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Exogenous intravascular nitric oxide enhances ventricular function after hemodilution with plasma expander

Surapong Chatpun and Pedro Cabrales

Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093, USA

Abstract

Aims—This study evaluated the hypothesis that exogenous nitric oxide (NO) supplementation during acute hemodilution with plasma expander (PE) provides beneficial effects on cardiac function.

Main methods—Acute hemodilution in golden Syrian hamsters was induced by a 40% of blood volume exchange with dextran 70 kDa. Intravascular NO supplementation after hemodilution was accomplished with a NO donor, diethylenetriamine NONOate (DETA NONOate). The test group was treated with DETA NONOate, while the control group received only vehicle. Left ventricular cardiac function was studied using pressure-volume measurements obtained with a miniaturized conductance catheter.

Key findings—Cardiac output increased to $122 \pm 5\%$ and $107 \pm 1\%$ of the baseline in the group treated with NO donor and the vehicle group, respectively. Stroke work per stroke volume (SW/SV) after hemodilution reduced to 90% of the baseline and the NO donor significantly reduced SW/SV compared to the vehicle. The minimum rate of pressure change ($dP/dt_{\min}$) was significantly lower in animals treated with the NO donor compared to vehicle treated animals. Systemic vascular resistance (SVR) decreased to $62 \pm 5\%$ of the baseline in the NO donor group, whereas the vehicle group SVR decreased to $83 \pm 5\%$ of the baseline. Using intravital microscopy analysis of microvessel in the dorsal skinfold window chamber, we established that the NO donor group induced significant vasodilation compared to the vehicle group.

Significance—NO supplementation in an acute hemodilution with PE has beneficial effects on cardiac performance. However, the NO supplementation effects with a NO donor are dose-independent and short-lasting.

Keywords

diethylenetriamine NONOate; cardiac function; vasodilation; hemodilution; microcirculation

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Corresponding author: Pedro Cabrales, Ph.D., University of California, San Diego, Department of Bioengineering, 0412, 9500 Gilman Dr., La Jolla, CA 92093-0412, Telephone: (858) 534-5847, pcabrales@ucsd.edu.

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Supplementary material

Supplementary material is available online.

Introduction

Microvascular studies using the hamster dorsal skinfold window chamber showed that an increase in plasma viscosity with high viscosity plasma expanders (HVPEs) increased wall shear stress and produced vasodilation compared to low viscosity plasma expanders (LVPEs) during an acute hemodilution (Cabrales and Tsai 2006, Tsai, et al. 2005, Cabrales, et al. 2006). This increase in plasma viscosity produced an increase in endogenous nitric oxide (NO) via vascular endothelial NO synthase, leading to an increased perivascular NO concentration and improved microvascular perfusion (Tsai, et al. 2005, de Wit, et al. 1997). Therefore, the improved microvascular function resulted by increased plasma viscosity after hemodilution is determined by the maintenance of NO production and availability. As the cardiovascular system is made up of microvascular subsystems arranged in parallel and series, it is expected that the beneficial effects observed in the skinfold microvascular tissue will produce an improvement in the entire circulatory system, thus affecting cardiac function. HVPE improved load-dependent cardiac function indices in combination with higher stroke work, compared to baseline and LVPE in an acute hemodilution model (Chatpun and Cabrales 2010). Therefore, increasing plasma viscosity enhances cardiac function and requires higher energy consumption, limiting the advantages of HVPE especially to situations where oxygen is a limiting factor.

