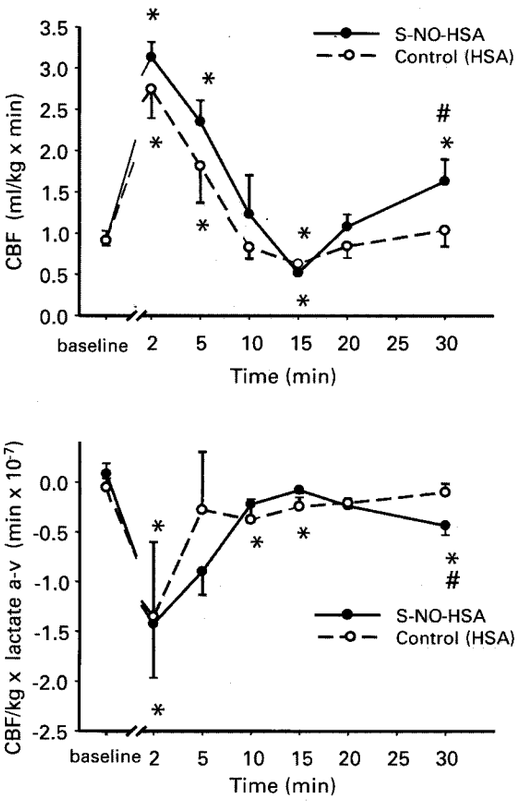

Fig. 2. Time course of coronary blood flow (CBF) and myocardial lactate production (CBF × a-v lactate) during controlled reperfusion after 15 min of severe ischemia of the heart. Measurements were made at baseline, before onset of ischemia, and at 2, 5, 10, 15, 20, and 30 min of subsequent reperfusion on cardiopulmonary bypass. S-NO-HSA = S-NO-HSA group; control = control group receiving human serum albumin (HSA). * $p < 0.05$ (vs. baseline), # $p < 0.05$ (S-NO-HSA vs. control group).

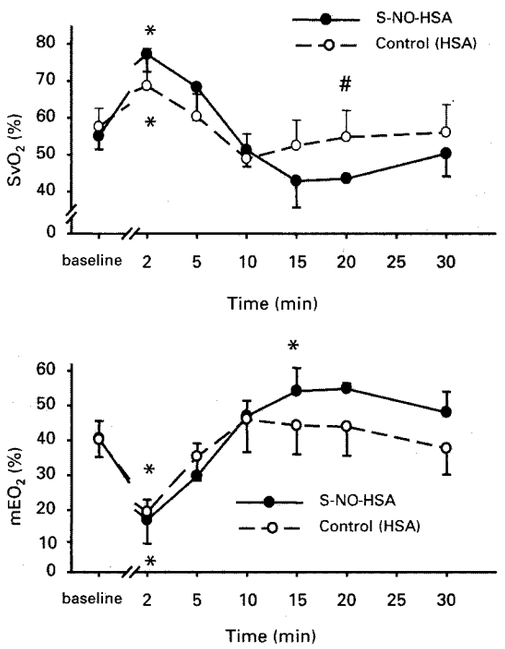

Fig. 3. Changes in coronary sinus oxygen saturation (SvO_2) and myocardial oxygen extraction (mEO_2) throughout the 30-min reperfusion phase on cardiopulmonary bypass. Parameters were determined at baseline and at 2, 5, 10, 15, 20, and 30 min of meticulously well-controlled myocardial reperfusion after 15 min of warm, global myocardial ischemia. S-NO-HSA = S-NO-HSA group; control = control group receiving human serum albumin (HSA). * $p < 0.05$ (vs. baseline), # $p < 0.05$ (S-NO-HSA vs. control group).

Despite a higher mDO_2 at 2 min of reperfusion, mEO_2 dropped significantly in both groups ($p \leq 0.05$) (fig. 3). This decrease in mEO_2 immediately after the start of reperfusion also coincided with a decrease of the arteriovenous oxygen content difference ($CaO_2 - CvO_2$) and an increase of SvO_2, in spite of constant SaO_2, CaO_2, and paO_2 ($p \leq 0.05$) (fig. 3). After 5 min, mEO_2 recovered again and, like the arteriovenous oxygen content difference, was nominally higher in the S-NO-HSA group between 15 and 30 min of reperfusion, which however did not reach statistical significance (fig. 3). SvO_2 at 20 min of reperfusion was significantly lower in the S-NO-HSA group ($p \leq 0.05$).

During reperfusion, arterial and venous lactate climbed above baseline in both groups ($p \leq 0.05$). Arterial lactate increased from 1 to 3 mg/ml immediately after opening the aortic cross-clamp and slowly decreased towards 2.5 mg/ml at the end of reperfusion in both groups. Myocardial lactate production increased at the beginning of reperfusion without any difference between groups but returned to almost baseline levels later on. It was however higher at the end of reperfusion on CPB in the S-NO-HSA group ($p \leq 0.05$) (fig. 2).

