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The Effect of Inhaled Nitric Oxide and Oxygen on the Hydroxylation of Salicylate in Rat Lungs

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ABSTRACT

Inhaled nitric oxide (iNO) is used as a selective pulmonary vasodilator, and often under conditions when a high fraction of inspired oxygen is indicated. However, little is known about the potential toxicity of iNO therapy with or without concomitant oxygen therapy. NO can combine with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$), which can in turn decompose to form hydroxyl radical (OH·). Both OH· and $ONOO^-$ are involved in various forms of lung injury. To begin evaluation of the effect of iNO under either normoxic or hyperoxic conditions on OH· and/or $ONOO^-$ formation, rats were exposed for 58 h to either 21% O_2, 21% O_2 + 10 parts per million (ppm) NO, 21% O_2 + 100 ppm NO, 50% O_2, 90% O_2, 90% O_2 + 10 ppm NO, or 90% O_2 + 100 ppm NO. We used a salicylate hydroxylation assay to detect the effects of these exposures on lung OH· and/or $ONOO^-$ formation measured as the appearance of 2,3-dihydroxybenzoic acid (2,3-DHBA). Exposure to 90% O_2 and 90% O_2 + 100 ppm NO resulted in significantly ($p < 0.05$) greater lung wet weight (1.99 ± 0.14 g and 3.14 ± 0.30 g, respectively) compared with 21% O_2 (1.23 ± 0.01 g). Exposure to 21% O_2 + 100 ppm NO led to 2.5 times the control (21% O_2 alone) 2,3 DHBA formation ($p < 0.05$) and exposure to 90% O_2 led to 2.4 times the control 2,3-DHBA formation ($p < 0.05$). However, with exposure to both 90% O_2 and 100 ppm NO, the 2,3-DHBA formation was no greater than the control condition (21% O_2). Thus, these results indicate that, individually, both the hyperoxia and the 100 ppm NO led to greater salicylate hydroxylation, but that the combination of hyperoxia and 100 ppm NO led to less salicylate hydroxylation than either did individually. The production of OH· and/or $ONOO^-$ in the lung during iNO therapy may depend on the ratio of NO to O_2. **(*Pediatr Res* 54: 337–343, 2003)**

Abbreviations

2,3-DHBA, 2,3-dihydroxybenzoic acid
SA, salicylate
NO, nitric oxide
NO_2, nitrogen dioxide
$ONOO^-$, peroxynitrite
O_2^-, superoxide
OH·, hydroxyl radical
O_2, oxygen
N_2, nitrogen
ppm, parts per million
V_T, tidal volume
Cdyn, dynamic compliance
Hct, hematocrit

NO inhalation has several clinical applications and is often used in conjunction with supplemental oxygen (1–7). Because NO has been shown to have both pro-oxidant and antioxidant properties (8–13), it is unclear how the combination of high NO and high O_2 may affect the lungs. We found previously that rats exposed to >95% O_2 and 100 ppm NO had lower mortality than rats exposed to >95% O_2 alone (13), suggesting that adding NO to a high inspired O_2 environment is protective against O_2 toxicity. However, the mechanism of this protection remains unclear (13).

OH· is involved in oxidative lung damage (14–17), and high O_2 can increase OH· production in lung tissue homogenates (18). In the presence of O_2, NO can form a variety of nitrogen oxides, including $ONOO^-$, which can also form OH· (8, 19, 20). Alternatively, NO may scavenge $ONOO^-$, with the resultant formation of less reactive oxides of nitrogen (21, 22). Thus, the overall effect of inhaled NO on $ONOO^-$ formation in a high O_2 environment within the lung tissue is difficult to predict. To determine the influence of inhaled NO on OH· and/or $ONOO^-$ production in the lungs during normoxia and hyperoxia, we measured the hydroxylation of SA (16, 17, 23–27) in the lungs of rats exposed for 58 h to various

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combinations of NO in O_2. The lungs were harvested 1 h after intraperitoneal (i.p.) SA injection, and physiologic variables, lung homogenate SA, and its $OH\cdot$ and/or $ONOO^-$ reaction product 2,3-DHBA were measured (15–17, 28).

