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Effect of Chronic Sodium Nitrite Therapy on Monocrotaline-Induced Pulmonary Hypertension

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Abstract

Pulmonary hypertension (PH) is a rare disorder that without treatment is progressive and often fatal within 3 years. The treatment of PH involves the use of a diverse group of drugs and lung transplantation. Although nitrite was once thought to be an inactive metabolite of endothelial-derived nitric oxide (NO), there is increasing evidence that nitrite may be useful in the treatment of PH, but the mechanism by which nitrite exerts its beneficial effect remains uncertain. The purpose of this study was to investigate the effect of chronic sodium nitrite treatment in a PH model in the rat. Following induction of PH with a single injection of monocrotaline, 60 mg; daily ip injections of sodium nitrite (3 mg/kg) starting on day 14 and continuing for 21 days, resulted in a significantly lower pulmonary arterial pressure on day 35 when compared to values in untreated animals with monocrotaline-induced PH. In monocrotaline-treated rats, daily treatment with ip nitrite injections for 21 days decreased right ventricular mass and pathologic changes in small pulmonary arteries. Nitrite therapy did not change systemic arterial pressure or cardiac output when values were measured on day 35. The decreases in pulmonary arterial pressure in response to iv injections of sodium nitroprusside, sodium nitrite, and BAY 41-8543 were not different in rats with monocrotaline-induced pulmonary hypertension and rats with chronic nitrite therapy when compared to responses in animals in which pulmonary arterial pressure was increased with U46619. These findings are consistent with the hypothesis that the mechanisms that convert nitrite to vasoactive NO, activate soluble guanylyl cyclase and mediate the vasodilator response to NO or an NO derivative are not impaired. The present data are consistent with the results of a previous study in monocrotaline-induced PH in which systemic arterial pressure and cardiac output were not evaluated and are consistent with the hypothesis that nitrite is effective in the treatment of monocrotaline-induced PH in the rodent.

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Keywords

pulmonary vascular bed; systemic vascular bed; pulmonary hypertension/therapy; right ventricular hypertrophy; sodium nitrite; nitric oxide; soluble guanylyl cyclase; monocrotaline

Introduction

Pulmonary hypertension (PH) is a rare disorder that without treatment is progressive and often fatal within 3 years [1; 2; 3; 4]. Current treatment of PH involves the use of a diverse group of drugs and lung transplantation [5; 6; 7; 8]. The beneficial properties of nitric oxide (NO) have been documented in experimental models and in human subjects [9; 10; 11; 12]. Inhaled NO has been shown to have beneficial, but transient effect, in patients with PH [13; 14; 15; 16]. Moreover, PH is associated with oxidative stress and poor responses to vasodilator agents [13; 14; 17; 18; 19; 20; 21].