The role of NO in the regulation of cardiac function and circulatory system has been remarkably studied in both normal and pathological conditions (Bolli, et al. 1998, Fellet, et al. 2003, Kojda, et al. 1997, Colley and Sivarajan 1984, Crystal, et al. 1988). The principal reported effect of NO on cardiac function describes bimodal effects on inotropy with positive inotropic effects at reduced NO availability, and negative inotropic effects at increased NO availability (Kojda, et al. 1997, Preckel, et al. 1997, Brady, et al. 1993). Since several studies have found no inotropic effects from NO availability manipulation by affecting endogenous production or administering exogenous NO in physiological conditions, the role of NO in pathophysiological conditions is even more complex and specific (Kojda, et al. 2001, Reading and Barclay 2002). Previously, cardiac function was studied using isolated heart preparation and isolated cardiomyocytes, but these experimental approaches did not truly represent operating conditions of the heart in its physiological environment. Several studies using an open-chest model have reported no statistically significant effects when using NO-releasing agents during isovolemic hemodilution on coronary vasodilation, although myocardial blood flow increased (Crystal, et al. 1988, Crystal, et al. 2008, Crystal and Salem 1991). Recently, real-time ventricular pressure volume measurements using closed-chest method have been introduced in small animals. In our studies we have implemented a miniaturized pressure-volume (PV) conductance catheter that provides the simultaneous measurements of ventricular pressure and volume in experimental small animal models, leading to a powerful approach to quantify cardiac function in physiological environment (Chatpun and Cabrales 2010, Shioura, et al. 2007, Pacher, et al. 2008, Pacher, et al. 2004).

The objective of this study was to evaluate the hypothesis that exogenous supplementation of NO positively enhances the effects of hemodilution with PE on cardiac function. Therefore, we hemodiluted our experimental model using 6% dextran 70 kDa to reach a systemic hematocrit of 28%, and NO was supplemented in the form of diethylenetriamine NONOate (DETA NONOate). DETA NONOate was selected based on its reliable decomposition rate (Maragos, et al. 1991). One set of animals was instrumented for left ventricular cardiac function using the indices derived from the PV measurements. A second set of animals was instrumented for vascular relaxation in terms of vascular diameter measurements. Both groups of animals underwent identical hemodilution and NO

supplementation and were analyzed at baseline, after hemodilution and after exogenous NO supplementation.

Materials and methods

Animal preparation for cardiac function measurement

Studies were performed in anesthetized male Golden Syrian hamsters (Charles River Laboratories, Wilmington, MA, USA) weighing 60 - 70 g. Animal handling and care followed the US National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals. The experimental protocol was approved by the University Animal Care Committee. Surgery was performed following intraperitoneal (i.p.) injection of sodium pentobarbital (50mg/kg). The left jugular vein was catheterized to allow fluid infusion and left femoral artery was cannulated for blood pressure monitoring, blood withdrawal, and sampling. Animals received a tracheotomy to facilitate spontaneous breathing using a polyethylene-90 tube. Animals were placed in the supine position on a heating pad and core body temperature was maintained at 37°C. Anesthesia state was evaluated every 20 min, and if the animals responded to a toe pinching, a small bolus of sodium pentobarbital (10-15mg/kg, i.p.) was administered.

Animal preparation for a dorsal skinfold window chamber and catheterization

The hamster dorsal skinfold window chamber model is widely used for microvascular studies in the unanesthetized state and the complete surgical technique for the preparation has previously been described in detail (Tsai, et al. 2005). Briefly, the animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) for dorsal hair removal and surgery to attach dorsal titanium frames. On one side of skin fold, tissues were removed following the outline of dorsal window frame until a thin layer of retractor muscle. Saline droplets were put on the exposed skin. Then a frame with cover glass was placed on the removed side held in place by the other frame of the chamber. The animal was put back in a cage for at least 2 days for recovery after window chamber implantation. A chamber was then visually checked under x650 magnification for any signs of edema or bleeding. Animals were administered with sodium pentobarbital before starting catheterization. Arterial and venous catheters (polyethylene-50 tube) were implanted in the carotid artery and jugular vein, respectively. Catheters were then tunneled under the skin and exteriorized at the dorsal side of the neck. After catheterization, animal was allowed at least 1 day for recovery before starting the experiment.

Inclusion criteria

Animals used for cardiac function were suitable for the experiments if 1) animals had no bleeding and 2) systemic parameters were within normal range, namely, mean blood arterial pressure (MAP) between 80-120 mmHg, heart rate (HR) above 320 beats/minute and systemic hematocrit (Hct) above 45%. Animals used for microvascular studies were included if 1) microscopic examination of the tissue in the chamber observed under a x650 magnification did not reveal signs of edema or bleeding; and 2) systemic parameters were within normal range, as described above.

