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In vivo bioassays of acute asbestosis and its correlation with ESR spectroscopy and imaging in redox status

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Abstract

In vivo electron spin resonance (ESR) spectroscopy and whole body imaging were used to investigate the toxicity of biological reactions and organ specific oxidative changes associated with the development of acute asbestosis. Pathogen-free mice were exposed to 100 μg of crocidolite asbestos suspended in 50 μL of a 0.9% NaCl solution by aspiration. The bio-assay group had broncho-alveolar lavage (BAL) and serum draws performed on control and treated mice at 1, 3, and 7 days post-instillation. The ESR spectroscopic measurements and whole body imaging were performed with a separate group of mice at the same time points. Bio-assays included measurements of albumin, lactate dehydrogenase (LDH), N-acetyl-β-D-glucoaminidase (NAG), and catalase in acellular lavage fluids, and total antioxidants status in blood serum. ESR spectroscopic and imaging measurements were performed after intraperitoneal injection of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-^{15}N-1-oxyl (TEMPOL) or 3-carbamoylproxyl (3-CP) nitroxides at a final concentration of 344 mg/kg body weight. Albumin showed a significant increase in BAL fluid at the 3 day exposure time point. The presence of this protein in lavage fluid indicates that the gas/blood barrier has been damaged in the lung. LDH in BAL fluid also exhibited a significant increase at 3 days post-exposure, an indication of enhanced cell membrane damage in the lung. Similar results were observed for NAG, a lysosomal enzyme, implying activation of phagocytic cells. Contemporaneously with the development of acute asbestosis at day 3 post-exposure, there were significant increases in the levels of total antioxidants in the serum and catalase in the BAL fluid. Significant impairment in the ability of asbestos exposed animals to clear TEMPOL radical during acute disease progression was evident at days 1 and 3 post exposure. ESR image measurements provided information on the location and distribution of the 3-CP label within the lungs and heart of the mouse and its clearance over time. Bioassays in concert with ESR spectroscopy and imaging presented in this study provide congruent data on the early acute phase of pulmonary injury and oxidant generation in response to asbestos exposure and their decline after 7 days. The increased levels of total antioxidants in the serum and catalase in BAL fluid correlated with the reduction in the clearance rate for TEMPOL, suggesting that a change in the redox status of the lung is associated with lung injury induced by asbestos. (Mol Cell Biochem **234/235**: 369–377, 2002)

Key words: electron spin resonance, ESR, ESR imaging, asbestosis, antioxidants, oxidative stress, lung damage, redox status

Introduction

Many biological reactions which cause cell injury and cell death can be attributed to reactive oxygen species (ROS) and the free radicals involved with them [1–3]. ROS are also involved in a number of pulmonary diseases caused by air pollutants, occupational exposures and tobacco smoke [4–8]. Examining ROS in living systems presents some obstacles

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due to the low concentrations and short half-lives of these radicals *in vivo*. Electron spin resonance (ESR) spectroscopy using spin traps can be used to measure these radicals. However, the spin trapping method is not suitable at this time for *in vivo* applications. In this study, we demonstrate the use of a low-molecular weight nitroxide, TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-^{15}N-1-oxyl), and 3-CP (3-carbamoyl-proxyl) nitroxides as probes to monitor oxidative stress in mice exposed to asbestos administered by asparation.

TEMPOL, a nitroxide, is known to function as an antioxidant in a variety of *in vitro* and *in vivo* animal models and disease [9–11]. This ability has been attributed to SOD mimicking, oxidation of reduced metals, and termination of free radical chain reactions [12, 13]. Nitroxides are also known to react with a variety of biological oxidants, including oxygen free radicals [14–17], and to protect against superoxide and hydrogen peroxide cytotoxicity [18, 19]. The nitroxides are ESR detectable (paramagnetic), and the one-electron reduced metabolite of TEMPOL (namely hydroxylamine) is diamagnetic and ESR-silent. Thus, nitroxides administered *in vivo* are converted using a cellular redox process to the hydroxylamine [20]. This process depends on the redox status (balance of oxidants to antioxidants) of the tissue being examined. The *in vivo* ESR method enables non-invasive determination of the nitroxide redox process in whole organs and tissues.