METHODS

Exposures. Sixty-two rats (male Sprague Dawley, 250–325 g body weight) were used in the study. The institutional review boards of both the Zablocki VA Medical Center and the Medical College of Wisconsin approved the study. The rats in groups of two or three were placed in a 44 × 22 × 20 cm Plexiglas chamber as previously described (13). The gas within the chamber was continuously recirculated through a 1.5-L soda lime canister at 72 L/min. The 21% O_2 atmosphere within the chamber was maintained by a flow of compressed air at approximately 10 L/min. Fifty percent or 90% O_2 atmospheres were maintained within the chamber by mixing a flow of compressed air and O_2 to achieve a total flow rate of 10 L/min. The NO concentration was set at either 0, 10, or 100 ppm by introducing the appropriate flow of NO—10,000 ppm NO in N_2—into the O_2-air mixture. The NO and NO_2 concentrations in the chamber were monitored as previously described (13). There were six rats in the 21% O_2 group, six rats in the 21% O_2 + 10 ppm NO group, six rats in the 21% O_2 + 100 ppm NO group, 10 rats in the 50% O_2 group, 11 rats in the 90% O_2 group, six rats in the 90% O_2 + 10 ppm NO group, and 17 rats in the 90% O_2 + 100 ppm NO group.

Lung isolation. After 57 ± 4 (SD) h of exposure, the rats were given 100 mg/kg SA i.p. as previously described (16, 17). After an additional 1-h exposure, each rat was anesthetized with sodium pentobarbital (50 mg/kg) i.p. A catheter was placed in a carotid artery and 1250 U/kg heparin was given. Blood was obtained for determination of Hct and plasma SA, and the rat was exsanguinated. A midline sternotomy was performed, and cannulas were placed in the pulmonary artery and left atrium. The lungs were removed and perfused with 0.9% saline at 15 mL/min in a nonrecirculating manner until the venous effluent appeared to be clear of blood (3–5 min). During the perfusion, the lungs were ventilated with 15% O_2, 5% CO_2, and balance N_2 using a piston-type ventilator with a V_T of 5 mL and an end-expiratory pressure of 2 mm Hg. The pulmonary arterial and airway pressures were measured continuously during the perfusion. The Cdyn was calculated as the V_T divided by the difference between the end-inspiratory and end-expiratory airway pressures. The lungs were then drained, weighed (wet weight), and homogenized in 10% trichloroacetic acid (TCA) (1.5 mL per gram lung) at 4°C, the homogenate was centrifuged at 4°C, and the supernatant was stored at −70°C for later determination of SA and 2,3-DBHA. The plasma was also stored at −70°C for later determination of SA.

2,3-DHBA and SA assays. The amount of 2,3-DHBA and SA in the lung homogenate supernatant, and the amount of SA in the plasma was determined as described previously (16, 17). Briefly, the supernatant was filtered through a 0.22-μm filter, and 25 μL was injected onto a C18 RP HPLC column (250 × 4.6 mm, model 2353229; Beckman Coulter, Inc., Fullerton, CA, U.S.A.) using an eluent of 30 mM citric acid, 1.8% acetic acid, and 1.5% methanol at a flow rate of 0.5 mL/min. 2,3-DHBA was quantified using an electrochemical detector (model 1049A, Hewlett Packard, Palo Alto, CA, U.S.A.) at an oxidizing potential of +0.5 V. The HPLC separation was such that there was no significant interference with the 2,3-DHBA peaks from any product in the lung supernatant samples. The lower limit of detection was approximately 80 pmol of DHBA per milliliter of rat lung supernatant. SA measurements in the lung homogenate supernatant and plasma were also carried out using a previously described spectrophotometric technique based on the formation of a SA-ferric iron complex that absorbs at 530 nm (16, 17).

Statistics. The results are shown as the mean ± SEM. Relationships between pairs of variables, such as plasma SA and lung SA, were examined using linear regression with Sigmaplot software (Jandel Scientific, Costa Madre, CA, U.S.A.). Groups were compared using one-way ANOVA. Differences between groups were identified using a Newman-Keuls posthoc test. Differences were considered statistically significant when $p < 0.05$.

RESULTS

The effects of exposure to 21% O_2, 50% O_2, and 90% O_2 on the measured physiologic variables are shown in Table 1. The measured variables are generally consistent with the deleterious effect of high oxygen exposure, primarily reflected in the body weight loss, increased lung wet weight, increased Hct, and decreased lung compliance. The effects of exposure to 10 and l00 ppm NO in 21% O_2 on these variables were small (Table 2). Exposure to 10 ppm NO in 90% O_2 was not significantly different from exposure to 90% O_2 alone (Table 3), whereas exposure to 100 ppm NO in 90% O_2 resulted in significantly greater lung wet weight and lower Cdyn compared with rats exposed to 90% O_2 alone (Table 3). This suggests that exposure to 90% O_2 + 100 ppm NO led to even greater lung injury than exposure to 90% O_2 alone.