There is evidence in the literature that inorganic nitrite can be used in the treatment of a variety of disorders including PH [22; 23; 24; 25]. It has been hypothesized that nitrite reduction to NO is catalyzed by deoxyhemoglobin and other heme proteins [26; 27; 28; 29; 30]. It has been reported that pulmonary vasodilator responses to inhaled and to injected sodium nitrite are enhanced by hypoxia although one study reported no effect of ventilatory hypoxia on the pulmonary vasodilator response to nitrite [31; 32; 33; 34]. Nitrite is formed by the oxidation of NO and was thought to be an inactive metabolite of endothelial derived NO [35; 36]. However, it has been reported that nitrite has vasodilator activity in humans and it is known that amyl nitrite was first used in the treatment of angina in 1867 [37]. The cardiovascular actions of nitrite in humans were studied by Weiss and coworkers and by Gladwin and associates [28; 29; 32; 38]. The vasorelaxant properties of nitrite in isolated arteries were reported by Furchgott and coworkers in 1953, and the observation that vasorelaxant responses to nitrite were associated with the activation of soluble guanylyl cyclase (sGC) and increases in cGMP levels were reported by Ignarro and coworkers and other investigators [39; 40; 41; 42; 43; 44; 45; 46]. It has been hypothesized that when sodium nitrite is administered in pharmacologic doses, NO derived from nitrite reduction can, in part, escape inactivation by hemoglobin and produce a vasodilator response although a recent study reported that infused nitrite does not induce vasodilation in the pulmonary circulation of the newborn lamb [31; 34; 47; 48; 49; 50; 51; 52; 53; 54]. Previous studies suggest that nitrite therapy may be useful in the treatment of PH [32; 53; 54; 55; 56]. It has been hypothesized that the beneficial effect of nitrite inhalation in PH results from the reduction of nitrite to NO by xanthine oxidoreductase (XOR) and by disproportionation in the acidic airways and alveoli [53; 54; 55; 57], however the mechanism by which nitrite exerts its beneficial effect is uncertain and the mechanism by which nitrite is reduced to vasoactive NO is uncertain [58; 59; 60; 61; 62]. The purpose of this study was to investigate the effect of daily ip injections of sodium nitrite in a rat model of PH induced by the plant alkaloid, monocrotaline. The findings in this study show that daily ip sodium nitrite therapy (3mg/kg for 21 days) in monocrotaline-treated rats reduced pulmonary arterial pressure, improved right ventricular/left ventricular + septum weight ratios, and reduced the development of medial hypertrophy in small pulmonary arteries without decreasing systemic arterial pressure or cardiac output. The present data show that pulmonary vasodilator responses to iv injections of sodium nitrite, sodium nitroprusside and BAY41-8543 were preserved in monocrotaline-treated rats. These findings are consistent with the hypothesis that the mechanisms that convert nitrite to vasoactive NO in the pulmonary vascular bed are not impaired, that vasodilator responses in the pulmonary vascular bed to NO donors, and sGC stimulators are not altered in monocrotaline-induced PH in the rodent. These data suggest that nitrite therapy can be useful in the treatment of PH.

Methods

Design of experiments: Monocrotaline-induced PH

The Institutional Animal Care and Use Committee of the Tulane University School of Medicine approved the experimental protocol employed in these studies and all procedures were conducted in accordance with institutional guidelines. All experiments were carried out in Sprague-Dawley rats weighing 300–350 g and for studies on monocrotaline-induced PH, the animals were injected with a single dose of monocrotaline 60 mg/kg into the tail vein and housed in the vivarium. The control animals were injected with an equal volume of 0.9% saline. Hemodynamic values were measured on day 28 in monocrotaline-treated rats and significant mortality was observed after day 28. Monocrotaline treated rats received daily ip injections of sodium nitrite (1 or 3mg/kg) starting on day 14 and continued for 21 days. The control animals received daily ip injections of 0.9% saline. On day 35, hemodynamic measurements were made in nitrite-treated rats, the animals were euthanized, and the heart and lungs were removed for histologic analysis.

Measurement of hemodynamic values

The animals were anesthetized with Inactin (100 mg/kg ip) (Sigma-Aldrich) and placed in the supine position on an operating table. Supplemental doses of Inactin were administered ip in order to maintain a uniform level of anesthesia. Body temperature was maintained with a heating lamp. The trachea was cannulated with a short segment of PE-240 tubing to maintain a patent airway. The animals spontaneously breathed room air. A femoral artery was catheterized with PE-50 tubing for measurement of systemic arterial pressure. The left jugular and femoral veins were catheterized with PE-50 tubing for iv injections and infusions of agents. For pulmonary arterial pressure measurement, a specially designed 3F single lumen catheter with a curved tip and with radio-opaque marker was passed from the right jugular vein and into the main pulmonary artery under fluoroscopic guidance (Picker-Surveyor Fluoroscope) as previously described [51]. Pulmonary and systemic arterial pressures were measured with Namic Perceptor DT transducers (Boston Scientific), digitized by a Biopac MP100 data acquisition system (Biopac Systems) and stored on a Dell PC. Cardiac output was measured by the thermodilution technique with a Cardiamax II computer (Columbus Instruments). A known volume (0.2 ml) of room temperature 0.9% NaCl solution was injected into the jugular vein catheter with the tip near the right atrium and changes in blood temperature were detected by a 1.5F thermistor microprobe catheter (Columbus Instruments) positioned in the aortic arch from the left carotid artery. The indicator dilution data were stored on the PC.