ESR imaging (ESRI) is performed using magnetic field gradients. It enables not only detection of free radicals and paramagnetic species, but also allows one to visualize the distribution of the species in an object. It has been shown that low-frequency (1.2 GHz or less) ESRI is capable of providing detailed mapping of free radicals in a variety of biological systems including tissues, isolated organs, and small animals [21, 22]. Recently, the technique has been reported to be useful for measuring spatial and metabolic profiling of topically applied nitroxides on human skin [23, 24]. This technique provides mapping and differential reduction rates of nitroxide in different part of the tissues [25–27]. The nitroxide, 3-CP, is reduced at relatively slower rates compared to TEMPOL in tissues [28]. Since imaging experiments take more time compared to spectroscopy, the slow clearance of 3-CP can be used advantageously for ESR imaging applications to obtain spatially resolved pharmacokinetic information. Thus, ESR imaging studies using nitroxides as redox sensitive spin probes might provide a non-invasive method of mapping metabolic information. The present study is intended to demonstrate the feasibility of detecting alterations in biochemical and cellular events caused during the development of acute asbestosis in mice. The results show major differences in nitroxide metabolism and distribution between normal and asbestos-exposed mice. It is further demonstrated that the ESR data correlate with bioassays of pulmonary damage and changes in antioxidant status.

Materials and methods

Reagents

TEMPOL, 3-CP, H_2O_2 and sodium formate were purchased from Sigma Chemical Company (St. Louis, MO, USA). Phosphate buffered saline (PBS), containing KH_2PO_4 (1.06 mM), Na_2HPO_4 (5.6 mM), NaCl (154 mM) at pH 7.4, was purchased from BioWhittaker Inc. (Walkersville, MD, USA). Chelex100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA, USA). The PBS was treated with Chelex100 to remove transition metal ion contaminants.

Animals

Healthy male BALB/c mice (4–6 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME, USA). They were acclimatized for 1 week before use in an animal care facility approved by the American Association for Accreditation of Laboratory Animal Care. The mice were housed in HEPA filtered ventilated cages on Betachip hardwood bedding and provided water and food *ad libitum*. Pulmonary exposure of mice to crocidolite asbestos was performed after the animals were anesthetized with a mixture of Ketamine and Xylazine (45 and 8 mg/kg i.p., respectively). Pulmonary exposure was by the aspiration technique [29]. The animals were placed on a board in a supine position, and the tongue extended with lined forceps. Then 50 µl of a NaCl solution was placed on the back of the tongue and subsequently aspirated.

Sham controls were exposed to NaCl solution only, while asbestos exposed animals had 100 µg crocidolite suspended in the NaCl solution.

Bronchoalveolar lavage (BAL)

At selected time intervals, asbestos-exposed and sham-exposed mice were euthanized with an intraperitoneal injection of 0.25 ml of pentobarbital sodium (EUTHA-6, Western Medical Supply, Arcadia, CA, USA). A tracheal cannula was inserted, and the lungs were lavaged through the cannula with ice-cold calcium and magnesium free PBS. Five lavages of 0.6 ml each were collected to obtain sufficient lavage fluid to perform all studies. The bronchoalveolar lavage fluid (BALF) obtained after the separation of cells was used for the albumin, lactate dehydrogenase (LDH), N-acetyl-β-D-glucoaminidase (NAG), and catalase assays. BALF and cells were isolated by centrifugation (500 g for 10 min at 4°C). The cell-free supernatants were collected and used for biochemical measurements.

Enzyme measurements

To determine the toxicity of instilled crocidolite, we measured BALF levels of a serum protein, albumin; activity of a cytosolic enzyme, lactate dehydrogenase (LDH); levels of a lysosomal enzyme, NAG; and levels of a heme enzyme, catalase which breaks down H_2O_2 into H_2O and O_2. In animals exposed to asbestos and sham controls BAL was performed, and the supernatant from the first lavage was used for enzyme and other measurements. Albumin was assayed by the measurement of binding of bromcresol green (BCG) to produce a blue green color with an absorbance maximum at 628 nm (Sigma Diagnostics, St. Louis, MO, USA) [30]. LDH activity was determined by monitoring the oxidation of pyruvate coupled with the reduction of NAD at 340 nm with a LDH assay kit (Roche Diagnostic Systems, Montclair, NJ, USA) [31]. NAG concentration was measured at 580 nm according to the method of Yakada *et al*. [32]. Catalase was measured at 550 nm by the methods outlined in Wheeler *et al*. [33, 34]. All the assays were performed on a Cobas FARA II analyzer (Roche Diagnostic Systems, Montclair, NJ, USA).