The mean plasma SA concentration 1 h after i.p. injection of 100 mg/kg SA in the exposure groups was 629 ± 31 μM. The ratio of lung SA concentration (nanomoles per liter lung homogenate supernatant) to plasma SA concentration (nanomolar) for each group studied is shown in Figure 1. There was a tendency for the ratio to increase with increasing O_2 concentration, and in both the 21% O_2 and 90% O_2 groups, the ratio increased with increasing NO concentration. There was a correlation between plasma SA concentration and the amount of SA in the lung for all the lungs studied ($r = 0.48$, $p < 0.05$; Fig. 2*A*), and between the amount of SA in the lung and the

Table 1. *The effect of oxygen tension on physiologic variables*

	21% O_2	50% O_2	90% O_2
Start weight (g)	284 ± 2	294 ± 3	299 ± 4*
60-h weight (g)	298 ± 3	300 ± 3	288 ± 4
Lung wet weight (g)	1.23 ± 0.01	1.48 ± 0.06	1.99 ± 0.14*
Hct (%)	40.0 ± 0.5	41.7 ± 1.0	44.0 ± 0.9*
Pa (Torr)	7.6 ± 0.2	7.1 ± 0.2	6.5 ± 0.4
Cdyn (mL/Torr)	0.77 ± 0.05	0.70 ± 0.04	0.56 ± 0.05*

Mean ± SE.

* Different from 21% O_2, $p < 0.05$.

Table 2. *The effect of NO in 21% O_2 on physiologic variables*

	21% O_2	+ 10 ppm NO	+ 100 ppm NO
Start weight (g)	284 ± 2	295 ± 2*	310 ± 2*
60-h weight (g)	298 ± 3	304 ± 2	317 ± 4*
Lung wet weight (g)	1.23 ± 0.01	1.45 ± 0.05*	1.43 ± 0.04*
Hct (%)	40.0 ± 0.5	43.4 ± 1.1	39.6 ± 1.1
Pa (Torr)	7.6 ± 0.2	6.6 ± 0.3*	7.8 ± 0.4
Cdyn (mL/Torr)	0.77 ± 0.05	0.64 ± 0.08	0.84 ± 0.08

Mean ± SE.
* Different from 21% O_2, $p < 0.05$.

Table 3. *The effect of NO in 90% O_2 on physiologic variables*

	90% O_2	+ 10 ppm NO	+ 100 ppm NO
Start weight (g)	299 ± 4	318 ± 4*	299 ± 2
60-h weight (g)	288 ± 4	302 ± 4*	275 ± 2*
Lung wet weight (g)	1.99 ± 0.14	1.79 ± 0.18	3.14 ± 0.30*
Hct (%)	44.0 ± 0.9	43.2 ± 1.1	49.7 ± 1.7*
Pa (Torr)	6.5 ± 0.4	6.3 ± 0.6	8.9 ± 0.8*
Cdyn (mL/Torr)	0.56 ± 0.05	0.73 ± 0.08	0.35 ± 0.02*

Mean ± SE.
* Different from 90% O_2, $p < 0.05$.

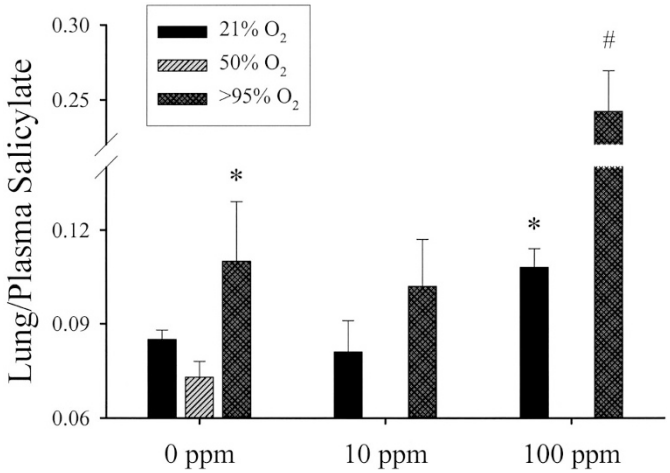

Figure 1. The mean lung to plasma SA ratio for each of the seven conditions studied. Notice the break in the y axis. Lung/plasma SA was greater with 100 ppm NO in both 21% and 95% O_2. *Different from 21% O_2, $p < 0.05$. #Different from 90% O_2, $p < 0.05$.

