Review

Inhaled nitric oxide in cardiology practice

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Received 1 December 1998; accepted 1 March 1999

Abstract

Inhaled nitric oxide allows selective pulmonary vasodilatation with rapidity of action. It is effective in the acute post-operative management of pulmonary hypertension in cardiac surgical patients and is also valuable in assessing the pulmonary vasodilator capacity in patients with chronic pulmonary hypertension. This review examines the current role of inhaled nitric oxide in cardiology medicine, discussing issues concerning its administration and toxicity, as well as a summary of clinical studies in cardiac patients. New roles, as a modifier of platelet and leukocyte function, post-thrombolysis and following lung transplantation are described. New agents and alternative therapies, which prolong pulmonary activity, are also discussed. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Nitric oxide; Pulmonary circulation

1. Introduction

One of the most remarkable discoveries made in modern medical science is that nitric oxide (NO), which has long been regarded as a toxic constituent of cigarette smoke and environmental pollutant [1], plays a critical role in a startling number of physiological processes. Examples include nitric oxide’s role as a modulator of vascular tone, platelet and leukocyte activation, its role as a neurotransmitter and as a mediator of immune and inflammatory responses. Its fundamental action within the blood vessel is activation of soluble guanylate cyclase, catalysing formation of cGMP from GTP [2]. Nitric oxide is a free radical that exists as an unstable gas at room temperature. A therapeutic role for inhaled NO (INO) was first suggested in 1991 in a lamb model of hypoxic pulmonary hypertension [3] and in a series of post-surgical patients with pulmonary hypertension [4]. From these studies, it was recognised that pulmonary vasodilatation could be achieved by low concentrations of INO (5–80 ppm), without systemic haemodynamic effects. The first widespread therapeutic role for NO was found in the management of persistent pulmonary hypertension of the newborn [5,6]. Since then, the role for INO has been further defined, not only in pulmonary, but also in cardiac disorders. This review will focus on the role of INO in cardiology medicine; its administration and pharmacology, a discussion of the haemodynamic consequences of INO (particularly with respect to left and right ventricular function), as well as non-haemodynamic uses of INO and recent developments in alternatives and supplementary therapies to INO.

2. Background

Following inhalation, due to its small size and lack of charge, NO diffuses rapidly across the alveolar cells to the subjacent vascular smooth muscle of the pulmonary arterioles to activate guanylate cyclase and increase cGMP levels [7] (see Fig. 1). This results most commonly in
muscle [13,14] and myocardial cell [15–17] preparations. Nitric oxide has also been implicated in the cardiac dysfunction of cardiomyopathy [18], cardiac allograft rejection [19,20] and sepsis [13]. In addition, increased inducible NO synthase expression and activity has been reported in states of dilated cardiomyopathy and myocarditis [18]. In vivo studies have suggested that NO may be involved in the downregulation of β-receptor responsiveness in patients with heart failure [21]. While all these reports suggest a negative inotropic effect of NO, it has recently been found that NO may have concentration-dependent effects on contractility with positive inotropic effects at very low concentrations [22,23]. As INO is typically used in hemodynamically unstable or critically ill patients, it is important to discern whether these experimental findings have clinical significance.

3. Pharmacology and toxicity

3.1. Delivery and monitoring of INO

Radiolabelling studies have shown that approximately 75% of the NO delivered by inhalation is absorbed [30,31]. Because it is rapidly transformed into higher oxides of nitrogen (NO₃⁻) in the presence of oxygen, NO is usually delivered mixed with the stable gas nitrogen, N₂. It has been shown that the concentration of NO₃⁻ delivered is dependent not only on the concentration of NO delivered, but also the concentration of oxygen with which it is mixed and the residence time in the delivery circuit [32]. This is of particular importance in critically ill and ventilated patients in whom the concentration of inspiratory O₂ is high and may have greater deadspace in the ventilatory apparatus. For this reason, recent guidelines recommend the NO/N₂ mixture is delivered into the ventilator circuit as close to the ventilator as possible, preferably with a mixing device in the inspiratory limb (to minimise peak gas concentrations) and synchronised inspiratory injection, where available [33].