Total antioxidant status

Total antioxidant status (TAS) assay uses the reaction between 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS), peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^{\cdot+}$, which was measured at 600 nm [35, 36]. This assay was performed on serum samples obtained from blood drawn from mice at 0, 1, 3 and 7 days post instillation. The TAS test measures the total antioxidant effect of three defense systems, primary, secondary and tertiary, in circulation. These systems include primary antioxidants, which work by preventing the formation of new free radical species. They involve superoxide dismutase (SOD), glutathione peroxidase and metal binding proteins. Secondary antioxidant defenses trap radicals thereby preventing chain reactions. These include vitamin E, vitamin C, beta-carotene, uric acid and bilirubin. Tertiary defenses repair biomolecules damaged by free radicals and include DNA repair enzymes [37].

ESR spectroscopy and imaging instrumentation

In vivo ESR spectroscopy and imaging measurements were performed using ESR imaging instrumentation consisting of a Bruker ESP 300E L-band (1.09 GHz) EPR spectrometer (Bruker Instruments, Billerica, MA, USA), three sets of custom-built water-cooled gradient coils and a personal computer-based data acquisition system [21, 22, 25, 38]. Each set of coils was independently powered by Hewlett-Packard DC power supplies (Model 6010A), interfaced to a personal computer via GPIB bus. The polarity of the currents was reversed using a relay device triggered by an IOTECH DAC488/4 Digital-Analog converter. ESR spectra were recorded using a Bruker bird-cage resonator with 34 mm access diameter.

In vivo *pharmacokinetics measurement of nitroxide in mice*

Crocidolite asbestos-exposed and control animals were anesthetized and intraperitonelly injected (i.p.) with the nitroxide spin label, TEMPOL, at a dose of 344 mg/kg. Pharmacokinetic ESR measurements were performed at body temperature using the Bruker ESP 300E L-band spectrometer. ESR spectra were acquired using custom-developed software (SPEX-300, U.S. EPR, Clarksville, MD, USA). The mice were placed in a 50 ml plastic centrifuge tube, which was then centered at the active volume of the bird-cage resonator. Spectral acquisitions were started within 3 min after injection of TEMPOL. The spectra were then acquired every 2 min for 20 min. The software was capable of performing fully automated data acquisition, baseline setting, integration of a selected segment, and quantification as a function of time [26]. The kinetic data were obtained by measuring the low-field line of the nitroxide triplet spectrum. The data acquisition parameters were: sweep width 10 G, microwave power 4.5 mW, modulation frequency 25 kHz, modulation amplitude 1.5 G, receiver gain 2.0×10^5, and time constant 0.16 sec. Double integration was performed on the measured first-derivative ESR spectra to obtain relative intensity of the spectra.

Projection acquisition and image reconstruction

Projection of data were acquired using an angular sampling method. The projections were acquired as single scans (1024 points/projection) using a constant sweep time. The measured projections were corrected for removal of hyperfine-based artifacts and deconvoluted with the corresponding zero-gradient projection [27]. The deconvoluted projections were then convoluted with a Shepp-Logan filter and subsampled to 128 points for back projection. A single-stage, filtered back projection reconstruction algorithm was used to recover the image. Projection data acquisition and subsequent image reconstruction were performed using a personal computer equipped with an IEEE-488 GPIB board (Capital Equipment Corporation, Burlington, MA, USA).

Statistical analysis

Each study group consisted of 4 or more animals. Statistical analysis between study groups was performed with paired

two-tailed Student's *t*-test. The level of significance was set at $p < 0.05$.

Results

Protein and enzyme levels in BALF and blood

To detect possible lung damage in crocidolite asbestos-exposed mice, albumin and several enzymes in the BALF of exposed and control animals were measured at days 1, 3 and 7 post exposure. Albumin levels in the BALF increased slightly over control at 1 day post exposure to asbestos and to a significant level after 3 days. Albumin levels in BALF dropped off to near baseline control levels at 7 days post exposure to asbestos (Fig. 1, panel A). These results indicate a significant damage to the gas/blood barrier in the lung at 3 days post exposure to asbestos.

LDH, a cytosolic enzyme, activity showed a significant increase in the BALF of animals at day 3 post exposure to asbestos. Similar to the drop in albumin post 7 days exposure to asbestos, LDH activity also leveled off to the base line control values at the 7 day post instillation time point (Fig. 1, panel B). The presence of increased activity of LDH in the BALF is an indication of cell membrane damage through release of cytoplasmic constituents. These observations show that significant cell damage occurred at the 3 day post instillation time point. The lysosomal enzyme NAG was only mildly increased at the 1 day post instillation time point. Increased levels were significant at the day 3 post instillation time point and declined to control baseline levels at 7 day post exposure to asbestos (Fig. 1, panel C). The increased level of NAG in the BALF is indicative of enhanced activity of phagocytotic cells as they attempt to internalize asbestos fibers.