Systemic parameters

MAP and HR were recorded continuously (MP 150, Biopac System, Santa Barbara, CA, USA). Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge, Clay Adams, NJ, USA).

Cardiac function measurement

Closed chest method was performed to assess cardiac function in this study (Pacher, et al. 2008). The right common carotid artery was exposed allowing a 1.4F PV conductance catheter (SPR-839, Millar Instruments, Houston, TX, USA) to be inserted. The PV conductance catheter was then advanced passing through the aortic valve into the left ventricle. At the baseline and the end of experiment, a bolus of 15% hypertonic saline (10 μ l) was intravenously infused to determine the parallel volume (Baan, et al. 1984). The pressure and volume signals were digitized and stored (MPVS300, Millar Instruments, Houston, TX, USA and PowerLab 8/30, ADInstruments, Colorado Springs, CO, USA). Cardiac function data analysis was performed offline with PVAN software (version3.6, Millar Instruments, Houston, TX, USA). The values of studied cardiac function indices were averaged from selected 8-12 cardiac cycles at each time point.

Estimation of left ventricular blood volume

Left ventricular blood volume was measured continuously in conductance units (RVU; relative volume unit) and converted to actual blood volume (μ l) at the end of the experiment (Chatpun and Cabrales 2010). The blood conductance at the baseline was estimated from baseline hematocrit, using the pooling data of blood conductance and hematocrit relationship.

Vascular diameter measurement

Hamsters instrumented with the dorsal skinfold window chamber were placed in a restraining tube with a longitudinal slit from which window chamber protruded and then were fixed to the microscopic stage of a transillumination intravital microscope (BX51WI; Olympus, New York, NY, USA). The tissue image was projected onto a charge-coupled device camera (COHU 4815, San Diego, CA, USA), connected to a videocassette recorder (AG-7355, JVC Tokyo, Japan) and viewed on a monitor. Measurements were carried out using a $\times 20$ water immersion objective (UMPlanFI 20 $\times$ /0.50, Olympus, New York, NY, USA). A video image-shearing method was used to measure the vessel diameter at the baseline and 5min after treatment with NO donor or vehicle (Intaglietta and Tompkins 1973).

Vascular resistance

Systemic vascular resistance (SVR) was calculated as the relationship between MAP and cardiac output (CO), $SVR = MAP/CO$. Microvascular resistance (MVR) defined the resistance to flow in the microcirculation and was calculated based on Poiseuille's equation that describes the pressure drop in a cylindrical pipe. MVR is determined by blood viscosity (μ) and microvascular geometry: length (L) and diameter (D). As the length of microvascular blood vessel remains unchanged, MVR was estimated to be proportional to the ratio of blood viscosity and diameter power fourth ($MVR \propto \mu/D^4$).

Nitric oxide (NO) donor

Diethylenetriamine NONOate (DETA NONOate; $C_4H_{13}N_5O_2$, Cayman Chemical, Ann Arbor, MI, USA) was used as NO donor. DETA NONOate liberates 2 moles of NO per mole of parent compound and has a nominal half-life of 20 hrs in physiological buffer at 37°C (Keefer, et al. 1996). DETA NONOate was diluted in normal saline solution with 10 mM NaOH before each infusion. DETA NONOate dose response was completed at three different concentrations (25, 10 and 1 mM), the NO donor was administered at 20 min after hemodilution.

Isovolemic hemodilution protocol

Acute anemia was induced by one isovolemic hemodilution step. Briefly, the volume of each exchange-transfusion was calculated as a percentage of the blood volume (BV), estimated as 7% of body weight. Hamsters were exchanged 40% of estimated BV using 6% dextran 70 kDa (Dex70; Pharmacia, Stockholm, Sweden), viscosity 3.0 cP. The systemic Hct was lowered by 45%. Dex70 was administered into the left jugular vein catheter at a rate of 0.1 ml/min with simultaneous blood withdrawal at the same rate from the left femoral artery catheter by using a dual syringe pump (33 syringe pump, Harvard Apparatus, Holliston, MA, USA). At 20 min after hemodilution, the NO donor or the vehicle (normal saline solution with 10 mM NaOH) was administered (0.1 ml, i.v.). Animals were followed over 30 min after NO donor or vehicle administration. Systemic parameters (MAP and Hct) were recorded and analyzed at baseline and after hemodilution. Blood samples were collected at the end of experiment for blood conductance calibration. Fig. 1 illustrates the experimental protocol.