While the commonest method for monitoring NO concentration delivered is the measurement of an electrochemical gradient induced by NO (with an accuracy of 1 ppm), the gold standard remains chemiluminescence monitoring (accuracy of a few ppb) [33]. Expiratory monitoring or scavenging is not considered necessary, as the effect of delivering NO on the ambient NO and NO₃⁻ concentrations is negligible [34]. As methaemoglobin (MetHb) is a by-product of the metabolism of INO (discussed subsequently), it has been recommended that serum MetHb measurements should be performed prior to, at 1 h, 6 h and daily following institution of INO [33].

3.2. Metabolism of INO

On arrival in the bloodstream, NO rapidly combines
with the haem unit of haemoglobin and thiol groups of serum proteins [35,36]. The affinity of NO for these binding sites is reflected in its in vivo half-life of only 100ms (approximately 30 fold faster than in vitro estimates) [37]. It has been estimated that the affinity of NO for haemoglobin is 1500 times higher than that of carbon monoxide [11]. Indeed, haemoglobin is involved in maintaining locality of action of endothelial derived NO [38] as well as inactivation of the high concentrations of NO inhaled non-therapeutically, as delivered by cigarette smoke [39]. The major pathway in the metabolism of NO involves the production of MetHb and nitrate ($NO_3^-$) from oxyhaemoglobin and NO [40], Fig. 1. An alternate pathway, nitrosylation of haemoglobin to form nitrosyl-haemoglobin, NOHb [30], may be more important in venous circulation due to the lower $O_2$ tension. Nitrosyl-haemoglobin has not been shown to increase in response to INO in arterialised blood [40,41]. While, in gaseous phase, NO will react with oxygen to produce $NO_2$ gas, in aqueous phase, the major product is nitrite ions, $NO_2^-$ [42]. Nitrite may then react with haemoglobin to form NOHb as an intermediary to the formation of MetHb and $NO_3^-$ [40], before the latter is excreted, mostly in the urine [30,40]. Using radiolabelled NO ($^{15}NO$), it has been shown that nitrate ($^{15}NO_3^-$), accounts for at least 70% of the end product of INO in healthy individuals [43].

It has been suggested that the formation of S-nitrosothiols by interaction with serum thiol groups may provide an important intermediary pathway in the activity of NO [35,44]. The half-lives of nitrosothiols are much longer (15–30 s) than free NO [41] providing a theoretical mechanism for activity at sites outside the pulmonary vasculature (Fig. 2). Quantitatively, the largest NO adduct is S-nitroso-serum albumin, which accounts for approximately 80% of all nitrosothiols [44]. It has been suggested that the mechanism of NO activity due to nitrosothiols may not be by spontaneous release, but via a catalytic reaction at the level of the smooth muscle cell wall [45], see Fig. 2. Notwithstanding this pathway, the majority of studies have not shown any systemic effects due to INO when delivered at therapeutic concentrations. One experimental trial at concentrations of INO up to 200 ppm found decreased right atrial pressure, as well as decreased LV end-diastolic pressure and volume in anaesthetised minipigs [46]. The proposed mechanism for such an effect was a decrease in systemic venous tone, without significant changes in systemic arterial parameters or resistance.

### 3.3. Effective concentrations

Initial reports of INO in the treatment of pulmonary hypertension used 5–80 ppm [5,6]. It has since been shown that there is little extra haemodynamic benefit obtained above 10–20 ppm in cardiac patients [24–26], and effective doses required in patients with acute hypoxic respiratory failure have been as low as 100 ppb [27,28]. When considering the appropriate dose, defining the desired endpoint is also important. Doses resulting in a haemodynamic response (usually defined by a decrease in pulmonary vascular resistance) may be higher than those required for an improvement in oxygenation [29]. Doses required for anti-platelet effect or inhibition of leukocyte adhesiveness, discussed later, may be even higher.