The enzyme catalase, which can serve as an antioxidant by reacting with H_2O_2 to form H_2O and O_2, also exhibited slightly increased levels (although not significant) at the 3 day post instillation time points (Fig. 1, panel D). Catalase can be produced by many cell types including macrophages in response to oxidative stress. Increased catalase would indicate an upregulation of antioxidant defenses induced by oxidative stress.

Total antioxidant status (TAS) in blood serum was measured and was shown to exhibit the same trend of a significant increase at the 3 day post instillation time point, while at 1 day and 7 day post instillation time points no significant difference was observed from the control mice (Fig. 1, panel E). This upregulation of whole body antioxidant defenses expressed at the systemic level may be an outcome of severe oxidative stress present in the lungs. This systematic increase paralleled with changes observed in lungs, i.e. albumin and enzyme levels in BALF also showed significant increases in pulmonary damage at 3 days post instillation.

TEMPOL metabolic clearance rates

TEMPOL clearance rates in both control and exposed mice were measured by following the decay process as evidenced as the change in ESR peak intensities over time. Figure 2 shows a typical ESR spectrum obtained from a mouse after injection with 344 mg/kg TEMPOL. The pharmacokinetics of nitroxide clearance in the thoracic region of the animals were followed using the low-field ESR peak of the nitroxide signal. Figure 3 shows representative decay curves (spectral intensity vs time) from control (sham treated) and asbestos-treated mice at 3 days after asbestos exposure. The plot clearly shows distinct differences in the rate constants (pseudo first order by least-squares best fit method) for decay between the control and treated mice at day 3. Similar results were obtained from 0, 1, 3, and 7 day post exposure groups (data not shown). Mean rate constants were calculated at 0, 1, 3, and 7 day post exposure (Fig. 4). Results from 1 day post exposure show a significant decrease in the rate constant indicating an impaired clearance of TEMPOL radical from the lungs. Data from three days post exposure show an even greater decrease in the rate constant, implying that in the animals exposed to asbestos the lungs were in oxidative stress and cleared radicals at a slower rate. Clearance at the seven day post exposure time point shows a return to control base line efficiency. At 1 day after aspiration of asbestos the clearance rate was 32% lower than in the sham exposed control clearance rate and at 3 days post exposure the clearance rate dropped to 45% lower than that in the control animals. These data imply that the mice at 1 and 3 days after asbestos exposure were seriously compromised and had some difficulty clearing TEMPOL radicals due to a change in redox state. This may have resulted from an enhanced radical production occurring in the inflamed lung during the development of acute asbestosis or as a result of an activated antioxidant radical defense system and the imbalance with radical generation. This inefficient radical clearance exhibited a time course similar to that for the enhancement of albumin and enzyme levels in BALF and total antioxidant status in blood serum. The three day time point post exposure to asbestos showed the culmination of these events as the most critical point of a change in redox status which correlated with acute asbestosis.

Imaging

Imaging data showed the same trend which was observed in the TEMPOL clearance rate measurements. In control animals, 3-CP radical clearance was rapid (Fig. 5, upper panel). Clearance occurred at a higher rate than in the animals exposed to asbestos post 3 days. The 1 day and 7 day post exposure mice showed similar clearance rates to the control

