Nitric oxide: a new role in intensive care

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ABSTRACT

Inhaled nitric oxide has been used for 30 years to improve oxygenation and decrease pulmonary vascular resistance. In the past 15 years, there has been increased understanding of the role of endogenous nitric oxide on cell surface receptors, mitochondria, and intracellular processes involving calcium and superoxide radicals. This has led to several animal and human experiments revealing a potential role for administered nitric oxide or nitric oxide donors in patients with systemic inflammatory response syndrome or ischaemia–reperfusion injury, and in patients for whom exposure of blood to artificial surfaces has occurred.

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Nitric oxide (NO) is a free radical[1] that acts as a signalling molecule, modulating functions across multiple body systems.[2] Endogenous NO is released by blood vessel endothelium in response to stimulation from the autonomic nervous system, shearing forces generated by increased blood flow, and a variety of ligands that act on endothelial cell surface receptors. Endothelial cell-derived NO inhibits platelet aggregation, inhibits white blood cell activation, and works directly on vascular smooth muscle to cause vasodilation[3] and alter regional blood flow. NO also affects cell wall permeability, cellular pumps that control entry of calcium into cells, and mitochondrial permeability transition pore (mPTP) receptors that affect access of superoxide radicals into mitochondria.

In cardiac myocytes, NO increases contractility and also increases heart rate.[4] Myocardial oxygen consumption is also reduced by endogenous basal NO, shown by an increased oxygen requirement after administration of a nitrogen oxide synthase inhibitor in patients, which is thought to be due to more efficient myocardial contractility.[5] Furthermore, NO is thought to improve myocardial distensibility; patients with higher levels of nitric oxide synthase gene expression have been shown to have less stiff left ventricles, and therefore a higher preload reserve that preserves stroke volume.[6] It has also been shown that NO synthesis and release is impaired in states of systemic inflammation associated with cardiopulmonary bypass (CPB).[7] In the same conditions, levels of NO inhibitor molecules are also raised.[8]

Despite an increase in case complexity, survival after paediatric and neonatal cardiac surgery continues to improve,[9,10] so there is an increasing focus on reducing long term morbidity. Globally, the incidence of congenital heart disease is estimated to be 1 in 100 live births.[11] In about half of cases, surgical intervention will be required,[12] and CPB is used in most of these cases. CPB provides cardiac and respiratory support via an extracorporeal system,[13] which creates a bloodless and non-pulsatile surgical field. A routine consequence of the procedure is systemic inflammation and ischaemia–reperfusion injury (IRi), due to blood contact with foreign surfaces, which causes injury to the heart, kidneys, brain and other organs. More recently, it has also been recognised that stored red blood cells rapidly develop a defect that limits oxygen delivery. NO also reverses the red cell storage defect.

For these reasons, exogenous NO may have a new role in any clinical scenario that includes systemic inflammatory response syndrome (SIRS) or IRi; this includes cardiac arrest, myocardial and cerebral ischaemia, sepsis, cardiac surgery and extracorporeal membrane oxygenation (ECMO). Some animal and human data from these scenarios have been published. Most human clinical data comes from patients who have undergone paediatric cardiac surgery, and we present these data in this review, along with a small amount of data from patients who have undergone ECMO.

Methods

Eligibility criteria

This review includes human data from adult and paediatric patients who underwent CPB and suffered IRi as a direct consequence of CPB, plus data from animal studies including IRi. Studies must have included exposure to exogenous NO during CPB or the peri-ischaemic period, and must have included a comparison with controls where NO was not administered. Outcomes examined encompass end-organ effects or biological markers. Pulmonary outcomes (eg, improved gas exchange or pulmonary hypertension) were excluded from this review as the effects of NO on pulmonary outcomes are already well known. Study designs that were considered eligible included randomised control

trials, cohort studies and case–control studies; conference abstracts describing small case series were excluded.

Search strategy

Electronic searches of MEDLINE, Scopus and EMBASE were conducted in May 2018, and updated in August 2019. A predefined search strategy was developed for MEDLINE using Medical Subject Headings (MeSH) terms and keywords and operating theatre/room to capture relevant citations. In addition, references were reviewed to identify potentially relevant papers missed by the searches. Results were limited to articles written in English but no date restrictions were applied. Separate databases of human and animal studies were constructed in Endnote (Clarivate Analytics, Philadelphia, PA, US) with duplicates removed. The results were reviewed, and full text articles were selected based on predefined criteria. If eligibility was unclear, the full text article was retrieved. Articles were reviewed and results were synthesised qualitatively.