#### 3.4. Toxicity of INO

Toxicity due to INO may be due both to the effects of NO itself or to its metabolites. Extreme concentrations of INO are associated with acute oxidation injury in the lung with consequent inflammation, desquamation and interstitial oedema [47]. It has been suggested that some of the damaging respiratory effects of cigarette smoke may be due to the high concentration of INO delivered (500 ppm) with consequent nitrosylation of proteins, production of peroxynitrites and depletion of antioxidants, interfering with fundamental enzyme and signalling systems [48]. Experimental and clinical studies have not revealed pulmonary histological abnormalities or evidence of oxidative damage due to continuous INO at therapeutic concentrations of 20–80 ppm, however [3,41,49]. In reports of the prolonged administration of INO (20 ppm for up to 53 days) in a group of patients with adult respiratory distress syndrome (ARDS) [50], and for 68 days at a mean 50 ppm [51] no evidence of lung toxicity has been detected. Genetic studies have not shown any increase in aberrant peripheral blood lymphocytes, indicative of chromosome abnormalities, in response to short duration INO (2 h at 40 ppm) [52].

A second line of toxicity is due to higher oxides of NO formed prior to inhalation. As the higher oxides of nitrogen ($NO_x$) are very toxic to humans, continuous monitoring of NO as well as $NO_x$ is required. During high oxygen flow
rates, these higher oxides may accumulate and themselves be associated with systemic hypotension. Adverse effects of NO\textsubscript{2} include depletion of antioxidants and lipid peroxidation at markedly lower concentrations (14 ppm) than are needed for NO toxicity [53]. While it has been recommended that inspiratory NO\textsubscript{2} levels be maintained at less than 1 ppm [54], United Kingdom guidelines for the use of INO in intensive care accept a maximum inspiratory concentration of NO\textsubscript{2} of up to 3 ppm [33].

A further toxic pathway relates to the metabolic by-products. While small increases in MetHb levels have been documented [4], there have not been any reports of significant morbidity due to this alone. With brief cessation of INO, MetHb levels rapidly return to normal. During continuous INO at 40 ppm, Jacob and colleagues found that MetHb levels stabilised while serum nitrates gradually climbed, consistent with MetHb being an intermediate in the NO metabolic pathway [41]. In a dose response study, Young showed that at usual therapeutic doses of INO (32 ppm) MetHb levels peaked at only 1%. Significant MetHb (5% of total haemoglobin) could be induced within one h of inhalation with extreme concentrations of INO (512 ppm) [55]. Concentrations lower than this resulted in plateauing of the MetHb concentration curves, suggesting that NADH MetHb reductase activity was not saturated at those levels in normal, healthy volunteers. Similar levels of MetHb have been obtained in patients with cardiac failure, following short inhalations, to a maximum of approximately 2% at 80 ppm [24].

An indirect adverse effect of NO is associated with its short duration of action. It has been reported on numerous occasions that abrupt cessation of delivery may be associated with deleterious increases in pulmonary pressures and right ventricular decompensation [29,56–58]. This may be inadvertent and corrected by recommencement of delivery [54,58], but further emphasises continuous monitoring of inhaled concentrations. One of the mechanisms postulated to account for this is suppression of the endogenous NO pathway in the pulmonary vasculature [59]. This has been shown to be true for bovine aortic endothelial cells exposed to NO and NO donors [60] and is further supported by experimental studies of chronic hypoxia combined with prolonged (3 week) INO administration which have shown impairment in pulmonary endothelial function [61]. Studies have generally shown that protein and transcription levels of NO synthase (NOS) are unaffected [62], but both guanulate cyclase and NOS activity may be decreased by NO [62,63]. Suggested mechanisms for this negative feedback include conformational changes of NOS due to NO binding [60], or peroxynitrite formation directly inhibiting NOS activity [63]. Whatever the mechanism of the rebound increase in pulmonary vascular resistance (PVR) following cessation of INO, it has been recommended, for ventilated patients, that a back-up system be available [33]. With respect to weaning of INO, it is worth noting that the rebound haemodynamic changes may be transient, and some investigators have suggested that a mild increase in pulmonary pressure need not mandate continuing INO therapy if it is haemodynamically tolerated [57].