The effects of iv injections of sodium nitrite (100 μ mol/kg or 6 mg/kg), sodium nitroprusside (3 μ g/kg) and BAY 41-8543 (100 μ g/kg) on pulmonary and systemic arterial pressures were investigated in monocrotaline-treated rats on day 28 and in nitrite-treated rats on day 35. The animals were then euthanized, and the heart and lungs were removed for histologic analysis. In control animals pulmonary arterial pressure was increased to ~30 mm Hg so that responses to sodium nitrite, sodium nitroprusside and BAY 41-8543, could be compared under high pulmonary vascular tone conditions. In another group of experiments, the conversion of iv injected nitrite to vasoactive NO or an NO-like compound was estimated by comparing decreases in pulmonary arterial pressure in response to iv injections of sodium nitrite and the NO donors sodium nitroprusside and DEA/NO when doses were expressed on a μ mol/kg basis in U46619-infused rats.

Right ventricular hypertrophy assessment

To measure ventricular weight, the hearts were excised following sternotomy and the atria and major vessels were carefully dissected free from the ventricles. The right ventricular

free wall (RV) was carefully dissected and separated from the left ventricle and septum (LV +S) and weighed. A ratio of RV to LV+S(RV/LV+S) was used as an index of RV hypertrophy.

Histological analysis of small pulmonary arteries

After the rats were euthanized, the pulmonary artery was cannulated and the lungs were perfused with heparinized 0.9% saline and infused with 10% paraformaldehyde and harvested. The tissues were fixed in 10% paraformaldehyde for 24 hours, paraffin embedded, and 5 μ m sections were obtained for hematoxylin and eosin staining. These were qualitative studies of the morphology of small arteries and a morphometric analysis was not carried out. Digital images of the specimens were photographed using a Nikon Eclipse E400 microscope and digital camera.

Drugs

Sodium nitroprusside, sodium nitrite, 2-(N,N-diethylamino)-diazene-2-oxide diethylammonium salt (DEA/NO) (Sigma Aldrich) were dissolved in 0.9% NaCl. Monocrotaline hydrochloride (Sigma Aldrich) was dissolved in 1 N HCl, neutralized with 0.5N NaOH, and diluted with PBS. The filtered solution was then injected into the tail vein in a dose of 60 mg/kg. BAY 41-8543 (2-[1-[2-fluorophenyl)methyl]-1H-pyrazolo[3,4-b]pyridin-3-yl]-5(4-morpholinyl)-4,6-pyrimidinediamine) was obtained from Dr. Johannes-Peter Stasch of the Institute of Cardiovascular Research, Pharma Research Centre, Bayer AG, D-42096, Wuppertal, Germany) and were dissolved in Transcutol®/Cremophor EL®/ 0.9% NaCl solution (10/10/80). U46619 (Cayman Chemical) was dissolved in 95% ethyl alcohol and diluted in 0.9% NaCl solution.

Statistics

The hemodynamic data and heart weight ratios are expressed as mean $\pm$ SE and were analyzed using ANOVA with repeated measures with paired and unpaired t-tests when indicated. The criterion used for statistical significant was $P < 0.05$.

