

Inhaled nitric oxide effects outside the lungs – experimental and clinical evidence

Tlenek azotu stosowany wziewnie – eksperymentalne i kliniczne dowody działania poza łożyskiem płucnym



Waldemar Goździk¹, Anna Goździk²

¹Klinika Anestezjologii i Intensywnej Terapii Uniwersytetu Medycznego we Wrocławiu

²Klinika Chirurgii Serca Uniwersytetu Medycznego we Wrocławiu

Kardiochirurgia i Torakochirurgia Polska 2012; 9 (4): 456–462

Abstract

New evidence indicates that nitric oxide inhalation leads to formation of new compounds which may be carried as thiol groups attached to protein in blood or act indirectly through nitrite and nitrate, metabolites which have been shown to elevate over time during exposure to inhaled NO. Additionally it has been shown that inhaled nitric oxide has no hemodynamic effects on normally perfused tissue, but increases blood flow selectively in ischemic tissue. It has a simple route of administration, safety profile and immediate action. Because of these properties, inhaled nitric oxide may serve as a rescue therapy for ischemic conditions in which collateral blood flow is important or until interventional or spontaneous reperfusion occurs. This review focuses on inhaled nitric oxide effects outside the lungs, and discusses its experimental and clinical applications, with particular attention to potential systemic effects of the gas.

Key words: nitric oxide, ischemia, reperfusion injury, vasodilatation.

Introduction

This review continues a discussion on extrapulmonary effects of inhaled nitric oxide (iNO). In the first part, presented earlier in this journal, we described a number of mechanisms in which nitric oxide can be kept and stored in the systemic circulation and mechanisms of its conversion to the active nitric oxide gas. Here we present experimental

Streszczenie

Doniesienia ostatnich lat wskazują, że wziewne stosowanie tlenku azotu prowadzi do powstawania szeregu aktywnych związków chemicznych, głównie z grupy S-nitrozotioili, oraz nitrozylacji grup sulfhydrylowych różnych białek. Aktywność tlenku azotu może być również uzyskiwana pośrednio poprzez azotyny i azotany, produkty jego metabolizmu. Wszystkie te związki mogą przenosić aktywność biologiczną tlenku azotu do krążenia systemowego i działać jako bardziej stabilne źródło jego magazynowania. Dodatkowo wykazano, że wziewny tlenek azotu selektywnie zwiększa przepływ krwi w tkankach niedokrwionych, jednocześnie nie wykazując działań hemodynamicznych w tkankach perfundowanych prawidłowo. Wydaje się, że leczenie wziewnym tlenkiem azotu, ze względu na prosty sposób podaży oraz natychmiastowe działanie, może być przydatne jako terapia ratunkowa w różnych stanach niedokrwienia, w których istotna jest poprawa przepływu naczyniowego do czasu wystąpienia naturalnej lub interwencyjnej perfuzji. W pracy przedstawiono szereg badań eksperymentalnych i klinicznych wskazujących na efekty działań systemowych wziewnego tlenku azotu, znacznie wykraczających poza łożysko płucne.

Słowa kluczowe: tlenek azotu, niedokrwienie, reperfuzja, wazodylatacja.

and clinical evidence of systemic action of iNO in various organs distant from the lungs and circumstances where NO inhalation can be considered as a rescue therapy for ischemic conditions in which collateral blood flow is important or until interventional or spontaneous reperfusion occurs. Additionally the rationale for iNO use in sepsis is presented.

Address for correspondence: dr n. med. Waldemar Goździk, I Klinika Anestezjologii i Intensywnej Terapii, Uniwersytet Medyczny we Wrocławiu, ul. Borowska 213, 50-368 Wrocław, Poland, tel. +48 604 601 212; e-mail: waldemar.gozdzik@gmail.com

Myocardial ischemia-reperfusion injury

Important questions concerning use of iNO for the modulation of ischemia-reperfusion injury (I/R injury) are implementation time, optimal dosage, and duration of the treatment. The most interesting observations elucidating the dosage and the mechanisms of iNO mediated protection come from experimental studies on myocardial I/R injury. Hataishi and colleagues examined the ability of iNO to decrease myocardial infarction (MI) size in mice subjected to 30, 60, and 120 min of left anterior descending coronary artery ligation followed by reperfusion. They observed that breathing NO for 20 min before and 24 h after reperfusion decreased MI size and improved systolic and diastolic function. Breathing 40 and 80 ppm (parts per million) NO decreased myocardial I/R injury equally, but 20 ppm was ineffective. Inhaled NO decreased cardiac neutrophil accumulation, and leukocyte depletion prevented the beneficial effects of iNO on MI size. NO inhalation increased arterial nitrite levels but did not change myocardial 3',5'-cyclic guanosine cyclic monophosphate (cGMP) levels. These results suggest that iNO concentrations required to elicit systemic effects are greater than those required to elicit pulmonary vascular effects [1]. In another study pigs breathing 80 ppm NO just before and during coronary reperfusion displayed improved endocardial and epicardial blood flow in the infarct zone, improved microvascular perfusion, reduced leukocyte infiltration, reduced infarct size, and decreased cardiomyocyte apoptosis in the infarct border zone [2]. Nagasaka and colleagues, using a similar cardiac I/R model, investigated the fate of 80 ppm iNO in mice, and quantified the formation of NO metabolites in blood and tissues. Studied rodents breathing NO rapidly increased a broad spectrum of NO metabolites. Levels of erythrocytic S-nitrosothiols, N-nitrosamines, and nitrosyl-hemes increased dramatically within 30 s of commencing nitric oxide inhalation. Marked increases of lung S-nitrosothiol (SNO) and liver N-nitrosamine levels were detected, as well as elevated cardiac and brain NO metabolite levels. Concentrations of each NO metabolite, except nitrate, rapidly reached a plateau and were similar after 5 and 60 min inhalation. They concluded that brief periods of NO inhalation can reduce infarct size in a murine model of cardiac I/R injury with similar efficacy as much longer periods of NO inhalation, suggesting that the concentrations of NO metabolites achieved in the target tissue may be more important for protection than the absolute amounts of nitric oxide absorbed or delivered [3]. In a recently published study the same research group demonstrated that the soluble guanylate cyclase (sGC α_1) subunit is required for inhaled NO to reduce MI size in mice subjected to cardiac I/R injury.