4. Uses of INO in adult cardiology practice

In adult cardiology practice, the main roles for INO are in the management of post-operative pulmonary hypertension after cardiac surgery and in the assessment of acute pulmonary vasodilator reserve in patients with isolated pulmonary hypertension. Other uses include the assessment of acute pulmonary vasodilator reserve in patients who are being assessed for heart transplantation and who have pulmonary hypertension secondary to left ventricular failure, and in the treatment of acute pulmonary hypertensive crises in patients with pulmonary hypertension of any cause. The commonest use for INO in adults in the United Kingdom is in the management of ventilated patients with ARDS [33], following early reports of benefit [50]. Subsequent studies in similar patients have confirmed a minor decrease in oxygen and ventilation requirements, but no improvement in mortality [64,65].

4.1. Use of INO in post-operative cardiac patients

4.1.1. Post coronary artery bypass grafting

Inhaled NO, at concentrations of 20 and 40 ppm, has been shown to be effective in decreasing pulmonary pressures in a number of studies following coronary artery bypass grafting (CABG), even in subjects without marked pre-existing pulmonary hypertension [26,66,67]. This is itself significant, in that subjects with normal pulmonary resistance do not usually respond to INO. These results suggest that there is a degree of reversible pulmonary hypertension, due to pulmonary endothelial dysfunction (discussed subsequently), or possibly increased levels of circulating cytokines associated with cardiopulmonary bypass [68]. Not all studies have demonstrated a benefit post CABG, however. In one surgical series, it was found that only those subjects undergoing cardiac surgery for a mitral valvular problem responded significantly to INO, not those undergoing CABG [69]. The differences in mean pulmonary artery pressures between these studies was not marked (29(1 mmHg [26]; 20(1 mmHg in [66]; median of 17 mmHg (range 12–24 mmHg) [69]), but still may have had a role in explaining the different results. Previous studies in patients undergoing cardiopulmonary bypass have shown that the degree of decrease in PVR due to INO is proportional to the pre-operative PVR [70].

The evidence for pulmonary endothelial abnormality associated with cardiopulmonary bypass is convincing. Wessel has shown that, in children undergoing repair of acyanotic congenital heart defects, whereas acetylcholine (ACh) induced significant pulmonary vasodilatation pre-
operatively, the same dose was much less effective post-bypass [71]. Pulmonary vasodilatation was still able to be achieved using INO, suggesting pulmonary endothelial defect. Furthermore, cGMP levels following ACh were not increased post-bypass, but again were increased following INO, suggesting normal vascular smooth muscle production capability. In a further series of 30 children, Beghetti documented a 75% decrease in exhaled nitric oxide following cardiopulmonary bypass [72]. This occurred despite a post-operative decrease in haemoglobin, which has previously been associated with increased exhaled NO. The possibility of increased NO metabolism is not excluded by this study, but in light of other evidence, it appears likely that pulmonary endothelial dysfunction is the major cause.

4.1.2. Pulmonary hypertension associated with repair of congenital and valvular heart disease

Because congenital heart disease is itself associated with pulmonary hypertension, and may be associated with pulmonary hypertensive crises immediately following corrective surgery, selective pulmonary vasodilatation has been shown to be beneficial, in infants [73,74], and children [75,76].

In adults, mitral valvular disease is also frequently associated with pulmonary hypertension. In a trial of only 10 min INO (37 ppm), Girard showed a 22% improvement in PVR immediately post-operatively in a series of 6 patients undergoing mitral valve replacement for mitral stenosis [77]. In a mixed series of surgical patients (predominantly mitral valvular disease), Rich showed a 35% decrease in PVR, which was dependent on the baseline PVR [70]. In a series of 9 patients undergoing mitral valve surgery with only mild resting elevation in PVR (median 274 dynes.s.cm⁻²), Snow found that INO reduced post-operative pulmonary vascular resistance, by 42% [69]. These studies are contrasted with a further study by Fullerton who found that in adult patients undergoing valvular surgery, no benefit of INO was seen [78]. The difference between these studies is likely to be related to the greater severity of the pulmonary hypertension in the latter group (mean PVR 622 dynes.s.cm⁻²). The vascular remodelling involved in these patients most likely involved a degree of fibrosis limiting any reversibility. Similar results are seen in cases of long-standing pulmonary hypertension due to other causes [79,80].

