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NITROFURANTOIN-INDUCED PULMONARY TOXICITY

IN VIVO EVIDENCE FOR OXIDATIVE STRESS-MEDIATED MECHANISMS

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Abstract—The present study was carried out to examine whether nitrofurantoin-induced pulmonary toxicity in normal rats was mediated via oxidant stress mechanisms. The relative importance of the cellular antioxidant enzymes in nitrofurantoin toxicity was also assessed. For this, the pulmonary toxicity induced by nitrofurantoin in rats was evaluated at various time intervals after a single subcutaneous injection. Data from this study showed that nitrofurantoin (200 mg/kg, s.c.) resulted in transient but measurable lung damage as evidenced by the increases in wet lung weight/body weight ratio and decreases in lung angiotensin converting enzyme activity. A transient decrease in GSH concentrations with a concurrent increase in GSSG concentrations as well as an increase in lipid peroxidation levels (measured by the formation of diene conjugates and thiobarbituric acid reactants) were also evident in lungs of nitrofurantoin-treated rats. In addition, nitrofurantoin did not alter the pulmonary superoxide dismutase and glutathione peroxidase activities, but it did produce transient decreases in catalase and glutathione reductase activities. These data indicate that impairment of the ability of the lung to detoxify reactive oxygen species may play an important role in the development of nitrofurantoin-induced pulmonary toxicity. The results of the present study suggest that nitrofurantoin can damage the lungs of rats, probably through oxidative stress-mediated mechanisms. Also, our data have provided *in vivo* evidence for substantiating lipid peroxidation as a possible cause of lung damage.

Nitrofurantoin (*N*-[5-nitro-2-furfurylidine]-1-amino-hydantoin), a commonly used urinary tract antimicrobial drug, has been shown to cause significant pulmonary toxicity in humans [1, 2] and experimental animals [3–5]. The exact mechanism(s) by which nitrofurantoin damages the lung remains unclear. Results from *in vitro* studies have shown that a cyclic single electron reduction/oxidation (redox cycling) of the parent molecule is a critical mechanistic event [6–8]. Nitrofurantoin activation under aerobic conditions may proceed via one electron reduction of the nitro group to the nitro-anion radical, catalysed primarily by the NADPH-cytochrome P450 reductase as well as other enzymes located in the cytosol and microsomal fractions [8–10]. This anion radical is autooxidized rapidly in the presence of molecular oxygen to regenerate the parent compound and the superoxide anion [8, 10]. The superoxide anion may dismutate, either spontaneously or in the presence of superoxide dismutase to form hydrogen peroxide [11, 12] which, in turn, may undergo an iron-catalysed Haber–Weiss reaction to form the highly reactive hydroxyl radical [8, 9] as shown schematically in Fig. 1. In general, these reactive oxygen species are thought to exert their damaging effects in biological systems by destabilizing cell membranes and disrupting critical cell functions [10, 13, 14].

The condition characterized by elevation in the cellular steady-state concentration of reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radical from the incomplete reduction of molecular oxygen has been defined as oxidative stress [14, 15]. This condition can lead to initiation of membrane lipid peroxidation, DNA damage, mutagenesis, and cell death [13, 16]. There is increasing evidence that oxidative stress is involved in various pathological and biological conditions including aging, cancer, atherosclerosis, inflammation, ischemic/reperfusion injury, and chemically-induced tissue injury [13, 15–17).

Generation of intracellular reactive oxygen species may not always lead to cellular injury, because of the presence of the cellular defence systems. Several cellular defence mechanisms such as superoxide dismutase (SOD†), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-R), vitamin E, and glutathione (GSH) are available for scavenging reactive oxygen species [13, 16, 18] (Fig. 1). However, when these defence systems are overwhelmed, such as in the presence of acute oxidative stress, it can lead to changes in the function and structure of cellular components. These changes include DNA damage, depletion of intracellular ATP and reducing equivalents, acceleration of lipid peroxidation, alterations in calcium homeostasis,

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† Abbreviations: SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; GSH-Px, glutathione peroxidase; GSH-R, glutathione reductase; DMF, *N*,*N*-dimethylformamide; ACE, angiotensin converting enzyme; BSA, bovine serum albumin; and TCA, trichloroacetic acid.