Shock states

Nitric oxide inhalation results in acute hemodynamic improvement when administered to patients with right ventricle MI and carcinogenic shock. During hemorrhagic shock and cardiovascular collapse, NO production is impaired mainly due to a drastic reduction of endotheli-

al shear stress and hypoxia related to uncoupling of endothelial nitric oxide synthase (eNOS). Nachuraju analyzed the systemic and microvascular hemodynamic changes related to decrease NO availability during the early phase of experimental hemorrhagic shock in hamsters. Exogenous NO in the form of S-nitrosoglutathione (GSNO) was given prior to the onset of hemorrhage. GSNO pretreatment in this study mitigated arteriolar vasoconstriction, increased capillary perfusion, venous return and prevented tissue ischemia [4]. The results of experiments with different intravenous NO donors in hemorrhagic shock have raised interest in possible inhaled NO impact on microcirculatory disturbances in the early stages of shock [5].

Neuroprotective effects of inhaled nitric oxide

Increasing experimental and clinical evidence suggests that iNO could have an impact on the developing central nervous system (CNS) and central nervous I/R injury [6]. Preterm infants treated for persistent pulmonary hypertension of the newborn (PPHN) are at high risk for neurodevelopmental impairment, including blindness, cerebral palsy, and global cognitive delay with educational and community functional disability [7]. The clinical impact of iNO on the development of the central system was for many years controversial, mainly because NO was demonstrated to increase bleeding time and inhibit platelet aggregation [8]. This could possibly result in an increase of the incidence of intracranial hemorrhage in critically ill preterm neonates. Later clinical studies have not confirmed these initial fears. Additionally, Schreiber and Kinsella showed a short-term neurologic benefit after iNO treatment. Schreiber reported a 47% reduction in severe intraventricular hemorrhage (IVH) or periventricular leukomalacia in the group that received iNO [9]. Kinsella *et al.* noted that iNO reduced neurological morbidity by 27%, an effect that was particularly striking in the very small premature neonates 750–999 gram weight [10]. A two-year developmental follow-up of the Schreiber study demonstrated that infants treated with iNO had approximately half the risk of having an abnormal neurodevelopmental outcome as those in the placebo treated group. Long-term benefits of iNO in premature infants were also demonstrated in a few recently published studies. Patrianakos-Hoobler performed a follow-up study of medical outcomes, neurodevelopmental assessment and school readiness in 135 of 167 (81%) surviving premature infants seen at 5.7 \pm 1.0 years. Inhaled NO treatment improved health status by decreasing the incidence of severe ongoing morbidities and also decreased the incidence of educational and community functional disability of premature infants at early school age [11]. In the Walsh randomized multi-center trial, it was demonstrated that iNO begun between 7 and 21 days and given for 24 days significantly increased survival without bronchopulmonary dysplasia in ventilated premature infants weighing < 1250 g. No adverse neurodevelopmental effects at 2 years of age were noted. [12]. However, it should be noted that the observations presented above were not supported by

the Van Meurs and Hinz studies on neurodevelopmental outcomes of premature infants with severe respiratory failure [13]. Whether iNO-mediated decreases in chronic lung disease, IVH and periventricular leukomalacia contribute to improved neurodevelopmental outcomes in this group of patients or if iNO may have an independent neuroprotective effect remain to be elucidated [7].

Numerous experimental studies have demonstrated the beneficial effect of iNO and its derivatives for the treatment of ischemic stroke. The possible mechanisms for neuroprotection with iNO are similar to the protection pathways of other organs, including downregulation of lung-derived cytokines and modulation of circulating neutrophils as they pass through the lungs. Neuroprotection may also be associated with delivery of NO-related compounds and metabolites, such as SNO, S-nitrosohemoglobin (SNOHb) or nitrites [7].