Hemodynamic parameters determined during the 30 min following termination of CPB are given in table 1. Despite similar right and left ventricular filling pressures, LVSP and MAP were significantly higher in the S-NO-HSA group ($p < 0.05$). Mean PAP also increased in both groups, however, without significant group difference. CO was significantly greater ($p < 0.05$) and CBF nominal-

Table 1. Temporal changes of hemodynamic parameters in NO-treated pigs and controls after separation from CPB

	Baseline	15 min after CPB	30 min after CPB
HR, 1/min			
S-NO-HSA	96±5	151±8*	137±5*
Control	96±4	146±7*	153±9*
MAP, mm Hg			
S-NO-HSA	74±5	74±11#	71±10
Control	77±4	52±7	56±4
mPAP, mm Hg			
S-NO-HSA	19±1	25±2*	27±3*
Control	19±1	24±2*	24±1*
LVSP, mm Hg			
S-NO-HSA	87±5	122±11*	123±7*,#
Control	88±4	112±6*	101±5*
LVEDP, mm Hg			
S-NO-HSA	10±1	9±1	10±1
Control	9±1	8±1	9±2
CVP, mm Hg			
S-NO-HSA	7±1	10±1*	10±1*
Control	8±2	10±1*	11±2*
CO, ml/kg·min			
S-NO-HSA	139±30	186±30*	173±10*,#
Control	107±10	153±20*	150±10*
CBF, ml/kg·min			
S-NO-HAS	0.94±0.16	2.28±0.30*	2.19±0.45*
Control	0.87±0.08	1.87±0.25*	1.67±0.21*

HR = Heart rate, MAP = mean arterial pressure, mPAP = mean pulmonary artery pressure, LVSP = left ventricular systolic pressure, LVEDP = left ventricular end-diastolic pressure, CVP = central venous pressure, CO = cardiac output, CBF = coronary blood flow, CPB = cardiopulmonary bypass.

* $p < 0.05$ vs. baseline; # $p < 0.05$ S-NO-HSA vs. control.

ly higher in animals treated with S-NO-HSA, although the difference in CBF did not reach statistical significance. Heart rate had already increased to 120 min^{-1} in both groups during controlled reperfusion due to DDD pacing. Inotropic stimulation after weaning from CPB further increased it to values greater than the set pacing frequency. Since heart rate after CPB was equal or less and MAP higher in the treatment group as compared to controls, stroke volume and stroke work index were higher in the S-NO-HSA group. As blood from the heart-lung machine had been retransfused prior to termination of CPB, hemoglobin levels almost reached baseline levels after weaning from CPB without any group difference over time. Furthermore, there was no group difference in pO_2 and SaO_2 during the 30 min following reperfusion on CPB. Therefore, mDO_2 after separation from CPB was only determined by CO and CBF, which were both higher in the S-NO-HSA group.

Discussion

Global cardiac ischemia is a common clinical situation but only during cardiac surgery and organ harvesting it is usually more or less effectively mitigated by various means of myocardial preservation. The setting applied here allowed us to study myocardial metabolic phenomena during early reperfusion on CPB after severe global normothermic myocardial ischemia under a meticulously well-controlled systemic blood flow and blood pressure and under load-independent conditions with the hearts beating at a constant heart rate. We thus avoided potential flaws that could have affected both myocardial metabolism and CBF. Additionally, systemic and pulmonary pressures and myocardial pump function was evaluated 15 and 30 min after weaning from CPB at similar filling pressures and inotropic support, to assess immediate postischemic contractile function.

The concentration of endogenously released NO in the coronary circulation under unstimulated conditions is in the nanomolar range. Therapeutic concentrations of NO donors are reported to be around 500 nmol/l and those after cytokine stimulation even in the micromolar range [9]. During the initial phase of reperfusion after prolonged ischemia NO levels drop below 1 nmol/l. However, concentrations necessary for preserved vasorelaxation are reported to be approximately 50 nmol/l [10]. The systemic administration of 0.1 μmol/kg/h S-NO-HSA prior to reperfusion in hindlimb ischemia in rabbits resulted in a 100 ± 15-nmol/l NO concentration measured in the wall of the femoral artery during reperfusion, which was double the baseline level [8]. This dose of the novel NO donor S-NO-HSA was also applied in the present study. It has previously been shown to preserve eNOS function in situations of intracellular calcium overload and reduces the formation of toxic oxygen radicals [8].

Despite a decrease of global DO_2, mDO_2 increased via augmentation of CBF during the first 5 min of controlled reperfusion when CO was merely determined by CPB flow. Myocardial hyperemia during the first 5 min after opening the aortic clamp could be augmented in the presence of S-NO-HSA that translated into a greater mDO_2. However, in spite of an enormous oxygen debt, the increased mDO_2 was accompanied by depressed myocardial oxygen utilization in both groups evidenced as a

decrease of mEO_2 and a concomitantly higher SvO_2. Similar results were reported by Pietersen et al. [11] and Smolenski et al. [12] after declamping of the aorta following CABG surgery and after reperfusion of the transplanted heart. Apparently, the myocardium is not able to metabolize the available oxygen and mVO_2 does not respond accordingly. This mechanism is obviously not amenable by NO supplementation, which may either be due to increased shunting or the inability of the myocardium to take up the resupplied oxygen. Therefore, early reperfusion metabolic derangements and mVO_2 can either not be altered by this regime or may not be under the sole control of NO. This would also account for the observation that there was no apparent difference in lactate production between groups in the early phase of reperfusion.