Experimental groups

After the initial hemodilution with Dex70, animals were randomly divided into two experimental groups, labeled: NO donor, 0.1 ml of 10 mM solution of DETA NONOate; and Vehicle, 0.1 ml of saline solution with 10 mM NaOH.

Statistical analysis

Results are presented as mean $\pm$ standard deviation. The Grubbs' method was used to assess closeness for all measured parameters values at baseline. Data between interested time points in a same group were analyzed using analysis of variance for nonparametric measures (ANOVA, Kruskal-Wallis test) and followed by post-hoc analysis with the Dunnett's multiple comparison tests. A two-way ANOVA followed by Bonferroni post test was performed to compare data between groups at time points of interest. Additionally, an unpaired t-test was performed to compare the vascular diameters and MVR between groups. A ratio of 1.0 means no change from baseline while lower and higher ratios are indicative of changes proportionally lower and higher than baseline (i.e., 1.5 would mean a 50% increase from the baseline level). All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Changes were considered statistically significant if $P < 0.05$.

Results

DETA NONOate dose response

The administration of 0.1 ml of 25 mM of DETA NONOate produced lethal hypotension (from 85 mmHg to 40 mmHg within 2 min after NO donor infusion). Infusion of 0.1 ml of 10 mM of DETA NONOate significantly decreased MAP to $73 \pm 8\%$ of the baseline, and then MAP increased toward baseline level within 10 min after infusion. On the other hand, infusion of 0.1 ml of 1 mM of DETA NONOate did not cause detectable changes in MAP. Based on these results, we selected 0.1 ml of 10 mM DETA NONOate for our experiment after hemodilution, and discarded using lower or higher dosages.

Group size and homogeneity

Twenty animals entered the study. All animals tolerated the isovolemic hemodilution protocol and NO donor supplementation without visible signs of discomfort. The animals were randomly assigned to the following experimental groups: NO donor ($n=10$; 67 ± 3 g); and Vehicle ($n=10$; 68 ± 2 g). Seven animals in each group were used for cardiac function study and the additional three animals were used to study blood vessel diameter and

microvascular resistance in the dorsal skinfold window chamber. All animals included in the study passed the Grubbs' test ensuring that all the measured parameter values at baseline were within a similar population ($P < 0.05$). Similarities between groups at baseline and after hemodilution were statistically verified among groups ($P > 0.30$).

Systemic parameters

Hct significantly decreased after hemodilution; in the NO donor group from $52 \pm 3\%$ to $27 \pm 3\%$ and in the vehicle group from $51 \pm 2\%$ to 29 ± 2 . MAP and HR during the experimental study are presented in Fig. 2. After hemodilution, there was no difference in MAP between groups. MAP instantly and dramatically decreased after NO donor infusion compared to vehicle treated animals and compared to baseline, indicating the hypotensive effect of NO donor. MAP in the vehicle group did not significantly change compared to baseline except only at 15 and 30 min after vehicle infusion. HR was unchanged after hemodilution compared to baseline. Infusion of NO donor slightly increased HR by 4% while infusion of vehicle did not affect HR compared to baseline and hemodilution, implying a chronotropic effect of NO donor. Furthermore, HR was significantly higher in the NO donor group compared to the vehicle group.