Several studies using models of hypoxic ischemic injury indicate that endogenous cytosolic NO produced in excess, or if a cell is in a pro-oxidant state, results in neuronal death.

It is well established that NO can react with superoxide anions produced by inducible NOS under inflammatory conditions or neuronal nitric oxide synthase (nNOS), as in the case of excitotoxicity, to form peroxynitrite, an anion with strong oxidant properties. Experimental data using isoform-specific NOS inhibitors have demonstrated a differential role for NO depending on the type and localization of NOS isoforms in the brain. Global inhibition of all NOS isoforms results in decreased infarct volume, whereas selective eNOS blockade increases infarct size, indicating its protective properties. Under conditions of hypoxia, eNOS can be downregulated, leading to dysregulation of cerebral flow and worsening injury. Inhaled NO treatment to the cerebral vasculature could, therefore, overcome down-regulated eNOS and improve cerebral blood flow and reduce ischemic injury. Nitric oxide confers neuroprotection by several mechanisms, nitric oxide S-nitrosylates caspase 3 and the subunits of the N-methyl-D-aspartate receptor (NMDAR). As a consequence of these reactions, Ca²⁺ influx through NMDARs and caspase 3 activities are both inhibited, leading to a decrease in cell death. Through the stimulation of the sGC, cGMP-protein kinase G pathway, NO activates cyclic-AMP-responsive-element-binding protein (CREB) and Akt kinase, two proteins that are mainly involved in neuroprotection. In addition to these pathways, NO induces the activity of hem oxygenase 1 (HO-1), which generates biliverdin, the precursor of the powerful antioxidant and antinitrosative molecule bilirubin [14]. In the Kuebler *et al.* study, cerebrovascular vasodilatory effects of iNO by using in vivo microscopy were reported. Nitric oxide inhalation exerts the properties of an ideal vasodilator: no significant effect on hemodynamics in normally perfused tissue (no effect on systemic blood pressure or blood flow in normal brain), whereas blood flow is exclusively increased in hypoxic-ischemic tissue [16]. This property of iNO has been shown recently in experimental stroke in mice and sheep. Inhaled NO exclusively dilated cerebral arterioles in the ischemic penumbra, thereby selectively increasing

cerebral blood flow in this critically hypoperfused area of the brain, whereas blood flow was not altered in the surrounding normal cortex. Under physiological conditions, iNO led to a significant dilatation of cerebral venules, most probably because of an oxygen tension dependent mechanism. Under hypoxic conditions, iNO also led to an arteriolar dilatation and cerebral perfusion improvement. In this study NO inhalation (50 ppm) was started 10 minutes and 1 hour after induction of transient cerebral ischemia or 10 minutes after permanent middle cerebral artery occlusion and maintained for 60 minutes, ischemic tissue injury was markedly attenuated and infarct volume was reduced by 40% as compared to control mice. Inhaled NO reduced lesion size and improved cerebral blood flow, cerebral metabolism, and neurological outcome without any obvious adverse effects [15].

The protective effects of inhaled NO on the outcome after cardiac arrest and CPR were studied by Minamishima *et al.* Inhalation of NO after cardiac arrest was associated with reduced water diffusion abnormality, caspase-3 activation, and cytokine induction in the brain and increased serum nitrate/nitrite levels. The authors postulated that NO inhalation after cardiac arrest and successful CPR improves outcome via sGC-dependent mechanisms [16]. Endothelial activation plays a central role in the pathogenesis of severe malaria, of which angiotensin-2 (Ang-2) has recently been shown to function as a key regulator. Nitric oxide is a major inhibitor of Ang-2 release from endothelium and has been shown to decrease endothelial inflammation and reduce the adhesion of parasitized erythrocytes. Recently Hawkes has suggested inhaled NO for the adjunctive therapy of severe malaria [17].

Renal effects of inhaled NO

Wright in 2001 showed that volunteers exposed to 20 ppm iNO significantly increased sodium excretion [18]. This is in line with a study in which pigs exposed to 40 ppm iNO displayed elevated glomerular filtration in association with a modest increase in renal blood flow [19]. We have studied prolonged up to 30 h effects of 40 ppm NO inhalation in healthy piglets, on renal function, and could confirm that a high dose of iNO indeed initially has a natriuretic effect in the pig. However, this effect disappears after about 12 hours and the phenomenon does not appear to be a serious challenge to the healthy kidney. An interesting additional finding was evidence of a slight but significant increase of tubular apoptosis after 30 hours of iNO, indicating that this exposure in some way can be considered a stress to some cell populations in this organ [20]. Additionally, according to a meta-analysis presented recently by Afshari and colleagues, iNO appears to increase the risk of renal failure in adults and children with acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) [21].