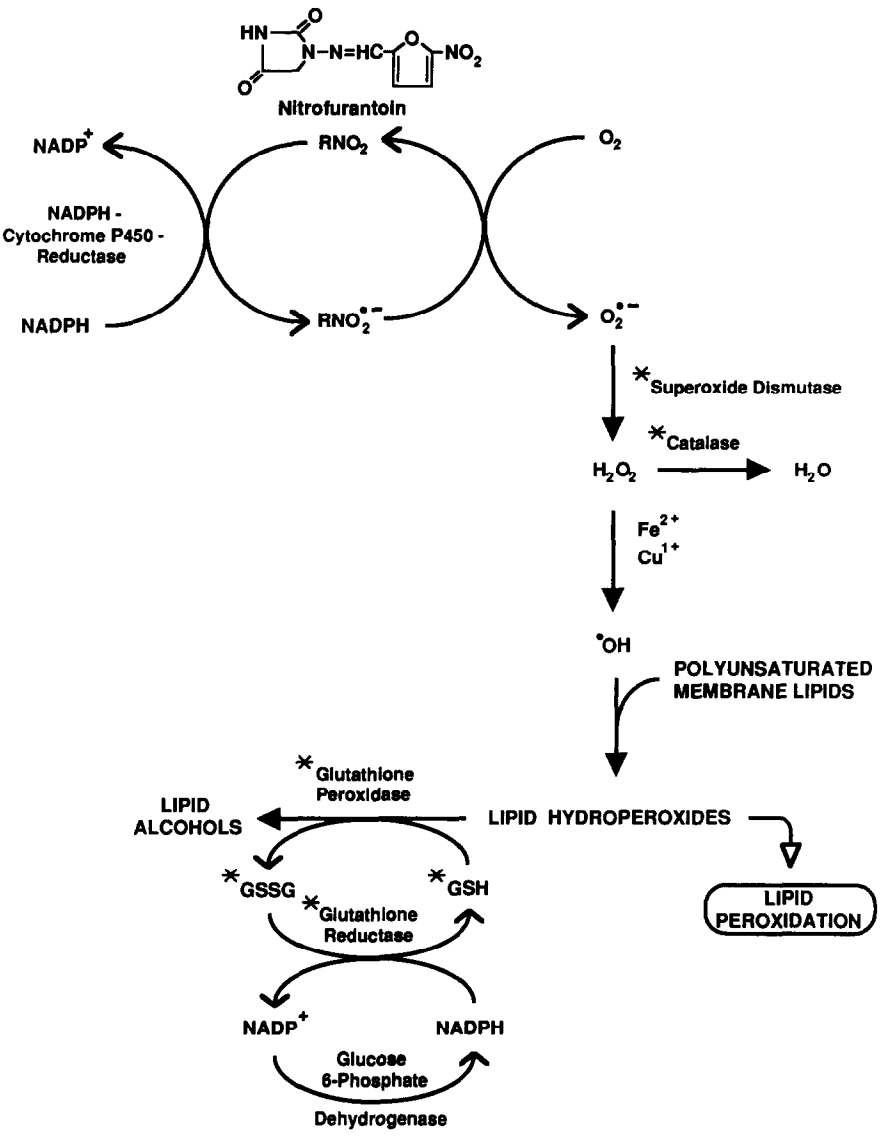

Fig. 1. Schematic representation of metabolic pathways involved in the redox cycling of nitrofurantoin. Nitrofurantoin (RNO_2) is reduced to its radical anion by an electron transfer from NADPH-cytochrome P450 reductase. In the presence of oxygen, this radical is rapidly converted back to RNO_2, generating superoxide anion radical ($O_2^{\cdot-}$). The superoxide anion may dismutate, in the presence of superoxide dismutase (SOD), to form hydrogen peroxide (H_2O_2) which, in turn, may undergo an iron-catalysed Haber–Weiss reaction to form the hydroxyl radical (·OH). These reactive oxygen species are capable of various cytotoxic effects including the initiation of lipid peroxidation. The generation of intracellular reactive oxygen species may not always lead to cellular injury, because of the presence of cellular defence systems such as glutathione (GSH), glutathione peroxidase, glutathione reductase, and catalase. In the present study, a number of possible contributing components (each identified by an asterisk) of this metabolic pathway were examined to elucidate the mechanism(s) of nitrofurantoin-induced pulmonary toxicity.

and increased formation of intracellular oxidized sulfhydryls, which can contribute to cell death [19–21].

Results from both *in vivo* [3–5] and *in vitro* [8, 11, 12, 22, 23] studies have suggested that nitrofurantoin-induced pulmonary toxicity is exerted via oxidant stress-mediated mechanisms. Most of these studies have focussed on experimental systems in which the defence mechanisms have been impaired prior to nitrofurantoin administration. However, there has been no direct evidence from *in vivo* studies so far to verify the role of oxidative stress in nitrofurantoin toxicity. The objective of the present study was to investigate whether nitrofurantoin-induced pulmonary toxicity in normal rats was mediated by oxidant stress mechanisms. The relative

importance of the cellular antioxidant systems in nitrofurantoin toxicity was also assessed. Accordingly, we have examined the activities of antioxidant systems, in particular SOD, CAT, GSH-Px and GSH-R as well as GSH levels in the lungs, at various times following subcutaneous administration of nitrofurantoin in rats. In addition, we have examined the extent of lipid peroxidation in the lungs of nitrofurantoin-treated rats.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (approximate body weight, 220–250 g) were purchased from Charles River Canada, Inc. (St. Constant, Quebec). All animals were housed in stainless-steel cages with free access to pelleted Purina laboratory chow and tap water. The animals were kept at room temperature (22–24°) and were exposed to alternate cycles of 12-hr light and darkness.

Rats were injected subcutaneously with a single dose of nitrofurantoin (200 mg/kg) to induce pulmonary toxicity. Injections were administered between 8:00 and 9:00 a.m. Nitrofurantoin was dissolved in *N,N*-dimethylformamide (DMF) and prepared shortly before use. Control animals were treated with an equivalent volume of the vehicle solution. It should be noted that DMF itself was ineffective in inducing any detectable damaging effects to the lung.

Experimental design. To investigate whether or not nitrofurantoin exerts its toxic effects on the lung by oxidative stress-mediated mechanisms, adult animals were treated with a single dose of nitrofurantoin and killed 0, 1, 2, 3, 6, 10, 14 and 21 days later. Nitrofurantoin-induced pulmonary toxicity was assessed biochemically by measuring the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and angiotensin converting enzyme as well as levels of glutathione and lipid peroxidation.

Tissue preparation. Lungs were removed from animals immediately after decapitation and were rinsed with ice-cold saline to remove excess blood. All subsequent steps were carried out at 0–4°. Following rinsing, lungs were quickly weighed and finely minced. Approximately 1 g of lung sample was homogenized with a Brinkmann Polytron in a sufficient volume of ice-cold 0.01 M potassium phosphate buffer, pH 7.4 to produce a 20% homogenate. The homogenate was centrifuged at 9,000 *g* for 10 min in a refrigerated Sorvall RC-5B centrifuge. The post-mitochondrial supernatant was decanted and recentrifuged at 105,000 *g* for 60 min in a refrigerated Beckman L8-55 ultracentrifuge equipped with a Beckman 50.2Ti rotor to obtain the microsomal fractions. For the measurement of lipid peroxidation, homogenates were prepared as described previously except that the homogenizing medium contained 3 mM EDTA.