Results

A total of 1267 studies relating to NO use in animal models were initially identified, and these were narrowed down to seven by screening titles and abstracts and reviewing full text articles. A total of 499 studies of trials in humans were retrieved from the electronic search, of which four were included in the final review that followed screening of the results. One study on the safety of NO in patients receiving ECMO was also included. The human studies were categorised based on the patient population: adult ($n = 3$) or paediatric ($n = 2$). All five studies were prospective randomised trials, and blinding was used in three.

Review of animal studies

Experimental work in murine models of myocardial ischaemia–reperfusion injury has demonstrated a promising effect of NO on myocardial infarct (MI) size and function, and is summarised in Table 1. Hataishi and colleagues supplemented the ventilator with NO for 20 minutes before reperfusion following ligation of the left anterior descending coronary artery.[18] Twenty-four hours after surgery, animals in the intervention group had markedly better systolic function compared with untreated animals, based on echocardiogram findings. Also, heart slices were obtained and stained so that infarct size could be measured via fluorescence microscopy. In animals inhaling 80 ppm NO, the ratio of MI area to left ventricular area after 30 minutes of ischaemia decreased (64 ± 3% on air *v* 58 ± 5% on NO) and the ratio of MI area to area without microspheres after 30 minutes of ischaemia decreased (20 ± 3% on air *v* 9 ± 1% on NO). These findings were replicated when the duration of ischaemia was increased. In addition, NO was associated with reduced myocardial neutrophil infiltration.[18]

Results of further studies, using the same murine model, concur with these results. Nagasaka and colleagues showed a reduction in markers of myocardial injury in mice that inhaled NO before reperfusion, and a 32% decrease in size of MI when NO was inhaled for an hour before reperfusion ($P \leq 0.05$).[19] It should be noted that in these studies, NO was only administered during periods of ischaemia, meaning that NO metabolites did not enter ischaemic tissue until reperfusion had occurred. In a similarly designed study, Neye and colleagues also compared NO administration during the reperfusion; they found that NO inhalation that commenced during reperfusion produced non-significant effects, but continuous inhalation throughout ischaemia and reperfusion was associated with the greatest reduction in myocardial area at risk.[17]

Results of several studies indicate that NO may also confer protection against ischaemic injury in neuronal tissue. Although cerebral blood flow is not altered by NO in normal physiological conditions, there is evidence that NO has a vasodilatory effect in experimental models of cerebral ischemia. Terpolilli and colleagues subjected mice to 45 minutes of ischaemia, through occlusion of the middle cerebral artery, and then stained coronal brain slices with cresyl violet to highlight infarcted tissue.[20] Commencing with 50 ppm NO inhalation during ischaemia resulted in reduced ischaemic tissue injury as compared with the control group (no NO inhalation). In a similar trial, Li and colleagues observed a reduced infarct volume 24 hours after cerebral artery ischaemia, but only at 10 ppm NO; at higher NO concentrations, infarct volume was reduced up to 16 hours but no significant effect was observed at 24 hours.[15] Contrary to this, Kida and colleagues noted no increase in cerebral blood flow with NO,[16] indicating no significant vasodilatory effect but possible beneficial results owing to reduced water diffusion abnormality and cytokine induction.[14,16] Age- and weight-matched male C57BL/6J wild-type, sGCα1-deficient and NOS3-deficient mice were subjected to 7.5 minutes of potassium chloride-induced cardiac arrest followed by cardiopulmonary resuscitation and inhalation of air supplemented with NO for 23 hours.[14] The NO intervention group had higher 10-day survival rates than the control group (11/13 mice *v* 4/13 mice; $P = 0.003$) and higher neurological function scores 96 hours after induced ischaemia. Magnetic resonance imaging conducted at 24 hours showed areas of hyperintense diffusion-weighted imaging in the control group, a measure of cerebral oedema, while this was largely absent in the intervention group. In addition, statistical analysis showed average apparent diffusion coefficients that were universally lower