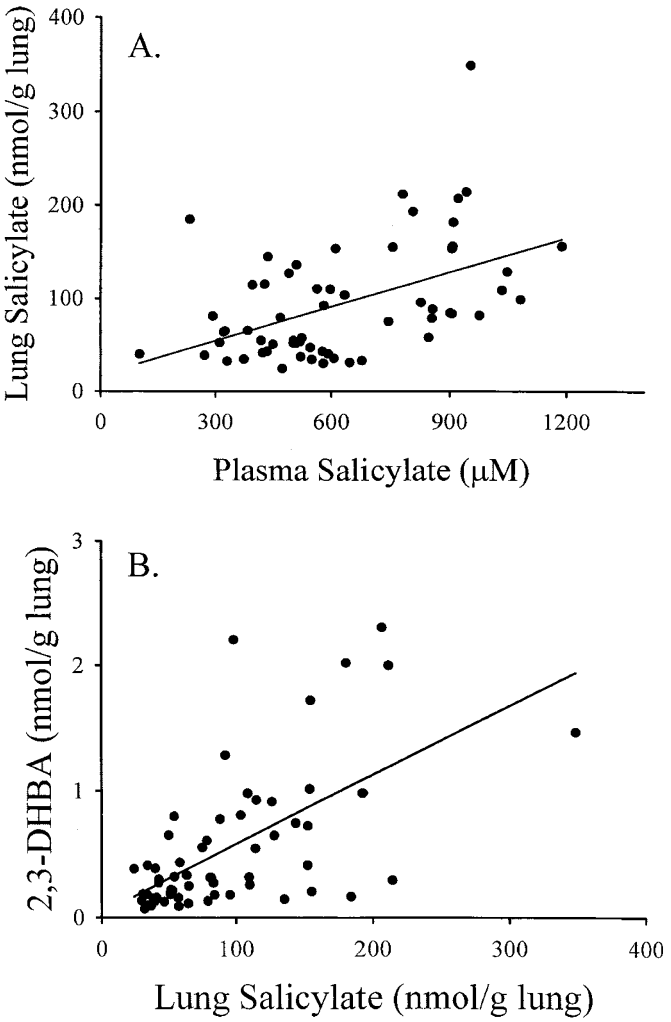

Figure 2. (*A*) The amount of SA in the lung *vs* the plasma SA. There was a significant correlation between plasma SA and lung SA. The line represents the linear regression of the points, and the resultant line is y = 0.12x + 17, $r = 0.48$, $p < 0.01$. (*B*) The total amount 2,3-DHBA in the lung *vs* the lung SA. There was a significant correlation between lung 2,3-DHBA and lung SA. The line represents the linear regression of the points, the resultant line is y = 0.005x + 0.03, $r = 0.61$, $p < 0.01$.

total amount of 2,3-DHBA in the lung ($r = 0.62$, $p < 0.05$; Fig. 2*B*). To remove the plasma SA concentration effect, the total 2,3-DHBA values presented in Figures 3, 4, and 5 were also normalized to total lung SA and expressed as a percentage of total lung SA.

Exposure to either 90% O_2 without NO or 100 ppm NO in 21% O_2 resulted in significantly ($p < 0.01$) higher lung 2,3-DHBA compared with the control 21% O_2 exposure (Figs. 3 and 4). The effect of exposure to 50% and 90% O_2 on SA hydroxylation is shown in Figure 3*B*, and 2,3DHBA/SA was significantly ($p < 0.01$) greater in 90% O_2 compared with 21% O_2. On the other hand, when NO was included in the 90% O_2, the lung 2,3-DHBA was less than with 90% O_2 alone (Fig. 5).

To determine the effect of lung wet weight on 2,3-DHBA, we plotted total lung 2,3-DHBA *versus* lung wet weight for only those animals exposed to 21% O_2, 50% O_2, and 90% O_2 and found a significant correlation between lung wet weight and 2,3-DHBA (Fig. 6; $r = 0.50$, $p < 0.01$). However, when the total lung 2,3-DHBA is plotted *versus* lung wet weight for all of the rats studied, as shown in Figure 7, the correlation is not significant ($r = -0.05$). Thus, changes in total lung 2,3-DHBA were not due solely to changes in lung wet weight.

DISCUSSION

The main findings of this study were that 1) increasing the fraction of inspired O_2 increased SA hydroxylation in the lung, and 2) adding NO under normoxic conditions increased SA hydroxylation in the lung, but 3) the combination of high O_2 and high NO prevented the increase in SA hydroxylation seen with either high O_2 or high NO alone. Using the lung wet weight as an index of lung injury, increased SA hydroxylation was correlated with lung injury upon exposure to the hyperoxic environment without NO. However, the lung injury was great-