Enzyme measurements. Superoxide dismutase in pulmonary homogenates was measured using the technique based on inhibition of pyrogallol (1,2,3-benzenetriol) autooxidation as described by Marklund [24]. Catalase activity in sonicated lung homogenates was determined spectrophotometrically by following the disappearance of hydrogen peroxide at 240 nm as described by Claiborne [25]. Glutathione peroxidase activity in homogenates was determined spectrophotometrically using *tert*-butyl hydroperoxide as the substrate [26]. Glutathione reductase activity was measured according to the method outlined by Karlberg and Mannervik [27].

The activity of angiotensin converting enzyme (ACE) was determined using the Sigma diagnostic procedure as described by Jurima-Romet and Shek [28]. One unit of ACE activity was defined as the amount of enzyme that catalysed the formation of 1 μmol furylacryloylphenylalanine per min at 37°.

Protein determinations were estimated by the method of Lowry *et al.* [29], using crystalline bovine serum albumin (BSA) as the standard.

Determination of lipid peroxidation. Homogenates from treated and control animals were assayed for the presence of lipid conjugated dienes and thiobarbituric acid reactants according to the method of Recknagel and Glende [30] and Buege and Aust [31], respectively. For the measurement of lipid diene conjugates, a 1.0-mL aliquot was extracted with 5.0 mL chloroform:methanol (2:1) and the extract was dried under a stream of argon. The chloroform-free residue was then redissolved in cyclohexane (1.5 mL spectrophotometric grade), and absorbance at 243 nm was recorded against a cyclohexane blank. For the measurement of thiobarbituric acid reactants, homogenate fractions (1.0 mL) were added to 10% trichloroacetic acid (TCA) and centrifuged at 600 *g* for 5 min. The resulting supernatants (1.0 mL) were added to 1% thiobarbituric acid solution, the mixture was incubated at 110° for 10 min and the absorbance of the solution was measured at 535 nm.

Reduced and total glutathione. Reduced and total glutathione, more precisely non-protein sulfhydryl, in pulmonary homogenates was determined as described by Kuo and Hook [32]. Briefly, the tissue was homogenized in 20% (w/v) TCA and centrifuged at 10,000 rpm for 20 min in a refrigerated Sorvall RC-5B centrifuge. An aliquot of the supernatant fraction in 0.3 M phosphate buffer was treated with 5,5-dithiobis-[2-nitrobenzoic acid] (NbS_2) and the absorbance at 412 nm was measured.

Chemicals. Nitrofurantoin, pyrogallol, catalase, glutathione, 2-thiobarbituric acid, and 5,5-dithiobis-[2-nitrobenzoic acid] were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were purchased from the Fisher Scientific Co. (Toronto, Ontario) or BDH (Toronto, Ontario).

Statistical analysis. Data from control and nitrofurantoin-treated groups were evaluated by one-way analysis of variance (ANOVA). If the F values were significant, the unpaired two-tailed Student's t-test was used to compare the treated group to the control group [33]. The level of significance was accepted at $P < 0.05$.

RESULTS

Body weight, wet lung weight and lung ACE activity. The effect of subcutaneously administered nitrofurantoin (200 mg/kg body weight) on body and

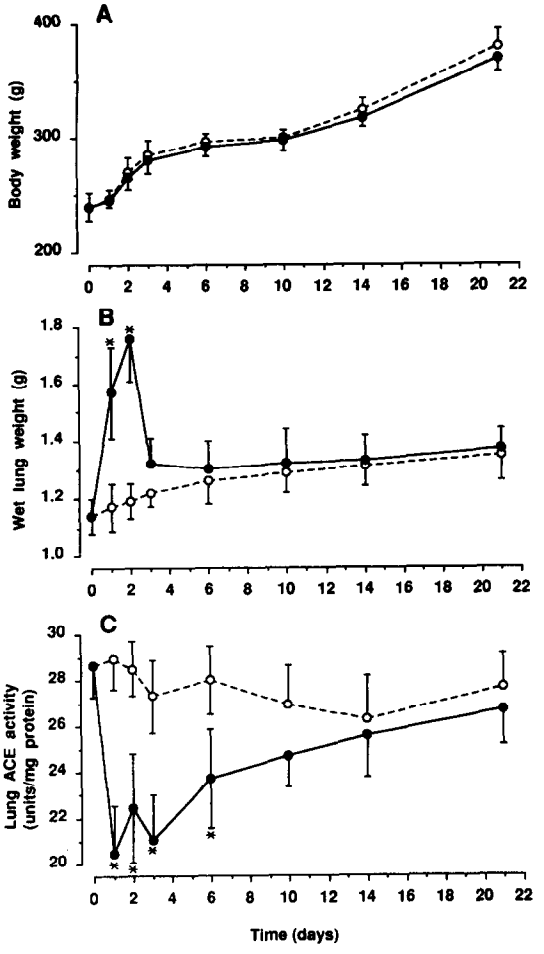

Fig. 2. Effect of nitrofurantoin on body weight (A), wet lung weight (B), and lung angiotensin converting enzyme (ACE) activity (C). Animals in the experimental group (●) were treated with a single subcutaneous injection of nitrofurantoin at a dose of 200 mg/kg and animals in the control group (○) were similarly injected with an equivalent volume of vehicle solution as described in Materials and Methods. Each data point is the mean ± SEM of 4 animals, and each asterisk indicates a statistically significant difference ($P < 0.05$) between the corresponding values obtained from experimental and control animals.

wet lung weights is shown in Fig. 2. The body weight of control animals increased progressively throughout the experimental period; no significant change in this parameter was observed in nitrofurantoin-treated rats when compared to their corresponding controls (Fig. 2A). The wet weight of the unperfused lungs was used as an index of pulmonary edema [28, 34, 35]. As shown in Fig. 2B, wet lung weight of nitrofurantoin-treated rats was significantly higher when compared to those of controls by 34% on day 1 and 48% on day 2; values returned to that of controls by day 3 of treatment and remained relatively unchanged for the rest of the experimental period.