Transplantation

Relevance of NO inhalation in prevention of reperfusion injury after lung and liver transplantation has been subject-

ed to clinical evaluation. The clinical trials analysis of iNO use in lung transplantation done by Tavatare *et al.* suggest reduction of morbidity or mortality in this group of patients. However, because of a very large number of methodological limitations in analyzed studies, the authors concluded that it is difficult to currently recommend the routine use of prophylactic inhaled NO in lung transplant surgery [22]. Varadarajan and colleagues studied the relationship between NO metabolism and I/R injury in human liver transplantation. They pointed to the role of reduced NO bioavailability caused by reduced eNOS in the early hours of reperfusion, which might contribute significantly to damage [23]. Lang provided 80 ppm iNO perioperatively and found improvement in post-transplantation liver function parameters and decreased hospital length of stay. This regimen did not affect inflammatory markers after reperfusion but significantly decreased hepatocyte apoptosis. Circulating nitrite increased significantly during nitric oxide inhalation, and A-V gradients were observed, indicating metabolism of this anion to NO [24]. A larger trial enrolling 80 patients is currently underway in order to confirm these results.

Ischemia-reperfusion injury in other organ systems

There is also evidence indicating the potential to modulate I/R injury in other organ systems. Inhaled NO in a human model of I/R injury (knee surgery) has shown attenuation of the inflammatory response measured as reduced expression of CD11b/CD18, P-selectin, and lipid hydroperoxidase. These effects were accompanied by increased plasma nitrite concentrations [25]. Ng *et al.* evaluated the effects of 80 ppm iNO on feline intestinal ischemia-reperfusion blood flow. NO inhalation resulted in a significant A-V gradient for S-nitrosoalbumin (SNO-Alb). Concomitant with this loss of SNO-Alb across the intestinal vasculature was an increase in nitrite and significantly improved intestinal blood flow [26]. In Da and colleagues' study, the combination of iNO 30 ppm and intravenous corticosteroids modifies endotoxin-induced organ damage in a piglet endotoxin model [27]. Toll-like receptor 4 (TLR4) signaling is a critical modulator of cell survival and I/R injury in many organs. Based on the Da treatment model we evaluated a combination of iNO and corticosteroid therapies on renal and heart TLR4 mRNA activation determined by quantitative real time PCR after 90 minutes of suprarenal aortic cross clamping followed by 20 hours observation in an I/R injury piglet model. The combination of iNO and intravenous corticosteroids resulted in diminished kidney and heart TLR-4mRNA activation early after 3 hours of reperfusion [28].

Platelet function

Nitric oxide produced by both endothelial cells and platelets has important antithrombotic effects by decreasing platelet activation. Inhaled NO could theoretically modify systemic hemostasis either by direct effects on platelets in the pulmonary circulation or by release of NO outside of

the pulmonary circulation. These effects are becoming an important issue of iNO therapy in the context of hemorrhagic complications, especially in case of prolonged exposure and high concentrations used. Early studies in healthy human volunteers and animals demonstrated prolonged bleeding times following NO inhalation [8] and decreased thrombosis formation after thrombolysis. The potential of iNO to alter platelet function is of particular concern for immature infants since abnormal hemostatic activity increases the incidence of IVH and associated neurologic injury. These issues were discussed in more detail earlier. Similarly to neonatal studies, adult studies brought conflicting results. It was reported that NO inhalation causes only mild, if any, attenuation of platelet function in healthy subjects [29] and reports of bleeding time prolongation [30]. The disparities in assessing the impact of iNO on platelet aggregation might result from a number of reasons: inhaled NO exposure time and concentration, methodology of performed coagulation tests, underlying disease and its associated complications. Most of the human volunteer studies were limited by the fact that the iNO exposure time was relatively short and lasted about 60 minutes. In order to settle this issue we investigated the effects of 40 ppm NO inhalation prolonged to 30 h on bleeding time and coagulation parameters in healthy piglets. We consider it unlikely that clinical use of iNO would enhance the risk of bleeding even after prolonged inhalation [31]. Some platelet aggregation tests in different studies were performed using platelet rich plasma (PRP) in which the Hb is removed before the analysis. The use of PRP is problematic because plasma contains natural nitrates, which may be converted into NO after preparation of PRP and thereby artificially affect platelet aggregation and function. This may explain why some studies that have tested platelet function in PRP have documented effects of iNO. In line with this, Albert and colleagues previously showed that GSNO inhibits platelet aggregability in a dose-dependent manner and that this effect is markedly reduced in whole blood compared to PRP [32]. Increased bleeding time has been reported in adult ARDS patients and neonates with respiratory complications. Impaired platelet function is commonly observed in sepsis. All these observations underscore the difficulty in extrapolating clinical bleeding tendency from different studies, and may explain some of the present controversy over effects of iNO on hemostasis in a clinical setting [31].