Results

Effect of nitrite treatment on monocrotaline induced pulmonary hypertension

The effect of monocrotaline administration on hemodynamic values in the rat is shown in Table 1. The iv injection of monocrotaline in a dose of 60 mg/kg into the tail vein resulted in a significant increase in pulmonary arterial systolic, diastolic and mean pressures with no significant change in systemic arterial pressure or cardiac output when values measured on day 28 were compared with values in normal control animals (Table 1). In this model, the administration of a single dose of monocrotaline produced large increases in pulmonary arterial systolic, diastolic and mean pressures and pulmonary vascular resistance without changing systemic vascular resistance (Table 1). The effect of chronic sodium nitrite treatment on monocrotaline induced pulmonary hypertension is shown in Table 1 and in figure 1. The ip injection of sodium nitrite, 3 mg/kg for 21 days starting on day 14 after the administration of monocrotaline and continuing until day 35, resulted in a significant decrease in pulmonary arterial systolic, diastolic and mean pressures and pulmonary vascular resistance without a significant change in cardiac output or systemic arterial pressure (Table 1 and Fig. 1). Although significant mortality occurred after 28 days in rats treated with monocrotaline data was obtained on day 35 from 3 rats that survived and these data along with the data from 4 monocrotaline-injected rats that were treated with sodium nitrite in a dose of 1 mg/kg ip for 21 days are also shown in figure 1. The results shown in Table 1 and figure 1 demonstrate that chronic treatment with sodium nitrite in a dose of 3 mg/kg ip

significantly attenuated monocrotaline-induced PH. The additional results presented in figure 1 show that treatment with sodium nitrite in a dose of 1 mg/kg ip did not significantly reduce pulmonary arterial pressure in monocrotaline-treated rats. However all rats treated with the lower dose of sodium nitrite survived for 35 days (Fig. 1). In addition although monocrotaline administration resulted in significant mortality after day 28, pulmonary arterial pressure in the 3 rats that survived was very high with an average value of 62 mm Hg (Fig. 1).

Effect of chronic nitrite treatment on right ventricular hypertrophy and small vessel histology

In order to investigate the effect of chronic nitrite treatment on right ventricular mass and lung vascular histology, the heart and lungs were removed from normal control rats, rats with monocrotaline-induced PH and rats with monocrotaline-induced PH treated with sodium nitrite (3 mg/kg ip for 21 days). The rats with monocrotaline-induced PH had significantly higher RV/LV+S weights (RV mass) than did normal control rats (Table 2 and Fig. 2). The rats with monocrotaline-induced PH that were treated with ip nitrite injections (3 mg/kg for 21 days) had significantly lower RV/LV+S weights when compared to values in untreated rats with monocrotaline-induced PH (Table 2 and Fig. 2).

The histologic analysis of sections from the left lower lung lobe show the development of medial hypertrophy in the small pulmonary arteries from animals with monocrotaline-induced PH. Treatment with sodium nitrite, 3 mg/kg ip daily for 21 days, resulted in a reduction in the extent of medial hypertrophy in small pulmonary arteries when compared to the degree of medial hypertrophy in vessels from untreated animals with monocrotaline-induced PH (Fig. 3).

Responses to sodium nitrite, sodium nitroprusside and BAY 41-8543

Responses to sodium nitrite, sodium nitroprusside and BAY 41-8543 were compared in normal control rats infused with U46619 to increase pulmonary arterial pressure to a high steady value, monocrotaline-treated rats and monocrotaline-treated rats injected with sodium nitrite, 3 mg/kg ip, for 21 days. The comparison of decreases in pulmonary and systemic arterial pressure in response to iv injections of the three vasoactive agents provides information about vascular responsiveness in the pulmonary and systemic vascular beds in the three groups of animals. The data in figure 4 show that monocrotaline treatment did not alter responses to iv injection of SNP, sodium nitrite or BAY 41-8543 in the pulmonary or systemic vascular bed in the 3 groups of animals. These data are consistent with the hypothesis that monocrotaline treatment in the present study did not alter vascular responsiveness to NO, the bioactivation of nitrite or the responsiveness of soluble guanylate cyclase to a heme dependent NO independent enzyme stimulator (BAY 41-8543).

Comparison of responses to sodium nitrite, sodium nitroprusside and DEA/NO

The comparison of responses to sodium nitrite, sodium nitroprusside and DEA/NO provides information about the extent of bioactivation of injected nitrite to vasoactive NO or an NO-like compound in the intact chest rat model. In U46619 infused animals to increase pulmonary arterial pressure to a high steady level the iv injection of sodium nitrite, sodium nitroprusside and DEA/NO produce dose-related decreases in pulmonary arterial pressure (Fig. 5). The dose response curve for sodium nitrite is approximately 3 log units to the right of the dose-response curves for the NO donors (Fig. 5). These data indicate that the potency of nitrite was 3 orders of magnitude lower than that of the true NO donor, DEA/NO. These data suggest that approximately 0.1% of iv injected nitrite is converted to vasoactive NO or an NO-like derivative in the circulation of the intact-chest rat model.