Since angiotensin converting enzyme has been used as a marker of lung injury induced by a number of xenobiotics [36, 37], the effect of nitrofurantoin on the activity of angiotensin converting enzyme in lung tissue was also examined in this study. As shown in Fig. 2C, angiotensin converting enzyme activity was reduced significantly by day 1 (29% of control value) of treatment and remained relatively low up to day 6; thereafter, enzyme activity gradually returned to control levels.

Enzyme activities of lung SOD, CAT, GSH-Px and GSH-R and total protein concentration. To assess the relative importance of the antioxidant system in nitrofurantoin toxicity, the effects of nitrofurantoin on lung superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activities were examined. The activities of superoxide dismutase (Fig. 3A) and glutathione peroxidase (Fig. 3B) were not affected significantly by nitrofurantoin treatment throughout the experimental period. In contrast, catalase activity was decreased significantly on days 1, 2, 3 and 6 of treatment, returning to control levels by day 10 of treatment (Fig. 3C). Similarily, the changes in glutathione reductase activity (Fig. 3D) were qualitatively similar to those observed for catalase activity (Fig. 3C). It should be noted that changes in protein concentrations did not account for the changes in enzyme activities, because the lung total protein concentrations between control and nitrofurantoin-treated rats were not significantly different throughout the entire experimental period (data not shown).

Lung GSH and GSSG concentrations. Since depletion of glutathione has been suggested as a mechanism whereby chemicals produce cell injury [18, 20, 38], the levels of glutathione in lungs of control and nitrofurantoin-treated animals were measured in the present study. Figure 4 shows the total concentration of GSH and GSSG in lung homogenates of control and nitrofurantoin-treated rats. In control rats, the respective concentration of GSH and GSSG remained relatively constant throughout the experimental period and 95% of glutathione was present in the reduced form (GSH). Treatment of rats with nitrofurantoin, on the other hand, resulted in significant decreases in GSH concentrations on days 2 and 3 of treatment. The decreases in GSH concentrations were accompanied by concomitant increases in GSSG concentrations. These data suggest that reduced glutathione was oxidized following the administration of nitrofurantoin.

Formation of diene conjugates and thiobarbituric acid reactants. Previous studies have shown that peroxidation of membrane lipids is a possible mechanism of acute oxidative stress-induced lethal injury [38, 39]. Therefore, in this study, the levels of lipid peroxidation in lung homogenates of control and nitrofurantoin-treated rats were also measured. As shown in Fig. 5, nitrofurantoin produced a transient increase in lipid peroxidation levels as measured by the formation of diene conjugates (Fig. 5A) and thiobarbituric acid reactants (Fig. 5B). The increase in diene conjugates was highest on day 1 of treatment (165% of control value) but gradually returned to control levels by day 6. With respect to thiobarbituric acid reactants, the maximum increase

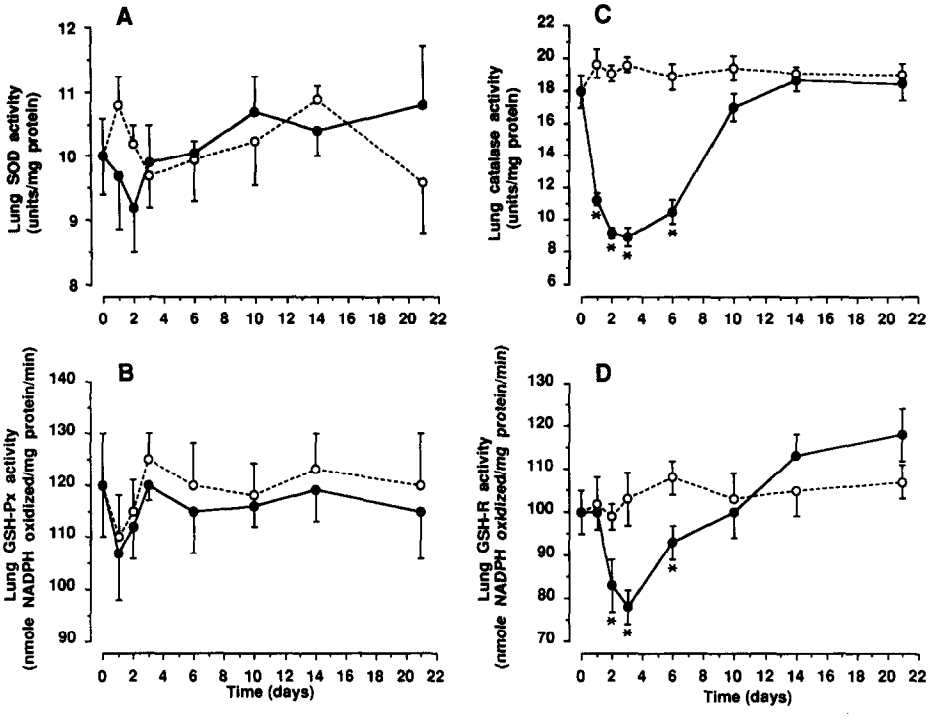

Fig. 3. Pulmonary superoxide dismutase (SOD) (A), glutathione peroxidase (GSH-Px) (B), catalase (C), and glutathione reductase (GSH-R) (D) activities in rats treated with nitrofurantoin. Animals in the experimental group (●) were treated with a single subcutaneous injection of nitrofurantoin at a dose of 200 mg/kg and animals in the control group (○) were similarly injected with an equivalent volume of vehicle solution as described in Materials and Methods. Each data point is the mean ± SEM of 4 animals, and each asterisk indicates a statistically significant difference ($P < 0.05$) between the corresponding values obtained from experimental and control animals.