4.1.3. Post cardiac transplantation

Because of immediate morbidity and mortality due to pulmonary hypertension following cardiac transplantation, INO has a particular role to play [81,82]. The donor heart has to adapt to a new haemodynamic state, during which time the PVR may need to be modulated selectively. As with other conditions, traditional vasodilator therapy often is associated with significant systemic hypotension limiting use [54]. In a study comparing conventional vasodilators and INO, Kieler-Jensen found that intravenous prostacyclin was the most potent in increasing (LV) cardiac index post transplantation, but that INO remained the only selective pulmonary vasodilator [83]. The PVR achieved by prostacyclin and INO were similar. It has been suggested that therapeutic response times were longer in the transplanted patient, possibly due to histological or structural changes in the lung [81].

4.1.4. Post insertion of left ventricular assist device

After insertion of left ventricular assist devices (LVAD), pulmonary hypertension and right heart failure is associated with inadequate LVAD filling and decreased survival. In one early series, over 20% of patients developed right heart failure or required a right ventricular assistance following LVAD implantation [84]. Inhaled NO has been used successfully in these situations to decrease pulmonary pressures and support the right ventricle until cardiac outputs are equilibrated or transplantation performed [85,86]. For this reason, INO has been recommended prior to consideration of implantation of an adjunctive right ventricular assist device, as this may be able to be avoided if there is a good response to INO [87]. Such haemodynamic benefits have been confirmed in a small randomised trial, supporting this use [88].

4.2. Use of INO in patients with congestive cardiac failure

Inhaled NO is frequently used in patients with congestive cardiac failure as part of their evaluation for cardiac transplantation [79]. As fixed pulmonary hypertension has been associated with increased morbidity and mortality immediately post transplantation [89], an assessment as to whether this can be corrected by vasodilators is required. Concern has been aroused, however, by a number of patients with stable congestive cardiac failure developing documented pulmonary oedema during administration of INO [90]. Furthermore, a consistent haemodynamic finding in patients with severe heart failure is not a decrease in mean pulmonary artery pressure, but an increase in mean pulmonary capillary wedge pressure or LV end-diastolic pressure during administration of INO [24,25,91,92]. These clinical observations, in combination with the in vitro experimental evidence of a direct negative inotropic action of NO [13,15–17] has led to concern that INO may exert a clinically important negative inotropic effect in patients whose ventricular function is already impaired.

4.3. Left ventricular effects of INO

In one study of patients with normal LV function, a negative inotropic effect of INO was suggested due to lack of increase in cardiac output despite a significant decrease in RV afterload [26]. We have also found a decrease in cardiac output in occasional patients with heart failure.
during administration of INO, despite the appropriate decrease in pulmonary resistance (reported in part [25]). While INO is inactivated rapidly in the presence of haemoglobin, the combination of NO with thiol groups on haemoglobin and plasma proteins to form active nitrosothiol adducts with longer half-lives [44], discussed earlier, makes it possible that INO could exert effects at more distal vascular beds such as the coronary circulation. In view of the experimental evidence for a negative inotropic effect of NO [13–17], and the possibility of action beyond the pulmonary circulation via nitrosothiols, it was proposed that INO might be having an adverse effect on LV function. A number of studies have subsequently been performed to address this very issue.