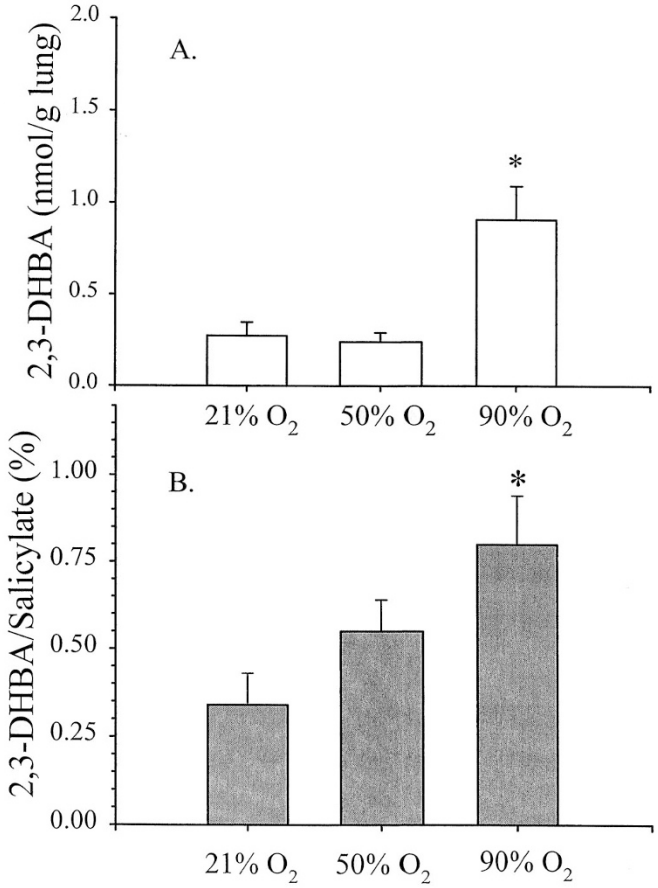

Figure 3. (*A*) Exposure to 90% oxygen resulted in more total 2,3-DHBA in the lung compared with exposure to the normoxic control (21% oxygen). (B) The formation of 2,3-DHBA was greater in 90% oxygen as demonstrated by a larger ratio of total 2,3-DHBA to total lung SA in 90% oxygen compared with 21% oxygen. *Different from 21% O_2, $p < 0.05$.

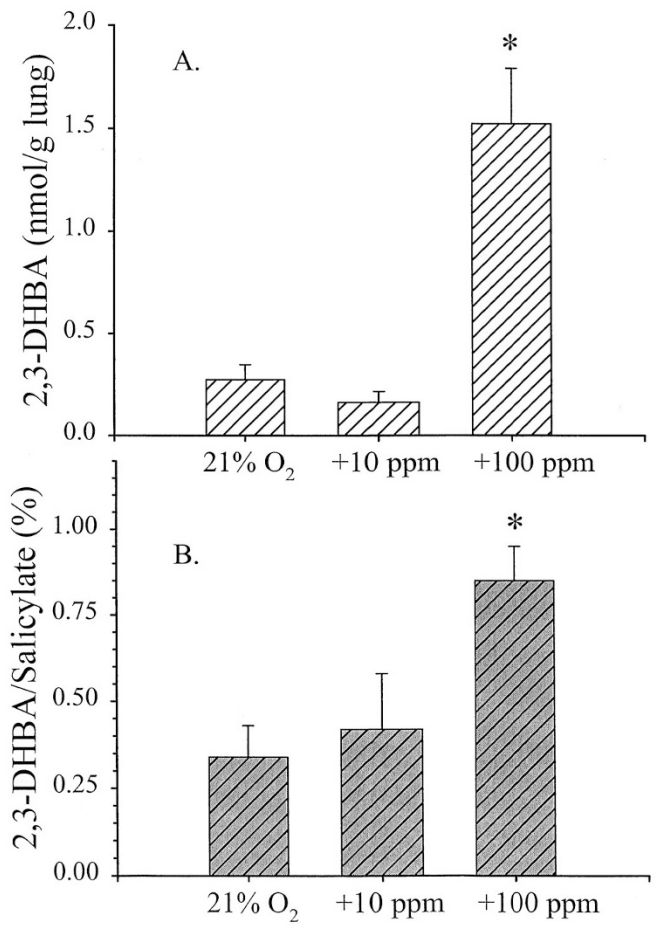

Figure 4. (*A*) Addition of 100 ppm NO to 21% oxygen resulted in more total 2,3-DHBA in the lung compared with exposure to 21% oxygen alone. (*B*) Addition of 100 ppm NO to 21% oxygen resulted in greater formation of 2,3-DHBA as demonstrated by a larger ratio of total 2,3-DHBA to total lung SA in 21% oxygen + 100 ppm NO compared with 21% oxygen alone. *Different from 21% O_2, $p < 0.05$.

est with hyperoxia plus NO, which produced no increase in SA hydroxylation.