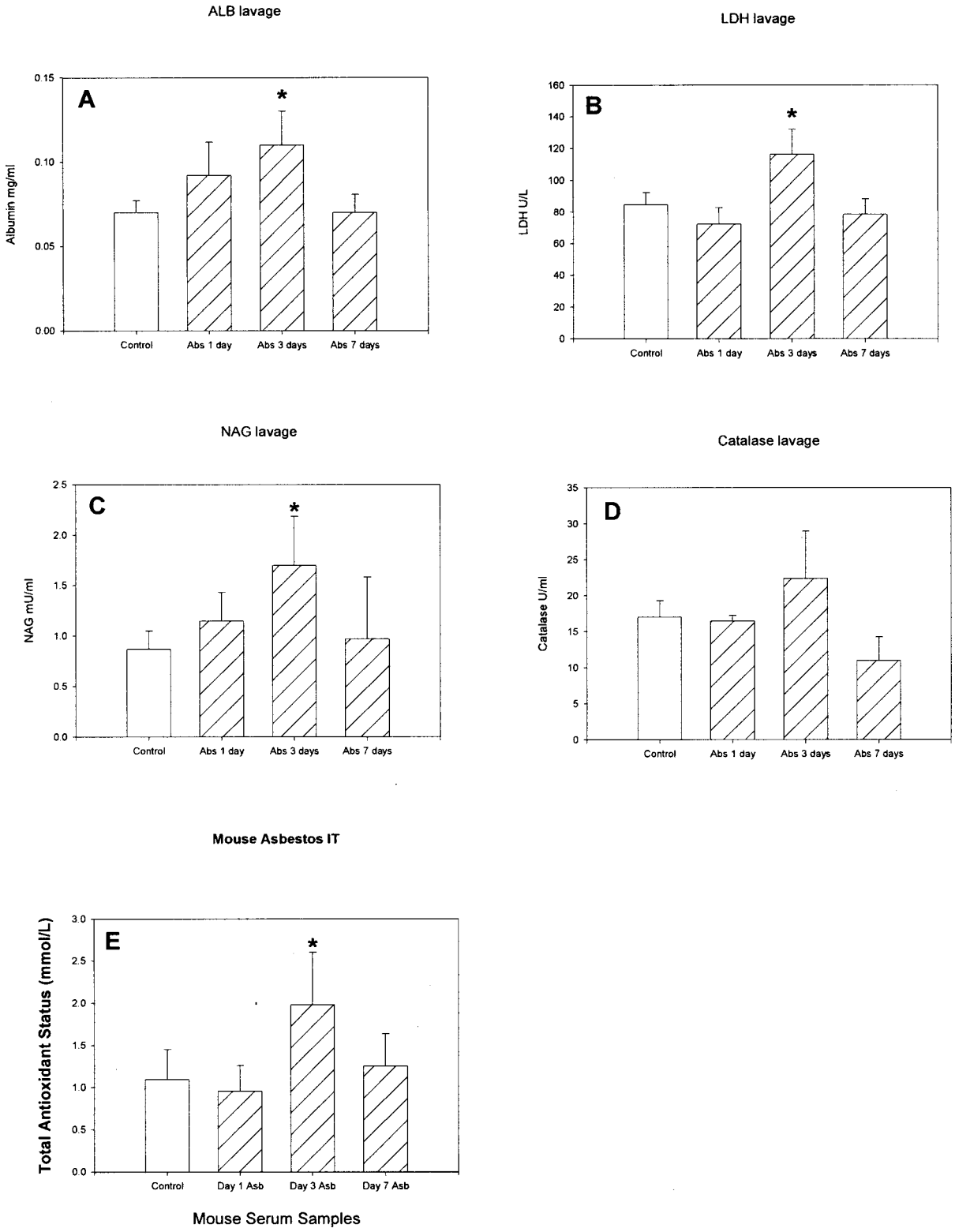

Fig. 1. Bioassay data from mice exposed by aspiration to asbestos (100 μg crocidolite) determined 1, 3, and 7 day post exposure: (A) albumin protein levels measured in lavage fluid from control and exposed mice; (B) lactate dehydrogenase (LDH) enzyme activity measured in lavage fluid from control and exposed mice; (C) N-acetyl-b-D-glucoaminidase (NAG) enzyme levels measured in lavage fluid from control and exposed mice; (D) catalase enzyme levels measured in blood from control and exposed mice; (E) total antioxidant status measurements from serum taken from control and exposed mice. *indicates a significant elevation from control ($p \leq 0.05$).

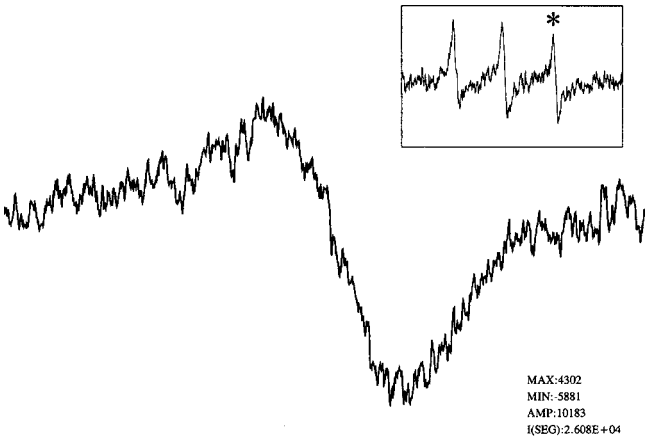

Fig. 2. An ESR spectrum measured *in vivo* from the thoracic region of a mouse. The mouse was intraperitoneally injected with 344 mg/kg TEMPOL and the spectrum was measured using L-band ESR after 5 min. The triplet, arising due to hyperfine splitting from the ^{14}N nucleus, is characterized by the coupling constant 15.8 G and peak-to-peak width 1.60 G. Measurement parameters are as described in the Materials and methods section. Insert shows triplet nitroxide radical spectra. The low-field peak (*) was used for pharmacokinetics studies.