Rationale for inhaled NO use in sepsis

Nitric oxide is a critical factor for protecting microcirculatory integrity and function in sepsis, since impairment of microcirculatory blood flow may be an early triggering event in the development of sepsis-induced multi-organ failure, a critical determinant of sepsis mortality. The use of iNO in sepsis at first glance appears at least controversial. However, it has to be emphasized that despite the fact that NO production is generally upregulated in sepsis, inducible NOS is differently expressed between organ systems, and NO can be consumed by reactive oxygen species (ROS), giving

the potential for localized areas of relative NO deficiency in microvascular beds despite a state of total body NO excess [33]. This can result in different tissue perfusion in various regions and explain pathologic microcirculatory shunting in sepsis. Optimized resuscitation of microcirculation in early stages of sepsis is now becoming a very important issue. These new concepts have even been suggested as an additional approach in severe sepsis early goal directed therapy. Early on it was assumed that NO is deleterious in sepsis and should be modulated by inhibiting excess formation. This point of view was mainly related to the assessment of its effects on the systemic circulation with concomitant arterial hypotension. Possible beneficial effects on the microcirculation often remained overlooked. NOS inhibition is clearly effective at raising arterial pressure in sepsis but it can simultaneously worsen the impairment of microcirculatory perfusion and oxygen transport to tissues [33]. Additionally, NO production blockade in sepsis facilitates leukocyte adhesion, platelet aggregation, promotes thrombus generation and promotes microvascular permeability. A very promising randomized controlled trial of nonspecific NOS inhibition was stopped early because of increased mortality in the NOS inhibition group [34]. Patients with septic shock have a shortage in the availability of arginine associated with slower production. Citrulline production is severely low in patients with sepsis and is related to diminished de novo arginine and NO production. These metabolic alterations contribute to reduced citrulline and arginine availability. Because NO preserves microcirculatory patency and function, upregulation of NO may be adaptive and in fact provide beneficial effects in sepsis. Taking an opposite approach to studying NO modulation in septic patients, administration of exogenous NO could potentially improve tissue perfusion. This alternative is supported by experimental models where NO donor administration results in decreased endothelial adhesion molecule expression and leukocyte adhesion and optimized tissue oxygen delivery. Clinical studies of sepsis patients have shown impairment of sublingual microcirculatory blood flow that could be reversed with topical administration of acetylcholine and intravenous nitroglycerin. The problem with administration of intravenous NO donors is a risk of causing arterial hypotension in this patient population. Inhaled NO has been reported to modulate apoptosis in sepsis, with SNO generation as one of the mechanisms underlying these effects. Inhibition of caspases, stimulation of anti-apoptotic activity of thioredoxin, and increased expression of heat shock proteins and Bcl-2 are the other anti-apoptotic mechanisms. The direct effect of iNO is likely dependent not only on the concentration of iNO but also on the specific redox state of the cells. Inhaled NO can also increase endothelial NOS activity in tissues. All of these mechanisms may limit organ damage in sepsis [35]. Nitric oxide endocrine activity occurs by formation of nitrite and SNOHb. Higher arterial vs. venous (A-V) concentrations of nitrite and SNOHb suggest consumption of the metabolite and NO release in the systemic vasculature. Nitrite and SNOHb A-V differences are diminished in patients with

severe sepsis and septic shock, and are associated with higher mortality [36]. In animal experiments nitrite therapy might protect against both TNF- α (tumor necrosis factor α) and LPS-induced toxicity in mice. Protection, measured as reduced hypothermia and mortality, was strongest when nitrite was administered as a pre-treatment, but therapeutic treatment could also provide significant protection if administered when hypothermia was not yet too serious [37]. In the Morgan *et al.* study on septic patients, in control healthy patients, arterial nitrite was higher than venous nitrite in plasma, whole blood, and red blood cells (RBCs), suggesting nitrite consumption in the systemic circulation. Arterial SNOHb was lower than venous SNOHb, suggesting SNOHb production in this group. Comparing sepsis patients to control subjects, there were no differences in arterial plasma nitrite in both groups. In contrast, venous whole blood and RBC nitrite were significantly higher in septic patients than controls, suggesting impaired intra-erythrocytic nitrite utilization. Opposite results were observed for venous SNOHb. Compared to controls, sepsis patients had significantly lower venous SNOHb, but arterial levels were similar. This narrowed the A-V (venous > arterial) SNOHb difference in septic patients, suggesting impaired SNOHb production. In addition, sepsis non-survivors are distinguished from survivors by an isolated rise in venous plasma nitrite. The authors hypothesized that these results reflect dysregulated intra-erythrocytic deoxyhemoglobin-mediated nitrite reduction, the interdependent system comprising the RBC membrane, membrane-associated proteins, deoxyhemoglobin, and nitrite [36]. Septic shock is characterized by abnormally high mixed venous hemoglobin (Hb) oxygen saturation, which could impair hemoglobin nitrite reductase activity. Sepsis-associated mitochondrial dysfunction or oxidative stress could also disrupt nitrite reductase activity in vascular and extra-vascular tissues independent of RBCs and hemoglobin. All these findings have implications for understanding the role of intravascular NO delivery in the pathophysiology of human sepsis. Inhaled nitric oxide therefore may play a pivotal role as a factor concomitantly improving conditions of microcirculation by leukocyte inhibition and vascular dilatation. Additionally it may take place without the risk of systemic pressure deterioration. Based on this concept a randomized clinical trial of inhaled nitric oxide to augment tissue perfusion in sepsis was undertaken [38].