Left ventricular function assessment

Cardiac output (CO) increased after hemodilution to compensate for a decrease in oxygen carrying capacity (Fig. 3A). Infusion of the NO donor instantly increased CO compared to baseline and lasted for 15 min, indicating a reduction of afterload. Despite higher CO in the NO donor group, there was no statistically significant difference between groups. Stroke volume (SV) after hemodilution was not different, and no significant difference was measured between the NO donor, and vehicle groups (Fig. 3B). Stroke work (SW) related to pressure-volume loop area, after hemodilution, was not different compared to baseline, and infusion of either the NO donor or the vehicle did not affect SW. However, the ratio of SW to SV (SW/SV) after hemodilution decreased significantly compared to baseline (Fig. 4A). Infusion of NO donor further decreased SW/SV compared to baseline and hemodilution. The NO donor induced significant changes in SW/SV compared to the vehicle during 5 min after treatment. The relationships between SW and CO for animals treated with NO donor or vehicle are plotted in Fig. 4B. CO linearly correlated with SW, the correlation coefficients were $r = 0.950$ for the NO donor group and $r = 0.956$ for the vehicle group. The plot indicates that animals administered with NO donor required SW less than animals treated with vehicle at the same CO. End-systolic pressure (P_{es}) after hemodilution was not different compared to baseline. However, administration of NO donor drastically decreased P_{es} compared to baseline and to animals treated with the vehicle (Fig. 4C). It was clearly seen that the decrease of P_{es} was parallel to a reduction in MAP. No significant changes in end-diastolic pressure (P_{ed}) were measured after hemodilution or after NO donor infusion (see supplementary material online, Fig. S1A). The relationship between P_{es} and end-systolic volume (V_{es}) for animals treated with the NO donor or the vehicle is plotted in Fig. 4D, the slope of this relationship represents the end-systolic elastance (E_{es}) for both groups. The E_{es} for animals treated with the NO donor was higher compared to the animals treated with the vehicle. The maximum rate of pressure change (dp/dt_{max}) did not change after hemodilution compared to baseline and was not affected by infusion of NO donor (see supplementary material online, Fig. S1B). The minimum rate of pressure change (dp/dt_{min}) did not remarkably change after hemodilution compared to baseline. However, infusion of NO donor significantly decreased dp/dt_{min} compared to baseline and hemodilution (Fig. 5A). dp/dt_{min} was lower for animals treated with the NO donor compared to the vehicle, indicating a negative effect on a cardiac relaxation phase. The significant decrease in dp/dt_{min} was also evident in the relaxation phase of pressure-volume loops, typical responses

observed in the experiment after the administration of NO donor and vehicle (1 min after the end of infusion) in Fig. 5B and 5C, respectively.

Vascular resistance analysis

Systemic vascular resistance (SVR) was not remarkably different after hemodilution compared to baseline. Infusion of NO donor significantly decreased SVR compared to baseline and hemodilution (Fig. 6). The NO donor infusion decreased SVR compared to the group treated with the vehicle, and resulted in decreased MAP and increased CO. The reduction in SVR by infusion of the NO donor was the result of peripheral vasodilation. Direct measurements of both arterioles and venules showed significant vasodilation in animals treated with NO donor compared to the vehicle (Fig. 7A). In our previous study, baseline blood viscosity (52% Hct) was 4.71 ± 0.62 cP and blood viscosity after hemodilution with dextran 70kDa (28% Hct) was 3.09 ± 0.09 cP (Chatpun and Cabrales 2010). These values were used to calculate MVR, thus it magnified the changes in blood vessel diameters. Arteriolar MVR was significantly lower for the group that received the NO donor compared to vehicle treated group, whereas venular MVR was not different between groups (Fig. 7B).