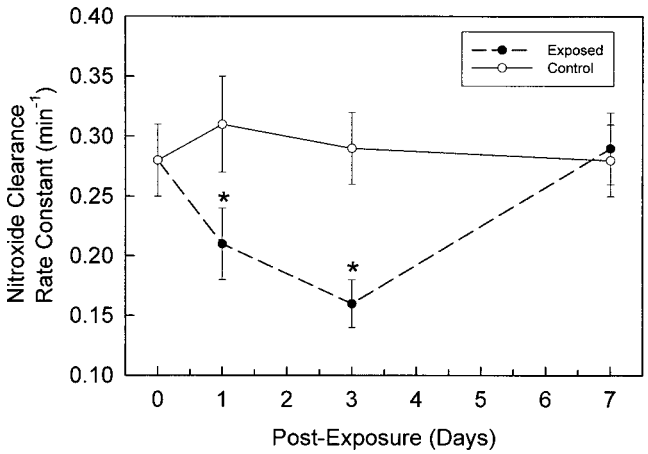

Fig. 4. Plot of rate constants of TEMPOL decay measured from control and exposed mice as a function of post-exposure time. The nitroxide clearance rates were measured at 0, 1, 3 and 7 days post exposure, and rate constants were then calculated as described in the text. Mice from the 1 and 3 day post asbestos exposure groups (●) showed significantly slower clearance rates than the control mice (O).

mice from the amount of radical observed in the imaging (data not shown). Mice exposed to asbestos and imaged after 3 days showed a dramatic decline in the ability to clear 3-CP compared to controls (Fig. 5, lower panel). These images were acquired for three 10 min time periods for a total of 30 min. The 5–15 time period images show an observable

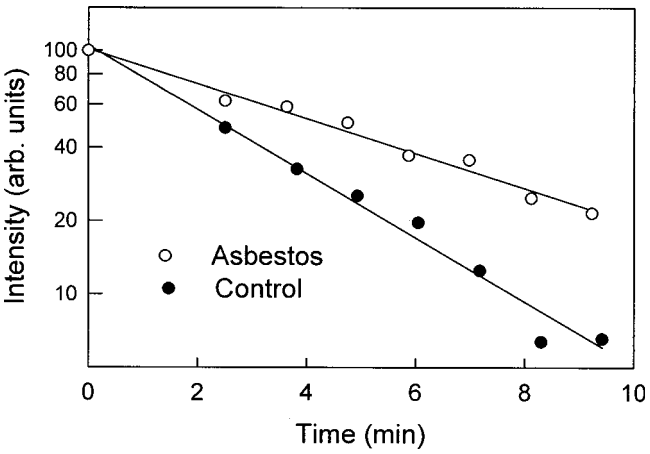

Fig. 3. Pharmacokinetics of nitroxide in control and asbestos-exposed mice. Time course of the ESR signal intensity of TEMPOL in the thoracic region of control and treated mice at 3-days post-exposure were measured following 344 mg/kg of TEMPOL injection (i.p.). Data shown are representative from a single animal of 4 tested. The semi-log plot shows the clearance of the nitroxide (in arbitrary units) as a function of time in the control and treated mice. The solid lines through the data points are linear fits to the respective data sets suggesting compliance with a pseudo first-order rate law. The rate constants were: control, 0.30 min^{-1}; treated, 0.16 min^{-1}.

3-CP image in the heart and lungs after it was first injected into the peritoneal cavity of the mouse and the spread out into the bloodstream. From 15–25 min it was observed that the control 3-CP image was much smaller than that observed in the 3 day post exposure mouse, showing that the radicals were being cleared more slowly in the exposed mouse. The 25–35 min images show further reduction in the size of images in both sample groups but again displayed the distinctly different clearance rates between the groups with the larger 3-CP image seen in the 3 day post asbestos exposure group.

These imaging results indicate that the 3-CP radicals were cleared more slowly in the 3 day post exposure group than in the control, 1 or 7 day post exposure groups. This shows an impaired clearance of radicals and could indicate an imbalance in the antioxidant defense mechanisms and a change in redox status in mice during an acute inflammatory reaction to asbestos.