Table 1. Summary of animal studies relating to use of nitric oxide (NO) in ischaemia–reperfusion injury

Author, year	Animal model	Ischaemia model	Intervention	Outcomes	
Minamishima, 2011[14]	Age- and weight-matched male C57BL/6J wild-type, sGCα1-deficient and NOS3-deficient mice	7.5 min cardiac arrest, induced by intravenous potassium chloride, followed by manual CPR	Weaned from mechanical ventilation 1 h after CPR, then air supplemented with 40 ppm NO for 23 h	10-day survival	4/13 air *v* 11/13 NO, $P = 0.003$
				Left ventricular end systolic pressure (mmHg)	58 ± 4 air *v* 73 ± 5 NO, $P < 0.05$
				Left ventricular end diastolic pressure (mmHg)	4 ± 1 air *v* 2 ± 0 NO, $P < 0.05$
				Preload recruitable stroke work (mmHg)	74 ± 9 air *v* 102 ± 11 NO, $P < 0.05$
Li, 2013[15]	Male Swiss Webster mice	Middle cerebral artery occlusion for 1 h followed by reperfusion for 47 h	NO at 10, 20, 40, 60 or 80 ppm in a Plexiglas chamber, starting immediately after artery occlusion	Mean % infarct volume	19.3 air *v* 14.0 NO
				Hippocampus GFAP immunoreactivity	13.41 ± 2.61 air *v* 5.69 ± 0.73 NO, $P = 0.05$
				Corpus striatum GFAP immunoreactivity	3.57 ± 0.64 air *v* 1.73 ± 0.23, $P = 0.05$
Kida, 2014[16]	Age- and weight-matched male C57BL/6J NOS3$^{-/-}$ and wild-type mice	6.5 min cardiac arrest, induced by intravenous potassium chloride, followed by manual CPR	40 ppm NO mixed in air started 1 h after resuscitation for 24 h in chambers	10-day survival (%)	0 air *v* 90 NO 30 min after resuscitation, $P < 0.05$
Neye, 2012[17]	Male Sprague-Dawley rats	Left anterior descending artery reversibly ligated for 120 min	50 ppm NO during 120 min of ischaemia and/or for either 3 or 5 h during reperfusion	Infarct size to AAR	0.59 ± 0.05 air *v* 0.45 ± 0.04 NO, $P = 0.05$
Hataishi, 2006[18]	C57BL/6J male mice	Myocardial ischaemia induced by ligation of left anterior descending coronary artery	80 ppm NO via ventilator 20 min before reperfusion	AAR to total infarct area without microspheres (%)	32 ± 4 air *v* 15 ± 2 NO, $P < 0.05$
				AAR to left ventricular volume (%)	64 ± 3 air *v* 58 ± 5 NO, $P < 0.05$
				Neutrophil count (mm^{-2})	18 ± 4 air *v* 4 ± 1 NO, $P < 0.02$
Nagasaka, 2008[19]	Male C57BL/6J mice	Myocardial ischaemia induced by ligation of left coronary artery	80 ppm NO delivered through the ventilator for duration of surgery	Infarct to AAR	32% decrease ($P = 0.05$) in NO group compared with control
Terpolilli, 2012[20]	Male C57BL/6J mice	Middle cerebral artery occlusion for 45 min	50 ppm NO in 30% oxygen during reperfusion	Pial arteriole diameter dilation compared with control (%)	22 ± 3.2, $P = 0.05$
				Cerebral infarct volume compared with control (%)	≥ 40, $P < 0.01$

AAR = area of myocardial tissue at risk; CPR = cardiopulmonary resuscitation; GFAP = glial fibrillary acidic protein.

in the NO group ($P \leq 0.05$). Furthermore, the induction of genes encoding inflammatory cytokines tumour necrosis factor-α, NADPH oxidase 2 (NOX2), interleukin-6 and interleukin-1β was prevented in wild-type mice subjected to NO inhalation.[14]