Discussion

The results of the present study show that chronic treatment with sodium nitrite in a dose of 3 mg/kg ip starting on day 14 and continuing for 21 days, attenuates monocrotaline-induced pulmonary hypertension in the rat. In the present study, the injection of monocrotaline in a dose of 60 mg/kg iv produced a large increase in pulmonary arterial pressure without a significant change in systemic arterial pressure or cardiac output when values were measured 28 days after treatment with the plant alkaloid. In this model of PH with a single injection of monocrotaline into the tail vein, systolic pressure in the pulmonary artery exceeded 100 mm Hg in some animals and significant mortality was observed after 28 days with 3 out of 6 animals dying, so that the 28 day time period was used as the control period for untreated PH. In this model of PH, right ventricular hypertrophy and increases in medial thickness in small intrapulmonary arteries were observed after 28 days. Based on an analysis of responses to vasodilator agents in this model of PH, it has been estimated that approximately 50% of the increase in pulmonary arterial pressure can be attributed to active pulmonary vasoconstriction and approximately 50% of the pressure increase produced by the treatment with a single 60 mg/kg iv dose of monocrotaline can be attributed to structural changes within the pulmonary vascular bed [63].

The results of the present study show that chronic treatment with sodium nitrite in a dose of 3 mg/kg ip starting on day 14 and continuing for 21 days was associated with a significant reduction in pulmonary arterial pressure and pulmonary vascular resistance when compared to values in untreated animals with monocrotaline-induced PH on day 28 or on day 35. The reduction in pulmonary arterial pressure with chronic ip nitrite therapy was associated with a smaller increase in right ventricular mass and an improvement in morphology of small intrapulmonary arteries and these beneficial effects occurred without a significant change in systemic arterial pressure or cardiac output. The analysis of histologic changes in small pulmonary vessels was only qualitative and a comprehensive morphometric analysis was not carried out. The results of the present study suggest that chronic nitrite therapy can have a beneficial effect on the pulmonary circulation that is selective and does not cause systemic hypotension.

The administration of a 1 mg/kg ip dose of sodium nitrite starting on day 14 and continuing for 21 days did not significantly attenuate monocrotaline-induced PH, but prevented mortality at the 35 day time period so that a therapeutic effect in terms of mortality was observed. It is possible that pulmonary arterial pressure may be reduced at later time points in animals treated with the 1 mg/kg ip dose of sodium nitrite. The results of the present study with chronic ip injections of sodium nitrite are consistent with the results of studies with intermittent inhaled sodium nitrite therapy in rodents and in the newborn lamb and are consistent with the hypothesis that nitrite therapy may be useful in the treatment of pulmonary hypertensive disorders [53; 54; 55; 57].

Nitrite therapy has been shown to have a beneficial effect in a wide variety of cardiovascular disorders in experimental animals [32; 55; 56; 64]. The mechanism by which nitrite exerts its beneficial therapeutic effect is uncertain [58; 61; 65; 66]. The beneficial effect of inhaled amyl nitrite in the treatment of angina pectoris was first reported by Brunton in 1867 [37]. The work of Brunton led to the use of glyceryl trinitrate (GTN) for the treatment of angina by Murrell and although GTN has been used for the treatment of cardiovascular disorders for more than 140 years, the mechanism of the therapeutic action of GTN remains uncertain [37; 67; 68; 69; 70]. It has been hypothesized that the therapeutic effect of GTN is due to the mitochondrial aldehyde dehydrogenase catalyzed conversion of GTN to GDN and inorganic nitrite that is subsequently reduced to NO or a S-nitrosothiol that activates soluble guanylate cyclase (sGC)[40; 41; 42; 71; 72]. However the mechanism of the bioactivation of nitrite to

vasoactive NO or an NO-like derivative is uncertain and it has been recently reported that the localization of the mitochondrial aldehyde dehydrogenase that is suggested to play an important role in the bioactivation of GTN is mainly cytosolic [34; 51; 52; 58; 61; 65; 66; 73; 74].