Discussion

Development of acute asbestosis is associated with an inflammatory response [5], which can promote and up regulate several mechanical events leading to secretion of cytokines, chemokines, growth factors, lytic enzymes, and ROS [39]. In addition these events can enhance the generation of ROS leading to an oxidative stress and an increase in antioxidant enzymes [40]. Development of acute asbestosis stimulates a complex sequence of signals and interactions which continue the toxic and inflammatory responses resulting in generation

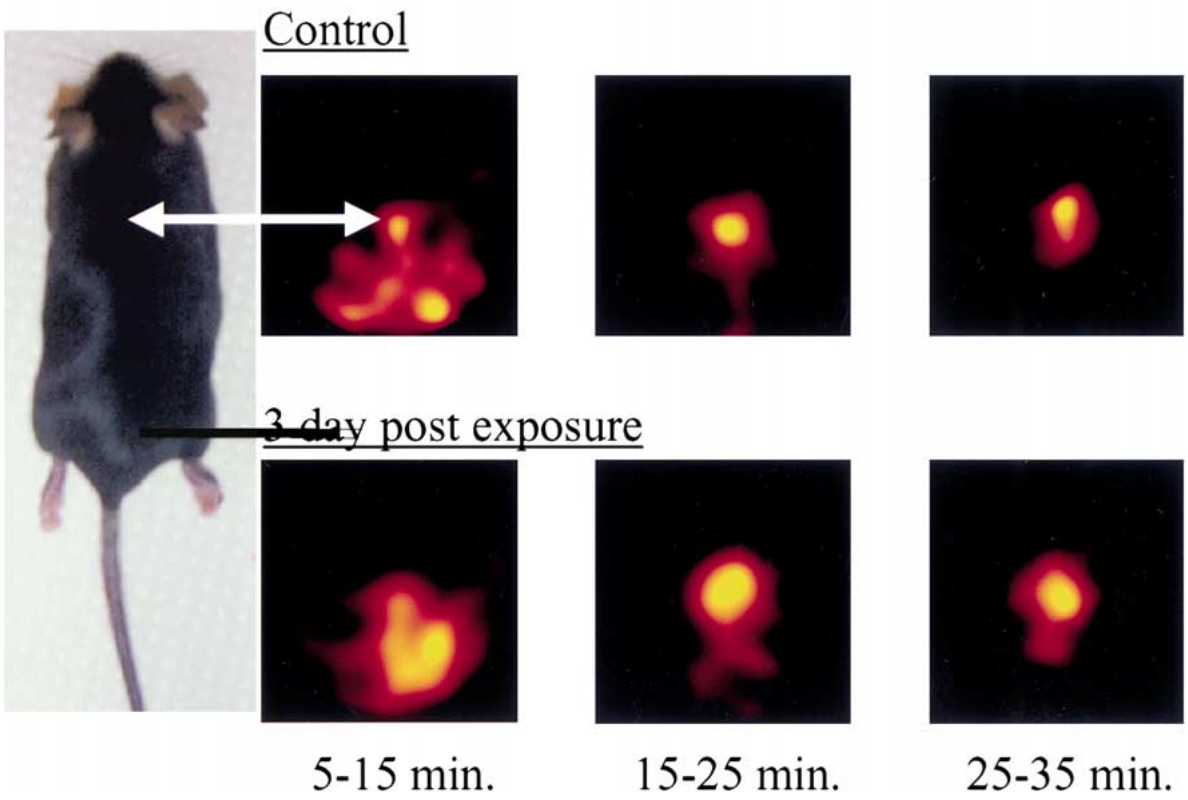

Fig. 5. Imaging of nitroxide distribution and clearance from control and 3 day post asbestos exposure mice obtained over 10 min time periods for 30 min. Images show 3-CP radical distribution at 5–15, 15–25 and 25–35 min post i.p. injection with 344 mg/kg 3-CP. The 3 day post asbestos exposure mouse shows slower reduction in radical signal strength as seen by imaging than the control mouse.

of ROS [40]. Previous studies have shown that the increase in antioxidant enzymes occurs after a lag time beyond the initiation of oxidative injury [41]. During this period antioxidant defenses and enzymes are produced at a much higher rate not only at the site of injury but elsewhere in the organism through cytokine and chemotactic signals.