Review of human studies

Adult patients

Studies that have translated this animal research to human patients are summarised in Table 2. A small non-blinded

study was conducted by Kamenshchikov and colleagues,[21] with 29 patients undergoing aortic valve replacement and aortocoronary bypass randomly assigned to receive 500 ppm NO through the ventilator circuit before transferring to delivery via the CPB pump prime solution. Surrogate biochemical markers of myocardial injury were measured, and each revealed a higher level of injury in the control group: creatinine kinase–muscle/brain (CK-MB) levels were significantly lower at 24 hours (P = 0.01) and 48 hours (P = 0.01) in the intervention arm, while cardiac troponin I (cTnI) levels (P = 0.04) and B-type natriuretic peptide (BNP) levels (249 ± 71 pg/mL *v* 311 ± 141 pg/mL; P = 0.02) also showed blunted responses. Results of this study are positive but limited in terms of generalisability owing to the small number of participants and short follow-up period.

Similarly, Kamenshchikov and colleagues measured CK-MB and cTnI levels as proxy markers for myocardial necrosis after ischaemia induced by CPB.[22] Thirty patients undergoing elective primary coronary artery bypass grafting inhaled NO via the CPB circuit during the perioperative period. Twenty-four hours after surgery, decreased cTnI levels (1.79 ± 0.39 ng/mL *v* 2.41 ± 0.55 ng/mL; P = 0.001) and CK-MB levels (47.69 ± 8.08 U/L *v* 62.25 ± 9.78 U/L; P = 0.001) were seen relative to the control groups, indicating a lesser extent of myocardial injury. This study is also of limited generalisability owing to small sample size and lack of long term follow-up. However, combining these results with those of Gianetti et al adds weight to the plausibility of NO having a cardioprotective effect.[21,22]

It has also been reported that the incidence of acute kidney injury (AKI) following CPB is lowered by concurrent NO administration.[23] A total of 244 adults with previously normal kidney function who were undergoing elective multiple valve replacement surgery with CPB were recruited into a double-blind randomised trial. Of them, 117 received 80 ppm NO and had a relative risk (RR) of AKI of 0.78 (95% CI, 0.62–0.99; P = 0.014). Intrahospital mortality (RR, 0.31 [95% CI, 0.07–1.46]; P = 0.068) and 1-year mortality (RR, 0.41 [95% CI, 0.11–1.50]; P = 0.088) were both decreased in the NO group as compared with controls. There was no difference in the length of ICU stay between the groups.

Paediatric patients

Only two studies to date have been conducted in paediatric populations. A cardioprotective effect was observed by Checchia and colleagues in a population of 16 children undergoing tetralogy of Fallot repair who were recruited to the randomised, blinded, placebo-controlled study.[24] In this study, 20 ppm of gaseous NO was added to the CPB circuit oxygenator in the intervention group, while the control group received placebo gas treatment. Twenty-four hours after surgery, children receiving NO had lower cTnI levels (8.9 ± 2.6 ng/mL *v* 12.5 ± 2.7 ng/mL; $P \leq 0.05$) and lower BNP levels (425 ± 154 pg/dL *v* 938 ± 511 pg/dL; $P \leq 0.05$), indicating an apparent cardioprotective effect. The study also analysed the inflammatory response following CPB, with no significant differences in levels of serum interleukin-6, interleukin-9 or tumour necrosis factor-α between groups. Contrary to this, levels of P selectin, which is released by endothelial cells in response to inflammatory cytokines, were markedly reduced following reperfusion in the adult NO patients studied by Gianetti et al (P = 0.02),[21] indicating a dampening of the inflammatory response.

James and colleagues examined low cardiac output syndrome (LCOS) as a specific primary endpoint, defined as lactate greater than 4 mmol/L and central venous oxygen saturation below 60%, or vasoactive inotrope score 10 or over or ECMO requirement.[25] In this study, the largest such trial conducted to date, 198 children who underwent any form of cardiac surgery with CPB were enrolled; 97 of them did not receive NO, and the remaining 101 children were randomly assigned to the NO intervention arm. The proportion of children developing LCOS was substantially lower in the intervention group (15% *v* 31%; P = 0.007); ECMO was used in one child in the treatment group, compared with eight in the control group. The results also showed a correlation between degree of benefit and age: the proportions of children < 6 weeks old developing LCOS in were 20% and 52%, respectively (P = 0.012), but no significant difference was seen for children aged more than 2 years (19% *v* 21%, respectively; P = 0.901). This difference may be explained by an increased susceptibility to LCOS in neonates owing to a reduced cardiac reserve, a more severe inflammatory reaction to CPB and immaturity leading to increased risk of end-organ damage,[27] compared with older children following cardiac surgery.[28,29]