The cardiovascular actions of nitrite in humans were first studied by Weiss and coworkers and later by Gladwin and associates and in experimental animals by numerous investigators [28; 29; 32; 34; 38; 51; 52]. The results of these studies suggest that when sodium nitrite is administered in pharmacologic doses; NO or a product derived from injected nitrite can, in part, escape inactivation or scavenging by hemoglobin and produce a vasodilator response [31; 34; 47; 48; 49; 50; 51; 52].

The mechanism of the bioactivation of nitrite in the pulmonary circulation is not well understood. It has been reported that nitrite can be reduced to NO by deoxyhemoglobin, XOR, ALDH2, and other hemeproteins or by disproportionation at acidic pH and it is known that NO has a beneficial effect in a variety of cardiovascular diseases [22; 23; 24; 25]. It has been hypothesized that that beneficial effect of nitrite inhalation results from the reduction of nitrite to NO by XOR in monocrotaline-treated rats or by disproportionation in the newborn lamb with hemolysis-induced PH [28; 34; 51; 52; 53; 54; 55; 57; 69; 75]. However, the mechanism by which NO exerts its beneficial effect on a cellular and molecular level is uncertain [68; 69; 70].

In the present study, the beneficial effect of chronic nitrite treatment may result from the conversion of nitrite into vasoactive NO or an NO-like compound and signaling through the sGC-cGMP pathway. NO has vasodilator and anti-inflammatory actions that contribute to the beneficial effects observed in the monocrotaline-induced PH model.

Nitric oxide binds to the reduced heme iron moiety of sGC [44; 76; 77]. Reactive oxygen species can oxidize the heme iron on sGC and reduce the catalytic activity of the enzyme [78; 79; 80]. In the present study, responses to the NO donor, SNP, were not impaired in rats with monocrotaline-induced PH suggesting that resistance vessels in the lung were responsive to NO. In addition, the pulmonary vasodilator response to sodium nitrite was not attenuated suggesting that the mechanism or mechanisms that converts nitrite to vasoactive NO or an NO-like compound were not impaired. The observation that responses to BAY 41-8543, a stimulator of normal reduced sGC were preserved in monocrotaline-treated rats when compared to responses in U46619-infused animals provides additional support for the hypothesis that sGC activity in pulmonary resistance vessels was not altered in this PH model. The present data are consistent with the hypothesis that nitrite is converted into vasoactive NO or an NO-like compound in monocrotaline-treated rats and that NO or an NO-like compound activates sGC to promote vasodilation, reduce right ventricular hypertrophy and inhibit remodeling in the pulmonary vascular bed by a cGMP dependent mechanism.

The mechanism by which inorganic nitrite is converted to vasoactive NO is uncertain. The comparison of decreases in pulmonary arterial pressure in response to iv injections of sodium nitrite and the NO donors SNP and DEA/NO provide an estimate of the conversion of injected nitrite into vasoactive NO. The extent of nitrite bioactivation in the intact chest rat model is estimated to be approximately 0.1% since the potency of nitrite was 3 orders of magnitude lower than that of the true NO donor, DEA/NO. The mechanism of the bioactivation was investigated in previous studies in the rat and it was suggested that about 50% of the reduction of nitrite to vasoactive NO was mediated by xanthine oxidoreductase (XOR) [34; 51; 52]. The results of studies in the rat are not in agreement with studies in the human in which forearm vasodilator responses to injected nitrite were not inhibited by the

XOR inhibitor oxypurinol [28]. In a recent study in the newborn lamb, inhaled nitrite induced pulmonary vasodilation whereas infused nitrite was without effect [53]. The reason for the difference in results of studies in the human subject, newborn lamb and intact-chest rat is uncertain but may involve differences in species [28; 53]. Although the results of the three studies are different, the suggested mechanisms of nitrite bioactivation: reaction with XOR in the rat, reaction with deoxyhemoglobin in the human forearm, and chemical disproportionation in the acidic airways and alveoli of the newborn lamb are all plausible.