Because analysis of ventricular function in response to pharmacological agents is frequently complicated by changes in loading conditions, we and others have used simultaneous pressure–volume relations to assess the effect of INO [93–96]. In a population with normal LV function, we found no effect of INO on indices of left ventricular performance [94]. Previously documented changes in LV preload were therefore attributed to changes in ventricular loading, and not indicative of a negative inotropic effect for INO. Indeed, modelling studies have suggested that an increase in LV preload can be predicted if the baseline pulmonary volumes are already elevated and the PVR decreases significantly in response to INO, independent of any change in inotropic status of the model ventricle [97]. The reason that such changes are seen in severe congestive cardiac failure is because of the elevated pulmonary volumes at rest. The lack of negative inotropy due to INO has been confirmed in animal models of heart failure and pulmonary hypertension [96] as well as in a human population [93]. Consistent with these results, is a further study by Hare and co-workers in subjects with cardiac failure dependent on mechanical left ventricular assistance [98]. Using an LVAD to assist cardiac output both on and off INO, it was found that INO increased left ventricular filling pressures only when cardiac output was not assisted. During cardiac assistance there was no increase in left atrial pressure. Despite the in vitro evidence, therefore, these in vivo studies are consistent in suggesting that an increase in pulmonary venous pressure only occurs when the ventricle is unable to respond to increased preload by increasing output, rather than the increased preload occurring as an expression of negative inotropy due to INO.

It has been suggested that the pulmonary hypertension associated with cardiac failure is not merely accumulation or ‘backpressure’ due to poor left ventricular output, but a reflex vasoconstriction, possibly to reduce left ventricular preload and protect the lungs from pulmonary congestion, at the cost of increased right ventricular work [91]. It remains important to note, however, that even in the absence of any inotropic effect, massive pulmonary vaso-dilatation may occasionally overwhelm the LV if it is significantly impaired [25,99]. By decreasing PVR in these patients, the failing LV is subjected to an excessive volume load with which it may not be able to cope.

4.4. Use of INO in pulmonary hypertension

While INO has been shown to be of benefit in reducing the PVR of acute pulmonary hypertension, independent of cause [3,100], its effects in patients with chronic PHT are less predictable. Although some patients with chronic PHT do show an acute vasodilator response to INO, others do not [80,101,102]. This most likely relates to the relative degree of medial hypertrophy versus intimal hyperplasia and luminal thrombosis that occurs in the small pulmonary arteries in chronic lung vascular disease [80]. Because of its potency and rapid onset of action, INO has been suggested as a screening tool for defining which patients with pulmonary hypertension may benefit from long term oral vasodilating therapy, such as calcium antagonism [101,102]. In these studies which used invasive haemodynamic monitoring, there was a strong correlation between response to INO and subsequent response to oral therapy. Because only 30–40% of the subjects respond to INO [101–103], this finding represents a significant step forward in directed therapy. An attempt to assess the response to INO using a 6-min walk as a non-invasive marker of reversibility of pulmonary hypertension was not successful in a small clinical trial [104]. In that study of 6 patients, there was no significant difference in distance walked or symptoms experienced during INO compared to placebo. Occasional patients with PHT who do have a vasodilator response have been ‘bridged’ to transplantation with INO for as long as 68 days [51]. Inhaled NO may also have a role in ‘bridging’ patients with a degree of fixed pulmonary hypertension during periods of acute pulmonary decompensation.

4.4. Right ventricular effects of INO

Because of the haemodynamic separation of the right ventricle from site of delivery of INO, any effects are indirect. As PVR is the main determinant of right ventricular afterload, the ventricle is very sensitive to any changes. A number of studies have suggested that right ventricular efficiency is improved in response to INO, mainly as a result of decreasing pulmonary input impedance rather than any change in right ventricular contractility [105,106]. One study, examining RV function in response to INO in experimental pulmonary hypertension, found improved RV dP/dt during INO [107], though this is a load-dependent measure and not well suited to comparisons made across differing volume and afterload states. The importance of this distinction is shown in a further study, which induced severe pulmonary hypertension, by microbead injections. The increase in PVR was itself associated with an increase in RV dP/dt, which was partially normalised (decreased)
by INO [100]. Stroke volume decreased due to increased PVR and again was partially restored by INO, consistent with improved haemodynamics, and independent of contractility changes.