Results from the study by Checchia et al indicated that although there was no difference in duration of hospital stay, there were significant reductions in time spent on mechanical ventilation (8.4 ± 7.6 *v* 16.3 ± 6.5 hours; $P \leq 0.05$) and time spent in the cardiac intensive care unit (53.8 ± 19.7 *v* 79.4 ± 37.7 hours; $P \leq 0.05$).[24] However, the larger trial by James et al found no differences in duration of ventilation (20.0 [range, 10–63] hours *v* 24.0 [range, 12–89] hours; P = 0.12), ICU length of stay (48.0 [range, 24–105] hours *v* 72.0 [range, 26–144] hours; P = 0.11) or hospital length of stay (9.0 [range, 6–17] days *v* 12.0 [range, 6–20] days; P = 0.164).[25] This difference could be a result of differences in baseline characteristics between the studies; for example, only surgery for a single indication (tetralogy of Fallot) was included in the study by Checcia et al while the study by James et al included all children undergoing any form of cardiac surgery with CPB.[24,25]

Table 2. Summary of human studies relating to nitric oxide (NO) use in adult and paediatric patients who underwent cardiopulmonary bypass (CPB) and suffered ischaemia–reperfusion injury and systemic inflammatory response syndrome as a direct consequence of CPB

Author, year	Population	Exclusion criteria	Country	Intervention	Comparator	Outcomes	Study design and enrolment	Intervention group size	Allocation concealment
Gianetti, 2004[21]	Patients > 18 years old, undergoing elective aortic valve replacement with aortocoronary bypass	Active infection, ejection fraction < 30%, malignancy, previous haematological, hepatic or renal disorders, corticosteroid or NSAID used within 7 days, post-operative use of nitrates	Italy	20 ppm NO introduced continuously to patient's ventilatory circuit during entire period of intubation	Standard CPB procedure	Myocardial injury — serum NO metabolites, CK-MB, cTnI, plasma BNP	Prospective, randomised non-blinded study	14	Inadequate
Kamenshchikov, 2019[22]	Adults undergoing planned primary coronary artery bypass graft with CPB	Non-elective surgery, > 70 years old, LVEF < 35%, history of MI within 3 months, chronic atrial fibrillation, diabetes mellitus, elevated cardiac markers 12 h before intervention	Russia	40 ppm NO administered during operation	Standard CPB procedure	cTnI and CK-MB as proxy biochemical markers for myocyte necrosis	Prospective, randomised study	30	Adequate
Lei, 2015[23]	Adults with normal kidney function undergoing elective multiple valve replacement surgery with CPB	Not reported	United States	80 ppm NO administered via a gas exchanger during CPB and up to 24 h after surgery	Nitrogen gas	Acute kidney injury and other post-operative complications	Prospective, randomised, double-blind trial	105	Adequate
Checchia, 2013[24]	Children undergoing tetralogy of Fallot repair between July 2007 and October 2010	Pre-operative increased vascular pulmonary resistance, cardiac arrest < 1 week before surgery, previous CPB, recent treatment with steroids	United States	20 ppm gaseous NO added to the CPB circuit oxygenator	Placebo gas	Markers of myocardial injury, inflammatory markers, clinical outcomes	Randomised clinical trial, only perfusionist unblinded	8	Adequate

(*Continues*)

Table 2. Summary of human studies relating to nitric oxide (NO) use in adult and paediatric patients who underwent cardiopulmonary bypass (CPB) and suffered ischaemia–reperfusion injury and systemic inflammatory response syndrome as a direct consequence of CPB (*continued*)