SA hydroxylation to 2,3-DHBA has been used in rats to measure OH· production in a wide variety of oxidant injuries, and 2,3-DHBA formation in the lungs has been found to increase with ozone exposure (15), ischemia-reperfusion injury (29), hyperoxia (30), and silica exposure (16, 17). In the present study, we found that exposure to 90% O_2 resulted in increased SA hydroxylation. An association between O_2 and OH· production has been described previously. For example, in isolated rabbit lungs, 2,3-DHBA formation after ischemia-reperfusion was greater in air-ventilated compared with nitrogen-ventilated lungs (29). Rats exposed to 100% O_2 for 60 h had significantly higher plasma 2,3-DHBA/SA compared with rats exposed to 21% O_2 for 60 h (30). Neonatal rats exposed to 95% O_2 had significantly higher lung 2,3-DHBA compared with normoxic controls (31). These studies suggest a P_{O_2}-dependent production of OH·, an interpretation that is consistent with our results in comparing 21% with 90% O_2 exposure.

It has also been demonstrated that, under some conditions, OH· may be formed from NO and O_2^- *via* $ONOO^-$ (8, 19, 20, 32), for which the second-order rate constant is near the diffusion controlled limit (19, 22, 31). $ONOO^-$ has a pKa of 6.6, which means that a substantial amount of $ONOO^-$ will be pronated at physiologic pH to yield peroxynitrous acid (ONOOH), and ONOOH rapidly decays to form NO_2· and OH· (8, 19, 20, 31). Thus, one would expect that increasing the NO concentration in the face of even normoxic O_2^- production might result in increased $ONOO^-$ and/or OH· production, an interpretation that is consistent with our results.

$ONOO^-$ is a potent oxidizing agent and may lead to SA hydroxylation without the formation of OH· (23, 24, 26). For example, Narayan *et al.* (27) found that SA was hydroxylated to 2,3-DHBA by $ONOO^-$, but were unable to find evidence for $ONOO^-$ decomposition intermediates, suggesting that $ONOO^-$ directly hydroxylated SA. Similarly, Kaur *et al.* (25) found that SA was hydroxylated to 2,3-DHBA by $ONOO^-$, but that OH· scavengers not only prevented hydroxylation but also nitration of SA, again suggesting that $ONOO^-$ directly hydroxylated (and nitrated) SA. Regardless, the increase in 2,3-DHBA in the 21% O_2 + l00 ppm NO group is compatible with increased formation of a potent hydroxylating agent. Whether the SA reactive species is OH·, $ONOO^-$, and/or some other decomposition product remains to be determined.

The perhaps surprising finding from this study was that adding NO to 90% O_2 resulted in less SA hydroxylation than

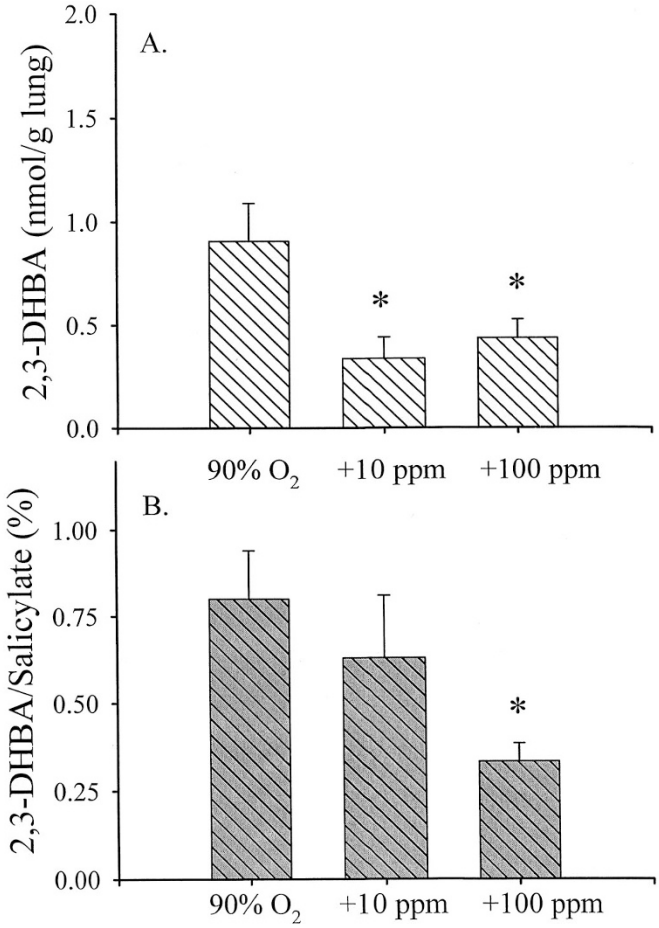

Figure 5. (*A*) Addition of 100 ppm NO to 90% oxygen resulted in less total 2,3-DHBA in the lung compared with exposure to 90% oxygen alone. (*B*) Addition of 100 ppm NO to 90% oxygen resulted in lower formation of 2,3-DHBA as demonstrated by a smaller ratio of total 2,3-DHBA to total lung SA in 90% oxygen + 100 ppm NO compared with 90% oxygen alone. *Different from 21% O_2, $p < 0.05$.