Another therapeutic concept of sepsis treatment was proposed by Da and colleagues, aiming to determine whether inhaled NO could stimulate glucocorticoid receptor upregulation in non-pulmonary tissues, thereby increasing the effectiveness of therapeutic glucocorticoid [28]. Severe sepsis is associated with relative adrenal insufficiency and glucocorticoid resistance, which resulted in a return to the concept of a replacement therapy with low doses of corticosteroids during longer periods. Some experimental data have demonstrated that during LPS-induced endotoxemia inhibition of glucocorticoid receptors occurs, which suppresses the effectiveness of endogenous and therapeutic steroids. On the other hand, glucocorticoid re-

ceptor expression in transgenic mice was correlated with increased resistance to LPS-induced endotoxemia. Using a 6-hour porcine endotoxin challenge model, Da and colleagues revealed that endotoxin infusion downregulated expression of glucocorticoid receptor in lung, liver, and kidney tissues concomitant with upregulation of inflammatory markers such as NF- κ B and TNF- α . Simultaneous administration of 30 ppm inhaled NO and glucocorticoid in this sepsis model blunted the inflammatory response not only in lungs but also in systemic organs. This demonstrated that inhaled NO stimulates upregulation of glucocorticoid receptors, making steroid therapy more effective in sepsis [28]. Although the results from this study were impressive, the effects were evaluated over a few hours only. We wished to investigate the treatments over a longer period of time, which is closer to the clinical reality for patients developing impaired organ function during sepsis. Accordingly we examined the effects up to 30 h of this combined therapy with iNO and steroids in a porcine endotoxin sepsis model, with special attention to organ function. We could not confirm all observations done by Da's group, but we found that combined early therapy with iNO 30 ppm and corticosteroids was associated with partial protection of organ functions after 30 hours of LPS infusion in piglets. The animals were evaluated summarizing individual organ failure, the method based on the Sequential Organ Failure Assessment score (SOFA), with some modification necessary to meet the requirements of the experimental model. The use of an "animal organ failure score" could facilitate the evaluation of complex therapies over time in an ICU-like setting. It is possible that prolonged and more massive LPS exposure in our study underlay much greater organ damage and partly undermined the beneficial effects of this combined therapy [39]. Since sepsis is increasingly recognized as a disease of the microcirculation, in which enhanced vasoconstriction and mitochondrial dysfunction cause irreversible damage and organ failure, novel therapies based on iNO and NO donors alone or combined with other therapies to rescue the microcirculation and reverse hypoxia and ischemia might be very promising [33, 36].

Future perspectives

The presented data support the concept that inhaled NO could be a novel treatment for a disease characterized by systemic endothelial dysfunction. It has been suggested that the dose of inhaled NO should be modified in relation to body size, concerning potential accumulation of bioactive NO metabolites that may affect the transduction of NO bioactivity to the target organ. A concentration of inhaled NO that is effective in a small animal may not be sufficient in humans. Conversely, a concentration that is effective and safe in adults may cause unwanted toxic effects in premature infants. The levels of circulating NO species will also depend on NO oxidation in blood, on renal excretion and on the activity of all the reductive systems influencing NO formation from the circulating NO metabolites [40]. Based on several studies and observations it is very likely now

that inhaled NO has no hemodynamic effects on normally perfused tissue but increases blood flow selectively in hypoperfused tissue without any effect on systemic blood pressure. This therapy has a simple and well-described route and type of administration, safety profile and immediate action. Because of these properties iNO may be easily implemented into primary care and serve as a rescue therapy for ischemic conditions in which collateral blood flow is important or until interventional or spontaneous reperfusion occurs.