Limitations

Although the present studies show a beneficial effect of chronic ip nitrite treatment in monocrotaline-induced pulmonary hypertension in the rat, there are limitations to the conclusions that can be drawn from these studies. The monocrotaline model of PH exhibits pulmonary arterial hypertension, right ventricular hypertrophy, and remodeling of the pulmonary vascular bed. This model is easy to use and when a single dose of monocrotaline is injected into the tail vein in the amount of 60 mg/kg a large increase in pulmonary arterial pressure in a period as short as 3 or 4 weeks is observed and significant mortality will occur. Although systolic pressure in the pulmonary arterial can reach 100 mm Hg and drugs that have efficacy in the treatment of PH reduce mortality, many agents that have a protective effect in the monocrotaline model in the rat are not effective in patients with PH. The finding of preserved vasodilator responsiveness in the single injection monocrotaline model should be taken into account when comparing this model to the typical human PH which often displays impaired vasodilator responsiveness. In addition although there is remodeling in the pulmonary vascular bed of the monocrotaline treated rat, the plexiform lesions that are characteristic of the human disease (but are not always seen) do not usually develop after a single injection of monocrotaline and “multiple” treatments may be needed to recapitulate human PH[80]. The differences in basic pathophysiologic mechanisms that are not well understood and the difference in treatment regimens suggest that a single dose monocrotaline model in the rat is not a good model for study of pulmonary hypertensive disease in the human which has a much different time course [80]. However, useful information about potential treatments for PH can be obtained from experiments in monocrotaline-treated rats. It should also be mentioned that the suggestion that prostacyclin (PGI₂) may be useful in the treatment of PH came from studies in anesthetized dogs and cats in which pulmonary vasoconstriction (hypertension) was induced by infusion of U46619 [81; 82].

In summary, results of the present study show that chronic treatment with sodium nitrite, 3 mg/kg ip, for 21 days attenuated monocrotaline-induced PH in the rat. These results are consistent with a previous study showing that inhaled nitrite has a beneficial effect in monocrotaline-induced PH in the rodent [55]. The present results extend previous work by showing that chronic nitrite therapy did not reduce systemic arterial pressure or alter cardiac output. The present results show that chronic nitrite therapy improved small vessel morphology in the lung and reduced right ventricular hypertrophy in monocrotaline-induced PH. The results of the present study showing that responses to iv injections of sodium nitrite, SNP and BAY 41-8543 are preserved in monocrotaline-treated rats are consistent with the hypothesis that the mechanisms mediating the bioactivation of nitrite and the activation of sGC by NO or an NO-like compound are not impaired. The present results are consistent with the hypothesis that nitrite therapy will be useful in the clinical management of pulmonary hypertensive disorders.

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Pulmonary hypertension is a fatal disease

Treatment involves a diverse group of drugs and lung transplantation

Nitrite anion may be useful in the treatment

Nitrite anion is converted into vasoactive nitric oxide and activates soluble guanylyl cyclase

Nitrite conversion is estimated to be 0.1%

The one injection model of monocrotaline-induced pulmonary hypertension is useful but may not be a good model of human pulmonary hypertension

Figure 1.

Bar graphs comparing mean pulmonary and systemic arterial pressures and cardiac output in control rats (28 days), monocrotaline-treated rats (28 days), and monocrotaline-treated + sodium nitrite (3 mg/kg ip, daily for 21 days) (35 days), monocrotaline-treated (35 days), and monocrotaline and sodium nitrite (1 mg/kg ip, daily for 21 days) (35 days). n = number of rats in the group. * P < 0.05.

Figure 2.