Human studies involving impaired RV function in association with pulmonary hypertension have also suggested benefit from INO [108]. In a study of 14 patients with severe congestive cardiac failure, Koelling found that those subjects with the most impaired right ventricular function responded best to INO in improving their exercise capacity [109]. From this, it was suggested that the pulmonary hypertension associated with the heart failure was limiting right ventricular output and contributing to their exercise intolerance [109]. A study of seven subjects with congestive cardiac failure performing upright bicycle exercise, also showed improved oxygenation and maximal oxygen uptake in response to INO associated with an improved exercise tolerance [110]. The results in this latter study were explained on the basis of improvement in ventilation perfusion matching. This is consistent with redistribution of pulmonary blood flow to better ventilated areas in response to INO, as has been shown in a study using scintigraphy in patients with severe cardiac failure [111]. A recent study found that the improvement in ventilation/perfusion matching may be associated with improvement in arterial oxygenation in a group of patients with Class II–III congestive cardiac failure [112].

4.5. Anti-platelet effects of INO

Anti-platelet effects due to NO activation of soluble guanylate cyclase in the platelet cytosol have been recognised for a number of years [113]. It has since been recognised that this activity is evident even with inhalation [114]. The main effects of INO on platelet function have been seen as an increase in bleeding time, with little effect on laboratory indices of platelet function in healthy subjects [115] or neonates [116]. In vitro studies have shown a dose-dependent inhibition of platelet aggregation, P-selectin expression and fibrinogen-glycoprotein IIb/IIIa binding [117]. That study used dose ranges from 100–884 ppm, but similar results have also been obtained in patients with ARDS while receiving INO [118]. No increase in bleeding time was seen in the latter study. A therapeutic role for INO in limiting platelet deposition and activation in massive pulmonary embolism has recently been suggested [119]. By both inhibiting platelet activation and decreasing pulmonary resistance, INO may have dual activity in this setting. Increased use of INO may introduce adverse reactions in unexpected situations. Two cases of increased bleeding in patients undergoing chemotherapy who required ventilation and INO therapy have been reported [120].

In a canine model of thrombolysis, Adrie and co-workers found that INO increased coronary patency post thrombolysis [121]. In this model, denuding an area of endothelium, occluding the coronary artery, delivering thrombus and then administering thrombolysis to restore patency was used to generate a platelet rich thrombus. While there was no effect of INO on measures of coronary flow, the fraction of time that the artery was open was increased by both 20 and 80 ppm INO. Other indices of anti-platelet activity were a decrease in the cyclic flow variations that have been associated with intermittent platelet deposition in similar models of thrombolysis. Despite the short half-life of INO, the anti-platelet effect lasted for at least 45 min after cessation of the 80 ppm INO. In this study, a higher concentration of INO (200 ppm) was not associated with any benefit. Inhaled NO, at concentrations of 200 and 400 ppm, did inhibit in vitro ADP-induced aggregation, but in vivo bleeding times were not affected. The lack of effect at the higher concentration of INO (200 ppm) may have been related to excess formation of peroxynitrite at the higher concentration, which may have a pro-aggregatory effect on platelets under certain conditions [122]. The anti-platelet effects of INO remain minor compared to aspirin [115], and it cannot be recommended as an alternative in this setting.

The possibility that INO could have long-lasting physiologic or even structural effects is suggested by an experimental model of restenosis following balloon carotid injury in rats [123]. In that study, rats given 80 ppm INO for 1 or 2 weeks starting 1 h prior to injury were found to have lower intima/media ratios compared to those breathing air alone. These results were not reproduced if only 1 week of INO was given followed by air for 1 week or if the rats were given a lower dose, 20 ppm, for 2 weeks. The ability of INO to block collagen induced in situ platelet activation in rats has also been shown by Nong and colleagues at concentrations of 40 ppm or greater [124]. In that study, the degree to which platelet aggregation could be blocked was closely associated with the intraplatelet cGMP levels, confirming activation of guanylate cyclase as the likely mechanism of action. A possible mechanism for persisting effect, despite only relatively short periods of inhalation, may be that INO may acutely alter platelet function blocking release of mitogens involved in migration of cells into the intima. This effect may be particularly important in diseased coronary arteries related to impaired release of NO in patients with coronary atheroma [125]. It is of note that the concentrations required for platelet effects are greater than those required for haemodynamic benefit. This may be understood in light of the inactivation of NO by haemoglobin as it traverses the bloodstream prior to interaction with platelets.