In addition, the unfavorable decrease of CBF that was obvious after the initial rise could not be prevented by S-NO-HSA as well. The decrease of mDO_2 from 10 to 20 min of reperfusion was once more related to a simultaneously lowered CBF. This is also the time range when maximum quenching of NO by oxygen radicals occurs [1]. Even despite enhanced NO generation, concomitant generation of O_2^- leads to inactivation of bioactive NO that is accompanied by endothelial dysfunction [13].

The differences between groups during the second half of reperfusion suggest that S-NO-HSA improves myocardial capillary perfusion and/or acts beneficially on cell metabolism. Energy supply to the cell in the form of ATP is closely related to oxygen uptake. During supply dependency of mVO_2, mEO_2 is at its upper limit. Increased mEO_2 in S-NO-HSA-treated animals 15 min after the start of reperfusion would support the assumption that the number of perfused capillaries was indeed higher and less myocardial shunting existed in the treatment group. Conversely, after blockade of NO synthase by intracoronary application of N^G-nitro-*L*-arginine methyl ester (*L*-NAME), a decrease of mVO_2 has been noted that was accompanied by a lower CBF and a higher SvO_2 in chronically instrumented dogs [14]. Augmented myocardial shunting can become predominant in determining mVO_2 after intracoronary application of *L*-NAME.

Continuous lactate washout in conjunction with supply dependency of mVO_2 indicates ongoing derangement of tissue oxygenation in both groups. This persisting imbalance emphasizes the severity of the ischemic insult and is consistent with observations after heart transplantation [12]. After severe ischemia of the heart, enhanced coronary reflow and higher mVO_2 have been associated with improved myocardial function, although a pronounced disproportionate imbalance exists between mVO_2 and contractile function following gross ischemic myocardial insults [15–17]. LVSP and MAP after weaning from CPB were significantly higher in S-NO-HSA-treated animals at similar end-diastolic pressures in this study. This finding, in combination with an increased CO at lower heart rates, suggests that myocardial performance, in spite of the severely compromised left ventricular function, was better preserved in the S-NO-HSA group. However, we cannot rule out a greater sensitivity of the myocardium to the applied inotrope and the vasopressor in S-NO-HSA-treated pigs.

Preliminary dose-finding studies in a group of pigs prior to these investigations revealed no hypotension when S-NO-HSA was applied at 0.1 μmol/kg/h as compared to 1 μmol/kg/h. This is important as during treatment with a number of NO donors, severe hypotension can become a serious problem that would also affect afterload. The beneficial effect of S-NO-HSA as it was applied here can be explained by two distinct mechanisms. First, it may have induced preconditioning [3, 4]. However, the window between application of the NO donor and potential protection seems to be too short in the present study. Secondly, and therefore more importantly, endothelial dysfunction was probably mitigated in the presence of resupplied NO resulting in a reduced no reflow phenomenon. It has previously been shown by our group that this can be mediated via product inhibition of eNOS by S-NO-HSA [8]. Subsequently, less O_2^- and sufficient NO generated by a protected eNOS can counteract a heterogeneous myocardial perfusion by increasing capillary density. This could augment CBF and result in a greater number of metabolically active cardiomyocytes being supplied with oxygen in the S-NO-HSA group. Heterogeneity of oxygen extraction in postischemic myocardium could indeed be linked to locally impaired blood flow by Stahl et al. [16]. An augmented and more homogeneous CBF would improve the supply/demand ratio for oxygen and metabolites with concomitant increased mVO_2 and mEO_2. As mVO_2 is tightly coupled with mitochondrial ATP production, a potentially greater energetic supply in the S-NO-HSA group resulted in the improved pump function in this group. Capillary density can be increased by endogenous NO produced by an intact eNOS either through depression of endothelin production, attenuation of leukocyte-endothelium interactions, or the additional scavenging of vasoconstricting oxygen radicals [5–7, 18, 19]. Application of S-NO-HSA before and throughout hindlimb ischemia in rabbits at the same concentration was able to reduce oxygen radical formation, preserve high-energy phosphates and prevented microvascular

constriction and edema formation [8]. Extended interstitial edema may additionally have compromised capillary perfusion in the control group.

In conclusion, this novel NO donor improved mDO_2 and thereby mVO_2 after severe global myocardial ischemia when mVO_2 is still supply dependent. However, early shunting or metabolic inhibition of the myocardium cannot be prevented. Enhanced washout of accumulated lactate, higher mVO_2 and the tendency towards a better oxygen utilization in S-NO-HSA-treated animals at the later stage of reperfusion may be indicative of an improved microvascular organ perfusion with earlier restoration of energy metabolism. This in turn translated in an improved contractile performance immediately after weaning from CPB.

Acknowledgments

This work was supported by the Austrian Science Fund (grant: FWF #P 15194) and by institutional funding from the Division of Cardiothoracic and Vascular Anesthesia and Intensive Care, University of Vienna, Austria.

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