In vivo ESR provides a direct method for studying free radicals. Unfortunately, previous attempts at *in vivo* ESR detection have proven to be challenging due to technical difficulties. The primary complication is the non-resonant loss of exciting frequency due to the presence of body fluids and the high water content of tissues. In order to detect free radicals directly from whole animals and to study their reaction *in vivo*, lower frequency ESR spectrometers with greater sensitivity have been developed [42]. Recently, considerable progress has been made in instrumentation, data acquisition and imaging for *in vivo* ESR, and this technique now has been applied successfully to the study of free radicals directly from living animals [25]. This *in vivo* ESR method has several advantages. (a) It measures non-invasively the free radicals in the whole animal at the specific site where they evolve. The result is much more physiological and less conjectural so that potential artifacts due to contribution by other organs and tissues can be deduced. (b) The formation and decay of free radicals can be monitored in 'real time' as it occurs. (c) It can provide conclusive evidence of direct scavenging efficiencies of infused antioxidants and free radical scavengers. Although *in vitro* model systems are very useful tools for testing the feasibility of the hypothesized pathway, only *in vivo* studies can answer the much sought-after question whether a particular antioxidant or scavenger is more effective.

Nitroxides are known to react with a variety of biological oxidants including free radicals and can react with oxyradicals to protect against superoxide and hydrogen peroxide cytotoxicity [18]. Studies suggest that nitroxides prevent biological ROS mediated oxidative damage at the molecular, cellular and whole-animal level, effectively interacting with ROS at the site of generation [43, 44, 45]. Nitroxides can act as redox sensitive probes which can help delineate tissue heterogeneity with respect to distribution, redox status and oxygen concentration [46]. Another possible outcome is that an increase in ROS can also induce antioxidants. This upregulation of antioxidants will reduce the number of free radicals present in the organism. The lower amount of ROS present in the system will reduce the rate of clearance of the nitroxide

radicals since there will be fewer radicals present to react with. Therefore an increase in antioxidants can reduce the rate at which the nitroxide is cleared.

ESR imaging studies using nitroxides as redox sensitive spin probes might provide a non-invasive method of obtaining valuable metabolic information. ESR imaging provided information on the location and distribution of nitroxide radicals within the mouse and their degradation over time [25]. The imaging and clearance rates from our findings were used to determine differential reduction rates of the nitroxide. ESR imaging has potential to provide, in a non-invasive manner, valuable physiological information in 2 and 3 dimensions. Functional imaging development could employ the physical architecture of a structure, to provide physiological/metabolic information about the structure.

The present study established the feasibility of detecting nitroxides in tissue exposed to asbestos and clearly shows major differences in nitroxide distribution and metabolism between normal and asbestos exposed mice. As the technique evolves and becomes more sensitive, ESR imaging may play a useful role in advancing functional imaging in clinical medicine.

We have previously shown that in an *ex vivo* lung model of silica-induced lung injury the lungs are in a state of oxidative stress with increased generation of ROS associated with enhanced levels of oxidative enzymes and lipid peroxidation [3]. In this isolated lung model the ability of the spin label TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl) to clear was substantially improved in silicotic animals. While the present study, involving the whole animal, shows the involvement of the whole body and activation of antioxidant defenses throughout the mouse. We have impaired the clearance of nitroxide radicals due to fewer ROS available at day 3 to react with the nitroxide. This *in vivo* model shows that the whole body of the mouse is in oxidative stress with the development of acute asbestosis.

Impaired radical clearance was found in asbestos treated animals after 3 days, which correlated with an elevation of the markers of lung damage and an increased antioxidant defense from the bioassay data. The present study used TEMPOL radical clearance rate to measure redox potential and was shown to be significantly reduced at the 3 day post exposure time point. These results could indicate that fewer biological radicals were present to react with the TEMPOL because the antioxidant defenses were activated after asbestos exposure at the 3 day post exposure time point.

Our bioassay results show an increase in damage to the lung tissue and cells from 1 day post exposure to at 3 days post exposure. After 7 days the damage decreased to control levels. The antioxidant response, seen in catalase and total antioxidant status, also showed increases at the 3 day post exposure. These results show increased damage and induction of antioxidant defenses in theory due to an decrease in oxidant radicals present in the lung. The reduced clearance of TEMPOL radicals at the 3 day post exposure time point may be due to having fewer radicals present in the lung to react with TEMPOL because these ROS are preferentially reactive with antioxidants. Antioxidant defenses were activated throughout the whole-body of the mouse due to cell signaling and the oxidative stress response. Increased catalase and total antioxidant status levels from the mouse serum show an increase in the whole mouse antioxidant defenses which would act to reduce the number of oxidant radicals available to react with and clear TEMPOL. The results of the present study clearly show a significantly different redox state in the control and 3 day post exposure mice. ESR imaging studies using nitroxides as redox sensitive spin probes may non-invasively provide valuable metabolic information and can be used to determine differential reduction rates of nitroxides, and in future studies, other radical species.

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