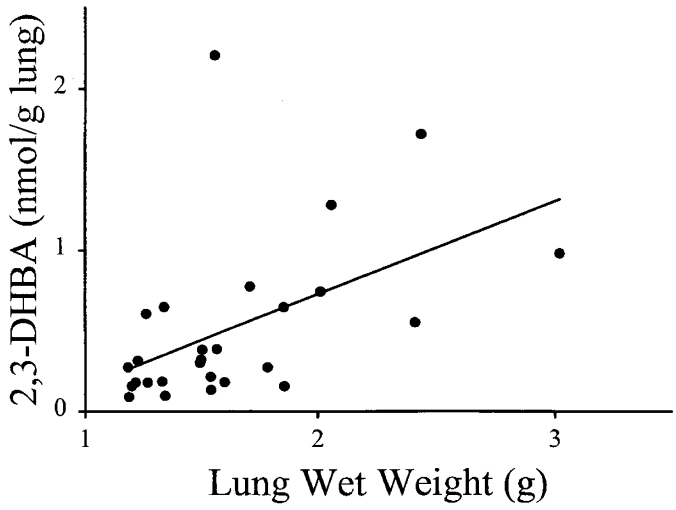

Figure 6. The total amount of 2,3-DHBA in the lung as a function of lung wet weight for the 27 lungs exposed to 21% O_2, 50% O_2, and 90% O_2. There was a significant correlation between lung 2,3-DHBA and wet weight in those lungs exposed to differing amounts of O_2. The line represents the linear regression of the points, the resultant line is $y = 0.6x - 0.4$, $r = 0.50$, $p < 0.01$.

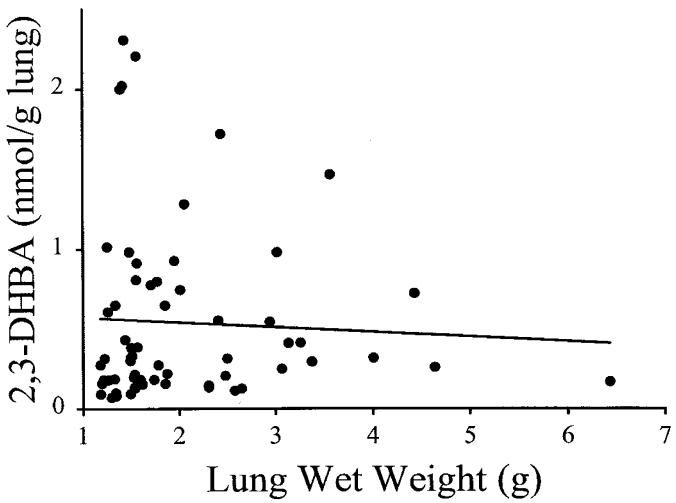

Figure 7. The total amount of 2,3-DHBA in the lung as a function of lung wet weight for all 62 lungs studied. There was no correlation between lung 2,3-DHBA and wet weight for all of the lungs studied, those exposed to differing O_2, and those exposed to differing O_2 with added NO. The line represents the linear regression of the points, the resultant line is $y = -0.03x + 0.6$, $r = 0.05$.

with either NO or hyperoxia alone. Although the exact mechanism was not identified, there are several possible reactions whereby a high level of NO may react with $ONOO^-$ and/or $OH\cdot$ to produce products that are not SA reactive (22). Turanlahti *et al.* (33) found that hyperoxia or NO added to room air increased lipid peroxidation as measured by expired pentane, whereas the addition of NO to hyperoxia decreased lipid peroxidation compared with hyperoxia alone. We found previously that lipid peroxidation as measured by lung tissue 4-hydroxy-2(*E*)-nonenal content was increased by hyperoxia but decreased when NO was added to the hyperoxic exposure (13). Thus, it may be that, in a hyperoxic environment, the relatively high concentrations of NO (40 and 100 ppm) act primarily as an antioxidant.