was observed on day 2 of treatment, declining to 138% of control value 4 days later, and returning to control levels by day 10. The formation of diene conjugates preceded that of TBA reactants because it is known that during the lipid peroxidation process, the diene conjugation assay measures an early stage in the process while the thiobarbituric acid assay measures the latter stages of lipid peroxidation [39]

DISCUSSION

The results of the present study showed that subcutaneously administered nitrofurantoin was toxic to the rat lung as evidenced by the measurable increases in wet lung weight and decreases in lung angiotensin converting enzyme activity. Changes in these parameters have been used successfully as indicators of lung injury induced by pulmonary toxicants such as bleomycin, paraquat, and thioureas [10, 28, 34, 35, 40]. The rapid increases in wet lung weight observed on days 1 and 2 of treatment perhaps reflect the accumulation of excessive intracellular fluid during the acute injury phase. Pulmonary edema, as a cause of increased lung weight, is a common condition following exposure to pulmonary toxicants [28, 34, 35, 40]. Although injury to pulmonary capillary endothelial cells, as evidenced in the present study by decreases in lung ACE activity, has generally been reported as a major cause of pulmonary edema [35, 37], the temporal dissociation between wet lung weight and decrease in ACE activity observed in our study would suggest that edema formation may not be due to direct damage of pulmonary capillary endothelial cells but perhaps due to alterations in capillary permeability.

The mechanism(s) by which nitrofurantoin causes lung injury is not clearly understood. Our data suggest that nitrofurantoin induces pulmonary toxicity, at least in part, via oxidant stress mechanisms. This is supported by our observations that nitrofurantoin treatment resulted in increases in lipid peroxidation and decreases in the GSH/GSSG ratio, both sensitive indicators of oxidative stress [41–43]. These findings are consistent with the known toxicological properties of nitrofurantoin. It has been reported that nitrofurantoin-induced lung damage was enhanced in animals exposed to vitamin E-deficient diets [3], selenium-deficient diets [5] or hyperoxic conditions [3]. Also, the depletion of GSH with concurrent increases in GSSG in nitrofurantoin-perfused lungs [44] or livers [23] supports the involvement of reactive oxygen species. In addition, *in vitro* studies have demonstrated the reduction of nitrofurantoin by pulmonary microsomes [10] or endothelial cells [12, 22] followed by the generation of reactive oxygen species. It is not clear, however, from the results of this study whether the generation

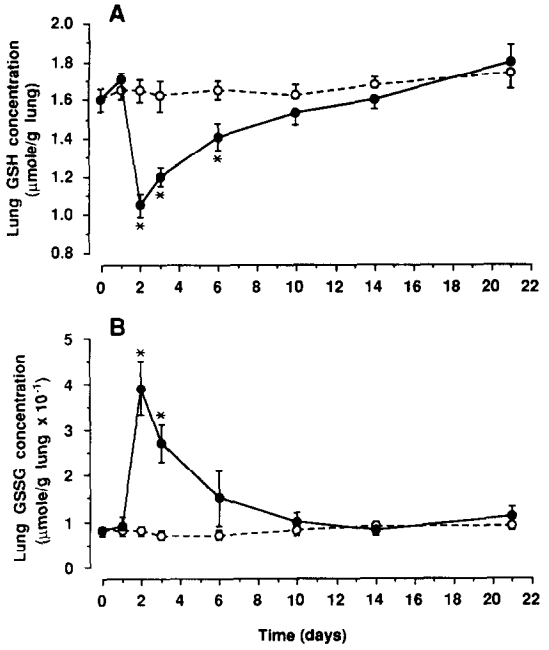

Fig. 4. Effect of nitrofurantoin on pulmonary GSH (A) and GSSG (B) concentrations. Animals in the experimental group (●) were treated with a single subcutaneous injection of nitrofurantoin at a dose of 200 mg/kg and animals in the control group (○) were similarly injected with an equivalent volume of vehicle solution as described in Materials and Methods. Each data point is the mean ± SEM of 4 animals, and each asterisk indicates a statistically significant difference ($P < 0.05$) between the corresponding values obtained from experimental and control animals.

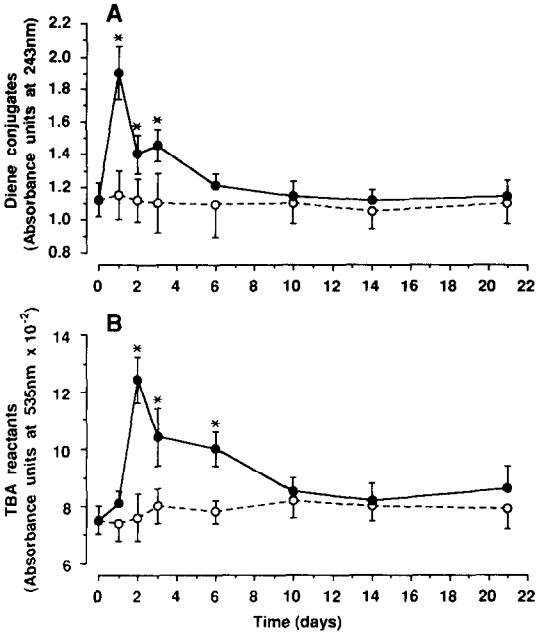

Fig. 5. Lipid peroxidation levels, as measured by the formation of diene conjugates (A) and thiobarbituric acid reactants (B), in lungs of rats treated with nitrofurantoin. Animals in the experimental group (●) were treated with a single subcutaneous injection of nitrofurantoin at a dose of 200 mg/kg and animals in the control group (○) were similarly injected with an equivalent volume of vehicle solution as described in Materials and Methods. Each data point is the mean ± SEM of 4 animals, and each asterisk indicates a statistically significant difference ($P < 0.05$) between the corresponding values obtained from experimental and control animals.

of reactive oxygen species was due to the direct actions of nitrofurantoin or to an influx of inflammatory cells such as neutrophils in response to the nitrofurantoin-induced pulmonary damage. Although the role of neutrophils in nitrofurantoin toxicity remains unclear at this point, increasing evidence suggests that neutrophils can generate reactive oxygen species within lung cells that may result in significant parenchymal cell injury [45, 46].