Bar graphs comparing heart weights in control rats (A), monocrotaline rats (B), and monocrotaline-treated + sodium nitrite (3 mg/kg ip daily for 21 days) (C). The atrium was removed, and the weight of the right ventricle relative to the weight of the left ventricle plus septum in the three groups of rats was compared. n = number of experiments. * indicates $P < 0.05$, when compared to control and ** when compared to monocrotaline treatment.

Figure 3.

Photomicrographs of hematoxylin and eosin stained sections of the left lower lung lobe from control rats (A), monocrotaline-treated rats (B), and monocrotaline + sodium nitrite (3 mg/kg ip daily for 21 days) (C). The small artery in B panel shows marked medial thickening when compared to the small artery in the control group in panel A. The section in panel C shows less medial thickening than the section in panel B. The bar indicates 25 microns.

Figure 4.

Bar graphs showing responses to iv injections of sodium nitroprusside, sodium nitrite and BAY 41-8543 in monocrotaline-treated rats, in control rats in which pulmonary arterial pressure was increased with U46619 and in monocrotaline-treated + sodium nitrite (3mg/kg ip, daily for 21 days). The vasoactive agents were injected iv and changes in mean pulmonary and systemic arterial pressures were measured. n = number of rats in the group. * indicates P < 0.05.

Figure 5.

Dose-response curves comparing decreases in pulmonary arterial pressure in response to iv injections of sodium nitroprusside, DEA/NO and sodium nitrite when doses are expressed on a $\mu\text{mol/kg}$ basis. Pulmonary arterial pressure was increased to ~ 30 mm Hg with an infusion of U46619. The dose-response curve for sodium nitrite is approximately 3 log units to the right of the dose-response curves for the NO donors. These data suggest that the conversion of nitrite to vasoactive NO is approximately 0.1% in the circulation of the intact chest rat model. n indicates number of animals.

Table 1

Effect of monocrotaline (MCT) and sodium nitrite treatment on pulmonary and systemic hemodynamic values.

	PAP, Systolic (mmHg)	PAP, Diastolic (mmHg)	PAP mean (mmHg)	PVR (mmHg/ml/min)	MAP (mmHg)	Cardiac Output (ml/min)	SVR (mmHg/ml/min)
Control	30 ± 2	9 ± 1	20 ± 0	0.18 ± 0.01	110 ± 5	105 ± 6	0.99 ± 0.07
MCT	86 ± 7 [*]	29 ± 4 [*]	54 ± 2 [*]	0.55 ± 0.04 [*]	99 ± 6	103 ± 7	1.06 ± 0.05
MCT + NaNO ₂ 3mg/kg	42 ± 1 ^{*#}	21 ± 3 ^{*#}	33 ± 3 ^{*#}	0.31 ± 0.02 ^{*#}	99 ± 5	92 ± 7	0.93 ± 0.04

Values are expressed as mean ± SE.

^{*} denotes a *P* value < 0.05 when compared to control,

[#] denotes a *P* value < 0.05 when compared to MCT n = 11, 9, and 11 for Control, monocrotaline, and monocrotaline+ NaNO₂ groups respectively.

PAP pulmonary arterial pressure, *PVR* pulmonary vascular resistance, *MAP* mean arterial pressure, *SVR* systemic vascular resistance.

Control rats received a saline injection via tail vein on day one. MCT rats received ip saline injections on day 14–35 as a control for ip NaNO₂ injections.

Table 2

Effect of NaNO₂ treatment 3mg/kg ip daily for 21 days on monocrotaline induced right ventricular hypertrophy

	RV/LV+S (grams)	RV/Total Heart (grams)
Control	221 ± 14	187 ± 11
Monocrotaline (day 28)	473 ± 32 *	336 ± 11 *
Monocrotaline + NaNO ₂ 3mg/kg ip (day 35)	319 ± 34 *#	272 ± 11 *#

Values are expressed as mean ± SE.

* denotes a *P* value < 0.05 when compared to control,

denotes a *P* value < 0.05 when compared to placebo.

n = 10, 7, and 11 for control, monocrotaline and monocrotaline + NaNO₂ groups respectively.