Discussion

The principal finding of this study was that intravascular supplementation of NO in the form of DETA NONOate positively enhanced the effects of hemodilution with a conventional LVPE (dextran 70 kDa) on cardiac function by increasing CO, SV and E_{es} , and lowering energy needed per unit of volume ejected (SW/SV) as well as SVR. MAP, P_{es} , and SVR after hemodilution combined with NO donor supplementation significant decreased, and these changes occurred in parallel to a reduction in MVR as revealed by the microvascular observation. The effects induced by the NO donor were not long lasting. It is worth noting that inhaled NO, which is increasingly used to treat pulmonary hypertension, shows similar effects in cardiac function (Noponen, et al. 2009, De Cruz, et al. 2009). In spite of the well-known vasodilator effect of NO donors, the activation of guanylyl cyclase leads the signaling pathway that affects the activation of various proteins involved in excitation contraction coupling, including the L-type Ca^{2+} channel (Hare, et al. 2008). Our data supports the important role of NO on cardiac function including chronotropic, mechano-energetic and vasodilatory, and presents the complex and diverse NO-mediated cardiac function regulation. Cardiac and microvascular benefits of NO supplementation during anemia have been observed in different tissues, including during anemia induced by plasmodium berghei ANKA. In previous studies, we showed that NONOate treatment increased cerebral blood flow of the in the pial microcirculation and prevented the occurrence of cerebral malaria (Cabrales, et al. 2011).

Intravascular supplementation of NO affects HR and myocardial contractility, due to NO released into the coronary circulation, directly affecting cardiomyocytes and endothelial cells (Hogan, et al. 1999, Muller-Strahl, et al. 2000). Generally, NO supplementation exhibits positive chronotropic effects, however Choate and Paterson found that exogenous NO decreased baroreceptor activity and inhibited chronotropic responses (Choate and Paterson 1999). In our study, HR transiently increased over a short period of time after NO donor administration. Similarly, Hogan *et al.* showed that NO donor increased HR in an anesthetized rabbit, an effect that was independent of the autonomic nervous system, as a result of a stimulation of the hyperpolarization-activated inward pacemaker current (Hogan, et al. 1999). NO rapidly binds to deoxyhemoglobin becoming inactivated and oxyhemoglobin promotes the oxidation of NO to nitrate and leads to formation of methemoglobin, in both situation NO reactions with the hemoglobin prohibits oxygen transport. In the present study, the stoichiometric amount of NO released from 0.1 mL of 10

mM of DETA NONOate correspond to 2.5% of the hemoglobin molecule in the blood of the animal. Experimentally, the IL482 CO-Oximeter System (Instrumentation Laboratory, Lexington, MA) and cyanomethemoglobin method (Sakata, et al. 1982) confirmed that less than 3% of the Hb of the animals treated with 0.1 mL of 10 mM of DETA NONOate was transformed to methemoglobin. Therefore, the dose of DETA NONOate used did not affect the oxygen carrying capacity.

The increase in CO observed in the NO donor group was the result of increased HR and SV. Although SV increased, SW almost remained unchanged after NO donor infusion, indicating NO enhanced blood affecting performance without increasing oxygen requirements. A study in an anesthetized open-chest canine model showed that sodium nitroprusside infusion increased myocardial blood flow while myocardial oxygen uptake decreased in order to maintain metabolic activity during hemodilution (Crystal and Salem 1991). Furthermore, the relationship between SW and CO showed the beneficial effect of NO on cardiac function, as the animals treated with NO donor showed an overall higher CO with similar stroke work done by the heart, compared to animals treated with vehicle. Similarly, the ratio of SW to SV in the NO donor group was lower compared to the vehicle group, which implies that less energy was used to eject the same amount of blood.

Despite an increased HR in the present study, the load-dependent systolic function index, dP/dt_{max} , was maintained, whereas P_{es} was lower relative to baseline after NO donor infusion, indicating that NO might not alter cardiac contractility. However, we found that E_{es} represented by the slope of the relationship between P_{es} and V_{es} was slightly higher in the group administered with NO donor, suggesting that NO had a positive inotropic effects. Some studies have reported that a positive inotropy caused by NO might be partially dependent on cGMP, which, when produced at low concentration, triggers cAMP production to stimulate protein kinase A, leading to the opening of sarcolemmal voltage-operated and sarcoplasmic ryanodine receptors (Ry/R) Ca^{2+} channels (Vila-Petroff, et al. 1999, Kojda, et al. 1996, Sarkar, et al. 2000). NO itself can also open Ry/R, L-type Ca^{2+} channels and the calcium release complex by S-nitrosylation or oxidation (Xu, et al. 1998). All hypotheses are worth exploring, in order to understand the complex mechanistic role of NO in cardiac failure and function.