It is well known that the maintenance of cell integrity in oxidative stress depends on the balance between the free radical generation and free radical defence systems. Imbalances may occur when increased radical production overwhelms the defence system or the defence system is compromised and incapable of detoxifying the normal flux of free radicals or when some combination of increased production and decreased detoxication occurs. The close temporal relationship between changes in the cellular defence system (catalase and glutathione reductase activities as well as glutathione concentrations) and pulmonary toxicity would suggest that the cellular defence system was compromised and unable to detoxify nitrofurantoin-mediated generation of reactive oxygen species. Similar findings have been reported by other investigators who showed that the toxicity of chemicals undergoing redox cycling was enhanced significantly in animals depleted of GSH [47, 48], GSH peroxidase [5, 48] and vitamin E [3, 5] or decreased significantly in animals with elevated lung activities of superoxide dismutase [49] and glucose-6-phosphate dehydrogenase [50].

The results of the present study suggest that an increase in intracellular hydrogen peroxide concentration may be responsible, at least in part, for the nitrofurantoin-induced lung damage. A decrease in catalase and glutathione reductase activities, as well as GSH concentrations, would allow significant increases in effective concentrations of hydrogen peroxide in the lungs of nitrofurantoin-treated animals. Rossi *et al.* [11], examining the nitrofurantoin-mediated oxidative stress toxicity in isolated rat hepatocytes, suggested that hydrogen peroxide plays a significant role in the cytotoxic effects of nitrofurantoin since the catalase inhibitors, azide or aminotriazole, markedly enhanced cytotoxicity. Results from *in vitro* studies with fibroblasts (WI-38) [51] or endothelial cells [12] exposed to nitrofurantoin suggested that the generation of hydrogen peroxide may be of critical importance since catalase had a protective effect against nitrofurantoin-induced cytotoxicity. Other studies have shown that erythrocytes with low catalase activity were sensitive to nitrofurantoin toxicity [52]. Furthermore, lymphocytes obtained from subjects

with glutathione deficiency demonstrated increased susceptibility to nitrofurantoin toxicity [53]. Hydrogen peroxide may exert its toxic effects either directly or, more likely, via the generation of the potentially toxic hydroxyl radical leading to initiation of lipid peroxidation and disruption of critical cell functions [13–18] (Fig. 1).

The role of intracellular glutathione (GSH) in the detoxication of reactive oxygen species has been well established [16, 18, 20]. GSH is known to serve as a reductant in the metabolism of both hydrogen peroxide and various hydroperoxides generated following the peroxidation of membrane lipids, a reaction catalysed by glutathione reductase [18, 38, 43]. During this reaction, GSH is oxidized to its disulfide, GSSG; oxidized glutathione is reduced back to GSH by glutathione reductase at the expense of NADPH (Fig. 1). The close temporal relationship observed in this study between changes in GSH concentrations and glutathione reductase activity would suggest that the decreases in GSH concentration with concurrent increases in GSSG concentrations appear to be due to decreases in glutathione reductase activity. It has been demonstrated in previous *in vitro* studies that nitrofurantoin, or its metabolites, inhibit glutathione reductase activity by interfering directly with the catalytic site of the enzyme [54, 55]. Although the effects of nitrofurantoin on lung GSH concentrations *in vivo* have not been reported as yet, GSH has been shown to be depleted in nitrofurantoin perfused rabbit lungs [44] and rat livers [23], in pulmonary endothelial cells [12, 22], and in hepatocytes [11].

It is clear from the results of this study that nitrofurantoin administration resulted in peroxidation of membrane lipids as evidenced by the formation of thiobarbituric acid reactants and diene conjugates (Fig. 5). Most of the evidence concerning the capacity of nitrofurantoin to induce lipid peroxidation *in vivo* has been obtained from studies in which alterations in host defence mechanisms responsible for controlling lipid peroxidation should cause correlative changes in nitrofurantoin toxicity [3, 5]. Thus, nitofurantoin toxicity is enhanced significantly in rats fed vitamin E-deficient diets and selenium-deficient diets [3, 5] or exposed to oxygen-enriched atmospheres [3]. The potential of nitrofurantoin to induce membrane lipid peroxidation has also been demonstrated *in vitro* [8, 9]. The mechanism(s), however, of nitrofurantoin-induced lipid peroxidation observed in our study is unknown. It is evident that the changes in lipid peroxidation corresponded very well with those of the host defence system suggesting the involvement of reactive oxygen species. This interpretation is confirmed by results from *in vitro* studies where stimulation of nitrofurantoin-induced lipid peroxidation was inhibited by superoxide dismutase, glutathione, ascorbic acid, catalase, or EDTA, agents which either scavenge and/or prevent the generation of reactive oxygen species [8, 9].