Literature

- Hataishi R, Rodrigues AC, Neilan TG, Morgan JG, Buys E, Shiva S, Tambouret R, Jassal DS, Raheer MJ, Furutani E, Ichinose F, Gladwin MT, Rosenzweig A, Zapol WM, Picard MH, Bloch KD, Scherrer-Crosbie M. Inhaled nitric oxide decreases infarction size and improves left ventricular function in a murine model of myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2006; 291: H379-H384.
- Liu X, Huang Y, Pokreisz P, Liu X, Huang Y, Pokreisz P, Vermeersch P, Marsboom G, Swinnen M, Verbeken E, Santos J, Pellens M, Gillijns H, Van de Werf F, Bloch KD, Janssens S. Nitric oxide inhalation improves microvascular flow and decreases infarction size after myocardial ischemia and reperfusion. *J Am Coll Cardiol* 2007; 50: 808-817.
- Nagasaka Y, Fernandez BO, Garcia-Saura MF, Petersen B, Ichinose F, Bloch KD, Feelisch M, Zapol WM. Brief periods of nitric oxide inhalation protect against myocardial ischemia-reperfusion injury. *Anesthesiology* 2008; 109: 675-682.
- Nachuraju P, Friedman AJ, Friedman JM, Cabrales P. Exogenous nitric oxide prevents cardiovascular collapse during hemorrhagic shock. *Resuscitation* 2012; 82: 607-613.
- Wang Y, You G, Kan X, Chen G, Zhao L, Zhou H. Nitric oxide inhalation, a proposed strategy for early treatment of hemorrhagic shock. *Med Hypotheses* 2011; 77: 182-184.
- Terpolilli NA, Kim SW, Thal SC, Kataoka H, Zeisig V, Nitzsche B, Klaesner B, Zhu C, Schwarzmaier S, Meissner L, Mamrak U, Engel DC, Drzezga A, Patel RP, Blomgren K, Barthel H, Boltze J, Kuebler WM, Plesnila N. Inhalation of nitric oxide prevents ischemic brain damage in experimental stroke by selective dilatation of collateral arterioles. *Circ Res* 2012; 110: 727-738.
- Marks JD, Schreiber MD. Inhaled nitric oxide and neuroprotection in preterm infants. *Clin Perinatol* 2008; 35: 793-807.
- Hogman M, Frostell C, Arnberg H, Hedenstierna G. Bleeding time prolongation and NO inhalation. *Lancet* 1933; 341: 1664-1665.
- Schreiber MD, Gin-Mestan K, Marks JD, Huo D, Lee G, Srisuparp P. Inhaled nitric oxide in premature infants with the respiratory distress syndrome. *N Engl J Med* 2003; 349: 2099-2107.
- Kinsella JP, Cutter GR, Walsh WF, Kinsella JP, Cutter GR, Walsh WF, Gerstmann DR, Bose CL, Hart C, Sekar KC, Auten RL, Bhutani VK, Gerdes JS, George TN, Southgate WM, Carriedo H, Couser RJ, Mammel MC, Hall DC, Pappagallo M, Sardesai S, Strain JD, Baier M, Abman SH. Early Inhaled Nitric Oxide Therapy in Premature Newborns with Respiratory Failure. *N Engl J Med* 2006; 355: 354-364.
- Patrianakos- Hoobler AI, Marks JD, Msall ME, Huo D, Schreiber MD. Safety and efficacy of inhaled nitric oxide treatment for premature infants with respiratory distress syndrome: follow-up evaluation at early school age. *Acta Paediatr* 2012; 100: 524-528.
- Walsh MC, Hibbs AM, Martin CR, Cnaan A, Keller RL, Vittinghoff E, Martin RJ, Truog WE, Ballard PL, Zadell A, Wadlinger SR, Coburn CE, Ballard RA; NO CLD Study Group. Two-year neurodevelopmental outcomes of ventilated preterm infants treated with inhaled nitric oxide. *J Pediatr* 2010; 156: 556-561.
- Van Meurs KP, Wright LL, Ehrenkranz RA, Lemons JA, Ball MB, Poole WK, Perritt R, Higgins RD, Oh W, Hudak ML, Laptook AR, Shankaran S, Finer NN, Carlo WA, Kennedy KA, Fridriksson JH, Steinhorn RH, Sokol GM, Konduri GG, Aschner JL, Stoll BJ, D'Angio CT, Stevenson DK; Preemie Inhaled Nitric Oxide Study. Inhaled nitric oxide for premature infants with severe respiratory failure. *N Engl J Med* 2005; 353: 13-22.
- Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, Giuffrida Stella AM. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. *Nat Rev Neurosci* 2007; 8: 766-775.