4.6. Effect of INO on leukocyte adhesion

While NO is involved in the mediation of the inflammatory response and massive release is associated with the hypotension and negative inotropy of sepsis [2], it has also been shown that inhibition of NO actually
increases neutrophil-endothelial cell interaction [126]. A subsequent study has shown that the increase in leukocyte adhesion with L-NAME can be abolished by INO [127]. In that study, inhaled NO had no effect on leukocyte adhesion in the absence of NO depletion, nor in states of NO excess, induced by endotoxemia. These results suggest that INO may be helpful in replenishing NO activity in states of pulmonary endothelial deficit. This mechanism may be important in explaining the benefit of INO in acute reperfusion injury seen in transplanted lungs [128]. Such lungs have been shown to have depleted endothelial function [129] as well as dense polymorph infiltration. Murakami showed that INO (80 ppm) given during the period of warm ischaemia and during reperfusion following lung transplantation from non-heart-beating donors was associated with a marked improvement, not only in lung function, but also a significant decrease in myeloperoxidase activity [130]. Inhaled NO maybe particularly beneficial in this setting, as it replenishes the pulmonary endothelial NO deficit thereby reducing PVR through vasodilatation, as well as limiting the infiltration and subsequent activation of inflammatory cells. Again, the higher concentration of INO required for inhibition of leukocyte adhesion compared to haemodynamic effects most likely relates to inactivation of NO in transit.

5. Future

Experimental studies of the effects of NO analogues or nucleophile adducts, modified primarily to prolong half-life, have been encouraging. Brilli and associates showed that intermittent inhalation of ethylputreanine NONOate and 2-(dimethylamino)-ethylputreamine NONOate for only 3 min was able to reverse pharmacologically induced pulmonary hypertension in a porcine model of pulmonary hypertension [131]. The pulmonary effect lasted for over 50 min despite the short inhalation time, without any evidence of systemic effects. Such aqueous agents, which release NO at predictable rates, are suitable for nebulised therapy and therefore do not require a complex ventilatory apparatus. Questions concerning repetitive dosing and metabolism of the NONOate molecules remain to be resolved. Cyclic GMP phosphodiesterase inhibitors such as dipyridamole [132] or nebulised zaprinast [133] have also been suggested as methods of prolonging INO effect. Inhalation of a cGMP analogue itself, 8-BrcGMP, has also been shown to have selective pulmonary activity [134]. Inhaled prostacyclin analogues have also been used in similar settings to those covered by INO. The rationale for these is that the INO delivery requirements, possible toxicity and need for close continuous monitoring make widespread use of INO difficult [135]. Preliminary studies suggest inhaled prostacyclin does not cause systemic vasodilatation at concentrations used, is easier than INO to administer and has no apparent toxicity [136]. Further studies are obviously needed if these statements are indeed borne out.

6. Conclusions

Despite concerns over possible toxicity, INO has been shown to be safe when closely monitored and has significant advantages as a selective pulmonary vasodilator. It has a particular role in post-operative management of pulmonary hypertension in cardiac surgical patients and the assessment of pulmonary vasodilator capacity, not only in assessment prior to cardiac transplantation, but also in planning therapy for other forms of pulmonary hypertension. While INO is rapidly inactivated by haemoglobin, the possibility exists for nitrosothiol production, which may be an important intermediary in INO effect. New roles, including modifying both platelet and leukocyte function, offer benefits after lung transplantation and possibly postthrombolysis. New agents show promise in maintaining pulmonary selectivity as well as an increased duration of action.

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