Although the addition of NO to 90% O_2 resulted in less hydroxylation of SA, all of the groups in high O_2 or high NO had evidence of lung injury. In fact, the group exposed to 90% O_2 and 100 ppm NO had the greatest lung injury, as evidenced by lung wet weight and lung compliance. We previously found that the inclusion of 100 ppm NO in >95% O_2 increased survival in rats compared with >95% O_2 exposure alone (13). However, in that study (13), indices of lung injury such as wet weight, pleural effusion volume, or Cdyn were either worse in the >95% O_2 + NO group or not different between the >95% O_2 and >95% O_2 + NO groups. Gutierrez *et al.* (10) also found an increased survival in rats exposed to high O_2 with added NO. Garat *et al.* (21), on the other hand, found that the addition of 100 ppm NO to >95% O_2 had no effect on survival or wet weight on the lung compared with >95% O_2 alone, and 100 ppm NO added to >95% O_2 increased vascular permeability to protein compared with >95% O_2 alone. Gries *et al.* (34) found that newborn guinea pigs exposed to >96% O_2 + 20 ppm NO had fewer symptoms of respiratory distress and a longer latency to symptoms than animals exposed to >96% O_2 alone, despite no statistical difference in measurements of

pulmonary dysfunction between the two groups. This led the authors (34) to conclude that the beneficial effects of NO on hyperoxia-induced respiratory distress were due to mechanism(s) other than protection from lung inflammation and edema. The beneficial effects of NO on survival in hyperoxia that we (13) and others (10, 34) have found appears to be due to factors other than the prevention of lung injury by NO. Thus, although the addition of NO to high O_2 decreased SA hydroxylation, in the present study it is not clear whether or to what extent the decrease in SA reactive species is involved in the increased survival effect of NO in high O_2.

In this study, we found that exposing rats to 50% O_2 and 90% O_2 resulted in increased lung wet weight compared with exposing rats to 21% O_2 (Table 1). The rats exposed to 90% O_2 had the highest 2,3-DHBA and the highest lung wet weight. This finding is consistent with previous studies that have demonstrated that a hallmark of hyperoxic lung injury is pulmonary edema (13, 35, 36). It is of interest to note that the rats exposed to 90% O_2 + 100 ppm NO had the greatest lung wet weight but did not have the greatest 2,3-DHBA. This suggests that, although adding NO to 90% O_2 prevented the formation of a hydroxylating species, this had no beneficial effect on pulmonary edema formation in the lungs. In fact, animals exposed to 90% O_2 + 100 ppm NO had the greatest pulmonary edema formation of all the groups studied. Previously, we found that the addition of 100 ppm NO to O_2 decreased lipid peroxidation, but the animals exposed to 100 ppm in O_2 had the highest lung wet weights of all the groups studied (13). This effect of NO on pulmonary edema formation is also consistent with previous studies. For example, Strome *et al.* (37) found, in newborn piglets, that the addition of 20 ppm NO to 100% O_2 resulted in greater lung vascular protein leak than 100% O_2 alone. In a model of acid aspiration in rats, Nader *et al.* (38) found that the addition of NO to O_2 resulted in more severe lung edema and protein leak than O_2 alone. Pulmonary edema is cleared by active Na^+ transport (39, 40), and it has been previously found that gene therapy with the Na^+/K^+-ATPase β_1 subunit improved alveolar fluid clearance and survival in rats exposed to 100% O_2 (40). When rats exposed to 100% O_2 were fed by gastrostomy tube, survival was improved, lung wet weight was decreased, and Na^+/K^+-ATPase mRNA was increased compared with rats exposed to 100% O_2 without gastrostomy tubes (39). These studies suggest that interventions that increase alveolar fluid clearance may improve lung injury and survival in hyperoxic exposure in rats. However, we have found that adding 100 ppm NO to the hyperoxic exposure increased survival in rats despite an increase in wet lung weight (13). Youssef *et al.* (41) found that exposing newborn piglets to hyperoxia increased lung wet weight and mRNA and expression of the Na^+/K^+-ATPase α_1 subunit, whereas adding NO to the hyperoxic exposure did not affect the increased lung wet weight but did decrease the expression of the Na^+/K^+-ATPase α_1 subunit. Thus, the relationship between the protective effect of NO in hyperoxia and factors related to lung fluid balance are not yet clear.

CONCLUSION

In conclusion, we found that increasing either O_2 or NO concentrations in the inhaled gas increased production of SA hydroxylating species within the lung. The increase was associated with an increase in physiologic indicators of lung injury. On the other hand, increasing both O_2 and NO concentration eliminated the increase in SA hydroxylating species, without improving the indicators of lung injury studied. Thus, although eliminating the increase in SA hydroxylating species may be involved in the improved survival of rats in >95% + NO (13), this effect does not appear to be *via* the prevention of the indicators of lung injury studied. However, this finding suggests that exogenous NO added to high inspired O_2 acts to scavenge $OH\cdot$ and/or $ONOO^-$ in the lung such that the production of $OH\cdot$ and/or $ONOO^-$ in the lung during inhaled NO therapy may depend on the ratio of NO to O_2.

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