In addition to the inotropic effect, NO also affects the myocardial relaxation. Our results showed that NO significantly affected lusitropy as evidenced by a prominent decrease of dP/dt_{min} and an early onset of relaxation phase, shown in the PV loop shapes, in the group administered with NO donor. Furthermore, the PV loop after NO donor administration was shifted to the right relative to the PV loop at baseline, indicating an increase on left ventricular diastolic distensibility or preload. The increase of myocardial distensibility by NO effect probably relates to the phosphorylation of troponin-I and the reduction of calcium-independent diastolic cross-bridge cycling. Despite increased preload due to NO donor, hemodilution causes an increase in CO and end-diastolic volume to maintain tissue oxygen metabolic needs, as oxygen carrying capacity is reduced. Pinsky et al. reported in their study in the beating rabbit heart, that an increase in LV preload enhanced the myocardial NO production (Pinsky, et al. 1997). Therefore, in our study, not only the NO was released from NO donor but also the myocardial NO may be produced, due to an increased LV preload by hemodilution. In general, the myocardial oxygen consumption per beat is determined by the ventricular pressure volume area (PVA), which represents the total mechanical energy generated during ventricular contraction. Previous studies have shown that the left ventricle PVA is linearly related to myocardial oxygen consumption per beat (Suga 1979). Therefore, in our study DETA NONOate decreased myocardial oxygen consumption by 15% compared to the vehicle, which can be explained by NO effects on

mitochondrial metabolism through a very potent reversible alteration in the activity of cytochrome c oxidase (Cleeter, et al. 1994).

Other evidence showing the beneficial effect of NO was the reduction in SVR. As the heart ejects against lower vascular resistance, it requires less energy to overcome the afterload. Therefore, during hemodilution, NO supplementation allows the heart to operate at a lower ratio between energy required, and energy needed to accomplish its function. Furthermore, our microvascular observation in the dorsal skinfold window chamber model showed that the reduction in SVR after NO donor infusion was a result of vasodilation. In contrast, Crystal et al. suggested that endothelial NO did not influence the coronary vasodilation caused by hemodilution, as well as no alternation in a vasodilatory effect of exogenous NO when performing hemodilution (Crystal, et al. 2008). The mechanisms whereby NO supplementation increased HR and CO are not ascertained in the present study. Additionally, data from other anesthetized animals models have suggested that at lower doses, similar to those used in our investigation, exogenous NO supplementation can influence the heart by directly affecting the sino-aortic node and/or direct coronary vasodilation with an associated increase of contractility (Girard, et al. 1996, Bohn, et al. 1995). Furthermore, since sino-aortic baroreceptor denervation abolishes NO-induced bradycardia in dogs, it has been suggested that the central effects of NO are modulated by an afferent baroreceptor activity (Ma, et al. 2005). Using blood viscosity and vascular diameter information, our study demonstrated that the decrease in SVR after the administration of NO donor was in parallel with the decrease in MVR, especially in arterioles, leading to lower MAP and P_{es} .

Although our study has shown the positive effects of the administration of NO donor in hamsters hemodiluted with LVPE, several studies elucidated that NO has bimodal effects; cardioprotective and cardiodepressive effects depending on the amount of NO (Kojda, et al. 1997, Preckel, et al. 1997, Brady, et al. 1993). As demonstrated in our study, a high dose of DETA NONOate (2.5 μ moles/animal) resulted in animals' death, whereas a low dose of DETA NONOate (0.1 μ moles/animal) did not have any effects on MAP. The dose selected for the study 1 μ moles/animal of DETA NONOate had significant effects on vascular resistance and cardiac function. Unfortunately, the exact dose of NO required for each specific situation is difficult to determine, since the effect of NO is influenced by the scavenging of NO by hemoglobin in the red blood cells and myoglobin concentration in cardiomyocyte (Muller-Strahl, et al. 2000, Massion and Balligand 2003). In our study, the effects of NO were clearly observed during 10 - 15 min after the administration of NO donor as similar to the previous finding with pirsidomine by Wainwright and Martorana (Wainwright and Martorana 1993). These studies suggest that the effects of NO are dose-dependent and not long lasting in vivo, which in part could limit chronic NO donor therapeutic application, however DETA NONOate has important acute therapeutic potential to enhance cardiac function and microvascular perfusion during anemia.