15. Kuebler WM, Kisch-Wedel H, Kemming GI, Meisner F, Bruhn S, Koehler C, Flondor M, Messmer K, Zwissler B. Inhaled nitric oxide induces cerebrovascular effects in anesthetized pigs. *Neurosci Lett* 2003; 348: 85-88.
16. Minamishima S, Kida K, Tokuda K, Wang H, Sips PY, Kosugi S, Mandeville JB, Buys ES, Brouckaert P, Liu PK, Liu CH, Bloch KD, Ichinose F. Inhaled nitric oxide improves outcomes after successful cardiopulmonary resuscitation in mice. *Circulation* 2011; 124: 1645-1653.
17. Hawkes M, Opoka RO, Namasopo S, Miller C, Thorpe KE, Lavery JV, Conroy AL, Liles WC, John CC, Kain KC. Inhaled nitric oxide for the adjunctive therapy of severe malaria: protocol for a randomized controlled trial. *Trials* 2011; 12: 176.
18. Wright WM, Young JD. Renal effects of inhaled nitric oxide in humans. *Br J Anaesth* 2001; 86: 267-269.
19. Troncy E, Francoeur M, Salazkin I, Yang F, Charbonneau M, Leclerc G, Vinay P, Blaise G. Extra-pulmonary effects of inhaled nitric oxide in swine with and without phenylephrine. *Br J Anaesth* 1997; 79: 631-640.
20. Gozdzik W, Albert J, Harbut P, Albert J, Zieliński S, Ryniak S, Lindwall R, Dziegiel P, Podhorska-Okolow M, Kübler A, Frostell C. Prolonged exposure to inhaled nitric oxide transiently modifies tubular function in healthy piglets and promotes tubular apoptosis. *Acta Physiol* 2009; 195: 495-502.
21. Afshari A, Brok J, Møller AM, Wetterslev J. Inhaled nitric oxide for acute respiratory distress syndrome and acute lung injury in adults and children: a systematic review with meta-analysis and trial sequential analysis. *Anesth Analg* 2011; 112: 1411-1421.
22. Tavare AN, Tsakok T. Does prophylactic inhaled nitric oxide reduce morbidity and mortality after lung transplantation? *Interact Cardiovasc Thorac Surg* 2011; 13: 516-520.
23. Varadarajan R, Golden-Mason L, Young L, McLoughlin P, Nolan N, McEntee G, Traynor O, Geoghegan J, Hegarty JE, O'Farrelly C. Nitric oxide in early ischaemia reperfusion injury during human orthotopic liver transplantation. *Transplantation* 2004; 78: 250-256.
24. Lang JD Jr, Teng X, Chumley P, Crawford JH, Isbell TS, Chacko BK, Liu Y, Jhala N, Crowe DR, Smith AB, Cross RC, Frenette L, Kelley EE, Wilhite DW, Hall CR, Page GP, Fallon MB, Bynon JS, Eckhoff DE, Patel RP. Inhaled NO accelerates restoration of liver function in adults following orthotopic liver transplantation. *J Clin Invest* 2007; 117: 2583-2591.
25. Mathru M, Huda R, Solanki DR, Hays S, Lang JD. Inhaled nitric oxide attenuates reperfusion inflammatory responses in humans. *Anesthesiology* 2007; 106: 275-282.
26. Ng ES, Jour'dheuil D, McCord JM, Hernandez D, Yasui M, Knight D, Kubes P. Enhanced S-nitroso-albumin formation from inhaled NO during ischemia/reperfusion. *Circ Res* 2004; 94: 559-565.
27. Da J, Chen L, Hedenstierna G. Nitric oxide up-regulates the glucocorticoid receptor and blunts the inflammatory reaction in porcine endotoxin sepsis. *Crit Care Med* 2007; 35: 26-32.
28. Gozdzik W, Olbromski M, Harbut P, Zielinski S, Ratajczak K, Albert J. Inhaled nitric oxide combined with corticosteroids attenuate kidney and heart toll-like receptors 4 expression in a porcine suprarenal aortic cross clamping ischemia reperfusion model *Brit J Anaesth* 2012; 108 suppl. 2: ii101-ii103.
29. Albert J, Norman M, Wallén NH, Frostell C, Hjemdahl P. Inhaled nitric oxide does not influence bleeding time or platelet function in healthy volunteers. *Eur J Clin Invest* 1999; 29: 953-959.
30. Beghetti M, Sparling C, Cox PN, Stephens D, Adatia I. Inhaled NO inhibits platelet aggregation and elevates plasma but not intraplatelet cGMP in healthy human volunteers. *Am J Physiol Heart Circ Physiol* 2003; 285: H637-H642.
31. Albert J, Harbut P, Zieliński S, Ryniak S, Gillis-Haegerstrand C, Lindwall R, Solski L, Lundberg JO, Svensson J, Gozdzik W. Prolonged exposure to inhaled nitric oxide does not affect haemostasis in piglets. *Intensive Care Med* 2007; 33: 1594-1601.
32. Albert J, Daleskog M, Wallén NH. A comparison of the antiplatelet effect of S-nitrosoglutathione in whole blood and platelet-rich plasma. *Thromb Res* 2001; 102: 161-165.
33. Trzeciak S, Cinel I, Phillip Dellinger R, Shapiro NI, Arnold RC, Parrillo JE, Hollenberg SM; Microcirculatory Alterations in Resuscitation and Shock (MARS) Investigators. Resuscitating the microcirculation in sepsis: the central role of nitric oxide, emerging concepts for novel therapies, and challenges for clinical trials. *Acad Emerg Med* 2008; 15: 399-413.
34. López A, Lorente JA, Steingrub J, Bakker J, McLuckie A, Willatts S, Brockway M, Anzueto A, Holzapfel L, Breen D, Silverman MS, Takala J, Donaldson J, Arneson C, Grove G, Grossman S, Grover R. Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock. *Crit Care Med* 2004; 32: 21-30.
35. Goldfarb RD, Cinel I. Inhaled nitric oxide therapy for sepsis: more than just lung. *Crit Care Med* 2007; 35: 290-292.
36. Morgan MA, Frasier LM, Stewart JC, Mack CM, Gough MS, Graves BT, Apostolakis MJ, Doolin KP, Darling DC, Frampton MW, Pietropaoli AP. Artery-to-vein differences in nitric oxide metabolites are diminished in sepsis. *Crit Care Med* 2010; 38: 1069-1077.
37. Cauwels A, Brouckaert. Nitrite regulation of shock. *Cardiovasc Res* 2011; 89: 553-559.
38. Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, Arnold RC, Colilla S, Zanotti S, Hollenberg SM. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Ann Emerg Med* 2007; 49: 88-98.
39. Gozdzik W, Paus S, Harbut P, Ryniak S, Zielinski S, Gillis Haegerstrand C, Kubler A, Hedenstierna G, Frostell C, Albert J. Inhaled nitric oxide and corticosteroids in endotoxemia, effects on animal organ failure score. *Intens Care Med* 2011; 237 suppl.1: S118 [0449].
40. Lundberg JO, Weitzberg E. Extrapulmonary effects of nitric oxide inhalation therapy: time to consider new dosing regimens? *Crit Care* 2008; 12: 406.