Previous studies have shown that peroxidation of membrane lipids and depletion of intracellular GSH concentrations are potential mechanisms of oxidant stress-induced toxicity [18–21, 38, 39, 42, 43]. Under our experimental conditions, membrane lipid peroxidation appears to be a major mechanism, whereby nitrofurantoin exerted its lung damaging effect. This interpetation is supported by our observation that the increases in lipid peroxidation occurred at the time of greatest acute injury. Depletion of GSH observed on day 2 of treatment may have also contributed to the observed toxicity of nitrofurantoin. It has been reported that decreases in the level of reduced glutathione are followed by extensive increases in lipid peroxidation and cell death [23, 38, 39]. Injury to the lung by a mechanism involving lipid peroxidation has also been shown to occur following exposure of animals to other redox cycling agents such as paraquat [10, 21, 47]. However, the precise role of lipid peroxidation as a possible mechanism of nitrofurantoin-induced pulmonary toxicity remains to be defined.

In summary, the results of the present study showed that pulmonary toxicity produced by nitrofurantoin was induced by oxidative stress-mediated mechanisms. Also, our results have provided *in vivo* evidence for lipid peroxidation as a possible cause of tissue damage. We believe that the significance of suggesting lipid peroxidation as a mechanism for nitrofurantoin toxicity could be important in providing useful information for the consideration of rational therapeutic approaches in treating patients suffering from nitrofurantoin toxicity.

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REFERENCES

1. Cunha BA, Nitrofurantoin—Current concepts. *Urology* **32**: 67–71, 1988.
2. Witten CM, Pulmonary toxicity of nitrofurantoin. *Arch Phys Med Rehabil* **70**: 55–57, 1989.
3. Boyd MR, Catignani GL, Sasame HA, Mitchell JR and Stiko AW, Acute pulmonary injury in rats by nitrofurantoin and modification by vitamin E, dietary fat, and oxygen. *Am Rev Respir Dis* **120**: 93–99, 1979.
4. Martin WJ, Nitrofurantoin. Potential direct and indirect mechanisms of lung injury. *Chest* **83** (Suppl): 51S–53S, 1983.
5. Peterson FJ, Combs GF, Holtzman JL and Mason RP, Effect of selenium and vitamin E deficiency on nitrofurantoin toxicity in the chick. *J Nutr* **112**: 1741–1746, 1982.
6. Rao RDN, Jordan S and Mason RP, Generation of nitro radical anions of some 5-nitrofurans, and 2- and 5-nitroimidazoles by rat hepatocytes. *Biochem Pharmacol* **37**: 2907–2913, 1988.
7. Biaglow JE, Varnes MB, Roizen-Towle L, Clark EP, Epp ER, Astor MB and Hall ET, Biochemistry of reduction of nitroheterocycles. *Biochem Pharmacol* **35**: 77–90, 1986.
8. Youngman RJ, Osswald WF and Elstner EF, Mechanisms of oxygen activation by nitrofurantoin and relevance to its toxicity. *Biochem Pharmacol* **31**: 3723–3729, 1982.
9. Trush MA, Mimnaugh EG and Gram TE, Activation of pharmacologic agents to radical intermediates. *Biochem Pharmacol* **31**: 3335–3346, 1982.
10. Boyd MR, Biochemical mechanisms in chemical-