Conclusions

Our findings demonstrate the positive and enhanced effects of NO supplementation on cardiac function by an increase in cardiac output, a reduction of vascular resistance and a lowering in stroke work per volume ejected, in an acute hemodilution with low viscosity plasma expander. This study suggests that the beneficial effects on cardiac function during an acute hemodilution can be obtained by NO supplementation without an increase of plasma viscosity using a high viscosity plasma expander. Furthermore, these results suggest that using NO donor has a therapeutic potential for the heart's performance in an early stage of an acute hemodilution.

The existence of positive regulatory functions mediated by NO suggests that modulation of these effects could have broader salutary effects. However, the effects of NO donors on cardiac function depend on the donor specie, dosage, release rate, pathological conditions, endogenous NO level, and sensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Schematic diagram of acute isovolemic hemodilution protocol. A bolus of NO donor (100 μ l i.v.) was infused at 20 min after hemodilution. BL, baseline.

Fig. 2.
 (A) Mean arterial pressure (MAP) and (B) Heart rate (HR) measured at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are presented as means $\pm$ SD. $\dagger$ $P < 0.05$ compared to BL. $\ddagger$ $P < 0.05$ compared to HD. $\S$ $P < 0.05$ between groups.

Fig. 3.

Left ventricular function indices derived by PV conductance catheter. (A) Cardiac output (CO) and (B) Stroke volume (SV) at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are relative to baseline and presented as means $\pm$ SD. † $P < 0.05$ compared to BL.

Fig. 4.

(A) Stoke work to stroke volume ratio (SW/SV) at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are relative to baseline and presented as means $\pm$ SD. † $P < 0.05$ compared to BL. ‡ $P < 0.05$ compared to HD. § $P < 0.05$ between groups. (B) Relationship between Cardiac output (CO) and stroke work (SW) during 30 min after treatment. Solid line is a linear regression for each experimental group (Vehicle: $y = 0.037x - 0.775$, $r = 0.956$; NO donor: $y = 0.038x + 1.301$, $r = 0.950$). (C) End-systolic pressure (P_{es}) at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are relative to baseline and presented as means $\pm$ SD. † $P < 0.05$ compared to BL. § $P < 0.05$ between groups. (D) Relationship between end-systolic pressure (P_{es}) and end-systolic volume (V_{es}) during 30 min after treatment. Solid lines are a linear regression for each experimental group (Vehicle: $y = 3.34x + 77.192$, $r = 0.898$; NO donor: $y = 7.706x + 40.205$, $r = 0.962$). Inset: overall slope values as mean $\pm$ SD of 7 experiments in each group

Fig. 5. Minimum rate of pressure change (dP/dt_{min}) at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are relative to baseline and presented as means $\pm$ SD. † $P < 0.05$ compared to BL. ‡ $P < 0.05$ compared to HD. § $P < 0.05$ between groups. Pressure-volume loops at the baseline and 1 min after treatment in (B) NO donor group and (C) Vehicle group. A thick line represents PV loop at the baseline and a thin dash line represents PV loop at 1 min after treatment.

Fig. 6.

Systemic vascular resistance (SVR) at baseline (BL), at 15 minutes after hemodilution (HD) and at 1, 2, 3, 4, 5, 10, 15 and 30 minutes after treatment. Values are relative to baseline and presented as means $\pm$ SD. [†] $P < 0.05$ compared to BL. [‡] $P < 0.05$ compared to HD. [§] $P < 0.05$ between groups.

Fig. 7. (A) Arteriolar and venular diameters relative to hemodilution. (B) Microvascular resistance (MVR) during 5 min after treatment relative to baseline. Values are relative values and presented as means $\pm$ SD.