Author, year	Population	Exclusion criteria	Country	Intervention	Comparator	Outcomes	Study design and enrolment	Intervention group size	Allocation concealment
James, 2016[25]	All children undergoing elective cardiac surgery with CPB in 2014	Administration of NO immediately before surgery	Australia	20 ppm NO added to oxygenator gas inflow throughout CPB	Standard conduct of CPB	LCOS, blood loss, use of blood products, cardiac arrest, ICU admission, mortality	Randomised, controlled trial	101	Adequate
Chiletti, 2018[26]	Children having ECMO in 2016	Receiving NO before ECMO	Australia	20 ppm NO added to oxygenator gas inflow throughout ECMO	Standard ECMO 3 years prior	Survival, bleeding, methemoglobin	Pre–post comparison	100 *v* 30	

BNP = B-type natriuretic peptide; CK-MB = creatinine kinase–muscle/brain; cTnI = cardiac troponin I; ECMO = extracorporeal membrane oxygenation; ICU = intensive care unit; LCOS = low cardiac output syndrome; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NSAID = non-steroidal anti-inflammatory drug.

Chiletti and colleagues describe their experience of 30 consecutive children supported with ECMO and receiving 20 ppm NO in the oxygenator of the ECMO circuit (which is similar to a CPB circuit).[26] Administration of NO into the ECMO circuit was not associated with methemoglobin rises nor was it associated with bleeding. In a follow-up poster from the same group, Pan et al[30] reviewed 334 ECMO runs from 2012 to 2018, 141 (41%) were after the introduction of routine circuit NO delivery. NO use was associated with a statistically significant reduction in the need for circuit changes (odds ratio [OR], 0.35 [95% CI, 0.16–0.77]; $P = 0.009$), but no changes in the incidence of neurological injury or hospital survival. However, in children aged 6 months or less (221/344, 64%), NO use was associated with less circuit changes (OR, 0.25 [95% CI, 0.09–0.71]; $P = 0.009$), less neurological injury (OR, 0.39 [95% CI, 0.16–0.96]; $P = 0.04$) and the same hospital survival rate (OR, 1.04 [95% CI, 0.54–1.99]; $P = 0.91$).

Discussion

This review highlights possible new uses of NO either inhaled through the lungs or delivered in the fresh gas flow of the oxygenator of a CPB or ECMO circuit. In children, NO delivery during CPB is associated with reduced levels of serum inflammatory markers and decreased incidence of LCOS. In adults, a reduced inflammatory response is also seen, and the likelihood of developing AKI after CPB is reduced. These clinical findings are consistent with results from both human trials using surrogate biochemical markers, to quantify organ injury, and evidence from animal models of ischaemia–reperfusion injury. For example, NO appears to convey neuroprotective and cardioprotective effects in mice. These results make the potential benefits in humans plausible. Previous studies have proposed roles for NO in preventing platelet aggregation, impact on white cell migration and function and also providing anti-inflammatory effects[3] which may also provide protection against SIRS, ischaemia–reperfusion injury and infarction.

The strength of this review is the rigor of the methods used — the search strategy was as inclusive as possible, and references were reviewed to ensure capture of all relevant articles. A limitation is the small number of relevant human clinical trials conducted to date. Nonetheless, these initial results show improved clinical outcomes with the use of NO, particularly in paediatric patients after CPB.

Most of the outcomes discussed in this review were based on surrogate markers that assess end-organ function, as opposed to clinical outcomes. While these results are promising, the full extent of the effect of NO remains unclear, so further clinical trials are needed.

Currently, it appears that NO is safe and could mitigate IRi and SIRS in patients after CPB and children receiving mechanical support. Its antiplatelet effect could lead to

increased circuit survival time and its potential benefits in brain injury and hypoxia ischaemia may lead to improved neurological outcomes.

Two large scale randomised trials looking at NO after CPB in children will compare NO to placebo, providing data on the effect of NO on LCOS, morbidity and mortality in children undergoing surgery for congenital heart defects.[31] Further work is also needed to examine the mechanism of action, serum NO concentrations and titration of treatment, and the timing of administration; such work will provide a full safety profile and optimise the use of this drug.

Conclusion

Nitric oxide administered to patients undergoing CPB appears to have several clinical benefits, including lower incidence of LCOS in infants and lower incidence of renal injury in adults. Further trials in different clinical situations involving SIRS, ischaemia–reperfusion, cardiac arrest and ECMO in cardiac surgery in both adults and children are ongoing.

Competing interests

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