induced lung injury: roles of metabolic activation. *CRC Crit Rev Toxicol* **7**: 103–176, 1980.
11. Rossi L, Silva JM, McGirr LG and O'Brien PJ, Nitrofurantoin-mediated oxidative stress cytotoxicity in isolated rat hepatocytes. *Biochem Pharmacol* **37**: 3109–3117, 1988.
12. Martin WJ, Nitrofurantoin: Evidence for the oxidant injury of lung parenchymal cells. *Am Rev Respir Dis* **127**: 482–486, 1983.
13. Halliwell B, Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. *Br J Exp Pathol* **70**: 737–757, 1989.
14. Kappus H, Oxidative stress in chemical toxicity. *Arch Toxicol* **60**: 144–149, 1987.
15. Sies H, Oxidative stress: Introductory remarks. In: *Oxidative Stress* (Ed. Sies H), pp. 1–8. Academic Press, New York, 1985.
16. Halliwell B and Gutteridge JMC, Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. *Lancet* **1**: 1396–1397, 1984.
17. Balentine JD, *Pathology of Oxygen Toxicity*. Academic Press, New York, 1982.
18. Chance B, Sies H and Boveris A, Hydroperoxide metabolism in mammalian organs. *Physiol Rev* **59**: 527–605, 1979.
19. Farber J, The role of calcium ions in toxic cell injury. *Environ Health Perspect* **84**: 107–111, 1990.
20. Pascoe GA and Reed DJ, Cell calcium, vitamin E and the thiol redox system in cytotoxicity. *Free Radic Biol Med* **6**: 209–224, 1989.
21. Bus JS and Gibson JE, Paraquat: Model for oxidant-initiated toxicity. *Environ Health Perspect* **55**: 37–46, 1984.
22. Martin WJ, Powis GW and Kachel DL, Nitrofurantoin-stimulated oxidant production in pulmonary endothelial cells. *J Lab Clin Med* **105**: 23–29, 1985.
23. Hoener B, Noach A, Andrup M and Yen T-SB, Nitrofurantoin produces oxidative stress and loss of glutathione and protein thiols in the isolated perfused rat liver. *Pharmacology* **38**: 363–373, 1989.
24. Marklund SL, Pyrogallol autooxidation. In: *CRC Handbook of Methods for Oxygen Radical Research* (Ed. Greenwald RA), pp. 243–247. CRC Press, Boca Raton, FL, 1985.
25. Claiborne A, Catalase assay. In: *CRC Handbook of Methods for Oxygen Radical Research* (Ed. Greenwald RA), pp. 283–284. CRC Press, Boca Raton, FL, 1985.
26. Del Maestro RF and McDonald W, Oxidative enzymes in tissue homogenates. In: *CRC Handbook of Methods for Oxygen Radical Research* (Ed. Greenwald RA), pp. 291–296. CRC Press, Boca Raton, FL, 1985.
27. Carlberg I and Mannervik B, Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J Biol Chem* **250**: 5475–5480, 1975.
28. Jurima-Romet M and Shek PN, Biochemical changes in rat lung during acute paraquat intoxication. *Biomed Environ Sci* **3**: 343–352, 1990.
29. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
30. Recknagel RO and Glende EA, Spectrophotometric detection of conjugated dienes. In: *Methods in Enzymology* (Ed. Packer L), Vol. 105, pp. 331–337, Academic Press, New York, 1984.
31. Buege JA and Aust SD, Microsomal lipid peroxidation. In: *Methods in Enzymology* (Eds. Fleisher S and Packer L), Vol. 52, Part C, pp. 302–310. Academic Press, New York, 1978.
32. Kuo C and Hook J, Depletion of renal glutathione content and nephrotoxicity of cephaloridine in rabbits, rats and mice. *Toxicol Appl Pharmacol* **63**: 292–302, 1982.
33. Gad SC and Weil CS, Statistics for toxicologists. In: *Principles and Methods of Toxicology* (Ed. Hayes AW), pp. 273–319, Raven Press, New York, 1982.
34. Giri SN, Hollinger MA and Sciedt MJ, The effects of paraquat and superoxide dismutase on pulmonary vascular permeability and edema in mice. *Arch Environ Health* **36**: 149–154, 1988.
35. Visscher MB, Haddy FJ and Stephens G, The physiology and pharmacology of lung edema. *Pharmacol Rev* **8**: 389–434, 1956.
36. Lazo JS, Lynch TJ and McCollister J, Bleomycin inhibition of angiotensin converting enzyme activity from serum, lungs and cultured pulmonary artery endothelial cells. *Am Rev Respir Dis* **134**: 73–78, 1986.
37. Hollinger MA, Giri SN, Patwell S, Zuckerman JE, Gorin A and Parsons G, Effect of acute lung injury on angiotensin converting enzyme in serum, lung lavage and effusate. *Am Rev Respir Dis* **121**: 373–376, 1980.
38. Boobis AR, Fawthrop DJ and Davis DS, Mechanisms of cell death. *Trends Pharmacol Sci* **10**: 275–280, 1989.
39. Horton AA and Fairhurst S, Lipid peroxidation and mechanisms of toxicity. *CRC Crit Rev Toxicol* **18**: 27–70, 1987.
40. Tom W-M and Montgomery MR, Biochemical and morphological assessments of bleomycin pulmonary toxicity in rats. *Toxicol Appl Pharmacol* **53**: 64–74, 1980.
41. Jenkinson SG, Marcum RF, Pickard JS, Orzechowski Z, Lawrence RA and Jordan JM, Glutathione disulfide formation occurring during hypoxia and reoxygenation of rat lung. *J Lab Clin Med* **112**: 471–480, 1988.
42. Girroti AW, Mechanisms of lipid peroxidation. *J Free Radic Biol Med* **1**: 87–95, 1985.
43. Mitchell JR, Smith CV, Lauterbourg BH, Hughes H, Corcoran GB and Horning EC, Reactive metabolites and the pathophysiology of acute lethal injury. In: *Drug Metabolism and Drug Toxicity* (Eds. Mitchell JR and Horning MG), pp. 301–319. Raven Press, New York, 1984.
44. Dunbar JR, Delucia AJ and Bryant LR, Glutathione status in isolated rabbit lungs: Effects of nitrofurantoin and paraquat perfusion with normoxic and hyperoxic ventilation. *Biochem Pharmacol* **33**: 1343–1348, 1984.
45. Craddock PR, Fehr J, Dalmasso AP, Brigham KL and Jacob HS, Hemodialysis leukopenia: pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes. *J Clin Invest* **59**: 879–888, 1977.
46. Martin WJ, Neutrophils kill pulmonary endothelial cells by a hydrogen-peroxide dependent pathway. *Am Rev Respir Dis* **130**: 209–213, 1984.
47. Bus JS, Aust SD and Gibson JE, Lipid peroxidation: A possible mechanism for paraquat toxicity. *Res Commun Chem Pathol Pharmacol* **11**: 31–38, 1975.
48. Cagen SZ and Gibson JE, Liver damage following paraquat in selenium-deficient and diethylmaleate-treated mice. *Toxicol Appl Pharmacol* **40**: 193–200, 1977.
49. Crapo JD and Tierney DF, Superoxide dismutase and pumonary oxygen toxicity. *Am J Physiol* **226**: 1401–1407, 1974.
50. Fisher HK, Cements JA and Wright RR, Enhancement of oxygen toxicity by the herbicide paraquat. *Am Rev Respir Dis* **107**: 246–252, 1973.
51. Michiels C and Remacle J, Quantitative study of natural antioxidant systems for cellular nitrofurantoin toxicity. *Biochim Biophys Acta* **967**: 341–347, 1988.
52. Lyng PJ, Kachel DL and Martin WJ, Importance of hydrogen peroxide in nitrofurantoin-induced cytotoxicity: Evidence from an inbred catalase-deficient strain of mice. *J Lab Clin Med* **112**: 301–306, 1988.

53. Spielberg SP and Gordon GB, Nitrofurantoin cytotoxicity: *In vitro* assessment of risk based on glutathione metabolism. *J Clin Invest* **67**: 37–41, 1981.
54. Grinblat L, Sreider CM and Stoppani AOM, Nitrofuran inhibition of yeast and rat tissue glutathione reductases. *Biochem Pharmacol* **38**: 767–772, 1989.
55. Buzard JA, Kopko F and Paul MF, Inhibition of glutathione reductase by nitrofurantoin. *J Lab Clin Med* **56**: 884–890, 1960.