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Differences in mRNA Expression, Protein Content, and Enzyme Activity of Superoxide Dismutases in Type II Pneumocytes of Acute and Chronic Lung Injury

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The lung is protected against oxidative stress by a variety of antioxidants and type II pneumocytes seem to play an important role in antioxidant defense. Previous studies have shown that inhalation of NO_2 results in acute and chronic lung injury. How the expression and enzyme activity of antioxidant enzymes are influenced in type II cells of different inflammatory stages has yet not been studied.

To elucidate this question, we exposed rats to 10 ppm NO_2 for 3 or 20 days to induce acute or chronic lung injury. From these and air-breathing rats, type II pneumocytes were isolated. The mRNA expression and protein content of CuZnSOD and MnSOD as well as total SOD-specific enzyme activity were determined.

For the acute lung injury (3 d NO_2), the expression of CuZnSOD mRNA was significantly increased, while MnSOD expression was significantly reduced after 3 days of NO_2 exposure. For the chronic lung injury (20 d NO_2), CuZnSOD expression was still enhanced, while MnSOD expression was comparable to control. In parallel to CuZnSOD mRNA expression, the protein amount was significantly increased in acute and chronic lung injury however MnSOD protein content exhibited no intergroup differences. Total SOD enzyme activity showed a significant decrease after 3 days of NO_2 exposure and was similar to control after 20 days.

We conclude that during acute and chronic lung injury in type II pneumocytes expression and protein synthesis of CuZnSOD and MnSOD are regulated differently.

Keywords: CuZnSOD; MnSOD; Type II pneumocytes; Acute and chronic lung injury

INTRODUCTION

Lung tissue and the epithelial lining fluid (ELF) of the alveoli have developed a system of chemical and enzymatic antioxidants to prevent lung from reactive oxygen species (ROS).[1,2] Many lung diseases are consequences of oxidative stress characterized by an increased exposure to ROS and/or decreased antioxidant capacities.[3,4]

One of the principal oxidants in the urban environment is nitrogen dioxide (NO_2). Apart from specialized working places, the highest NO_2 concentrations are found in cigarette smoke, which can reach up to 250 ppm.[5] Tobacco smoke is the major etiological factor in chronic obstructive pulmonary disease (COPD) a worldwide health problem that has an increasing prevalence and mortality.[6]

The NO_2 toxicity has also received special interest in clinical situations as NO inhalation has become a useful tool to induce pulmonary vasodilatation. Dependant on exposure time and oxygen concentration, NO_2 is formed during NO application. Because of its low water solubility and particle size ($\leq 2\,\mu m$) NO_2 can reach the alveoli.[7]

In the terminal respiratory tract, type II pneumocytes are an important cell population for protection against ROS. They are the proliferative pool of type I pneumocytes, one of the most sensitive cells to NO_2.[8,9] It has been shown that acute NO_2 exposure alters the metabolic function of type II pneumocytes

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resulting in increased phospholipid synthesis and a reduced secretion.[10,11] Recently, we could show that vitamin E treatment of NO_2-exposed rats improves the impaired secretion of phosphatidylcholine from type II cells.[12]

In aerobic organisms, the cytosolic copper–zinc superoxide dismutase (CuZnSOD) and the mitochondrial manganese superoxide dismutase (MnSOD) belong to the most important antioxidant enzymes that protect the lung from ROS-mediated injury.[13,14] They catalyze the dismutation of superoxide to form hydrogen peroxide (H_2O_2) and molecular oxygen.[15] Although their expression and enzyme activity were investigated in hyperoxia injured total lung tissue and bronchial epithelium, the expression and enzyme activity in type II cells is not well understood.

Different studies show that mRNA expression[16–18] and enzyme activity[17,19] of CuZnSOD is not influenced by O_2 exposure, while the protein synthesis is increased.[20]

Investigations on MnSOD show an increased mRNA expression in acute injured lungs after inhalation of O_2 or cigarette smoke.[16,17,19,21] In contrast, Erzurum *et al.*[18] did not find any changes in mRNA levels after exposure to 100% O_2. Data about MnSOD protein expression are also contradictory, describing unchanged[17] or decreased[21] MnSOD protein amounts after short-term exposure. Similar results are obtained referred to enzyme activity, where reduced[21] and enhanced[17] activities were found.

Studies on isolated type II cells exposed to O_2 indicate an increase in total SOD activity.

In this study, we investigated mRNA expression and protein content as well as enzyme activity of these antioxidant enzymes in type II pneumocytes from NO_2-induced acute and chronic lung injury. In acute injured lung, mRNA expression for CuZnSOD was significantly increased, while MnSOD expression was significantly reduced. Protein level was enhanced for CuZnSOD, while the MnSOD quantity was unchanged in acute and chronic lung injury compared to the control. Total SOD activity was significantly reduced in type II cells after short-term NO_2 exposure, while in the chronic phase activity was comparable to the control.

MATERIALS AND METHODS

Animals and Exposure Experiments

All experiments were done on Spraque–Dawley rats (Charles River, Sulzfeld, Germany; initial body weight 150 g) that were kept in cages with free access to food and water. The animals were either exposed to normal air as controls or to NO_2 atmospheres for different time periods.

For NO_2 exposure, the cages containing the animals were placed into gas-tight chambers and continuously exposed to 10 ppm NO_2 for 3 and 20 days. The NO_2 concentration was measured within the exposure chambers with a NO_2-sensitive electrochemical element (ECS 102-1; MPSensor System, München, Germany). Food and water were changed daily to keep their oxidation minimal.

Type II Cell Isolation

After NO_2 exposure, the animals were anesthetized with sodium pentobarbital solution (0.09 g/kg body weight; Narcoren®, MERIAL GmbH, Hallbergmoos, Germany) containing 500 IU heparin (Liquemin® N 25 000; Hoffmann-La Roch AG, Grenzach-Wyhlen, Germany) per rat by intraperitoneal administration. Type II pneumocytes were isolated as described in detail by Dobbs *et al.*[22] In brief, after bronchoalveolar lavage (BAL), the lungs were additionally washed with the described solutions before elastase solution was instilled. Digestion with elastase was allowed to take place at 37°C for 20 min. In the presence of deoxyribonuclease I (DNAse I; 250 μg/ml), the lungs were minced with scissors and the elastase reaction was then stopped by addition of 5 ml of fetal bovine serum (FBS; Gibco-BRL, Eggenstein, Germany) per lung. The final cell suspension was filtered several times through a nylon gauze and centrifuged for 10 min at 150*g*. The cell pellet was resuspended in Dulbecco's modified Eagles' medium (DMEM, Gibco-BRL) and finally transferred to rat immunoglobulin G (IgG)-coated bacteriologic Petri dishes to a density of 30×10^6 cells. After 40-min incubation in a 10% CO_2-air incubator, the macrophages were adherent to the plastic dishes. The unattached type II pneumocytes were removed, centrifuged, and were used to measure the enzyme activity or resuspended in Trizol® (Gibco-BRL) to a density of 5×10^6 cells/ml for total RNA and protein isolation.

Thus we obtained a type II cell population of 90% purity.

Cell Homogenization

For determination of superoxide dismutase enzyme activity, only freshly isolated type II pneumocytes were used. In brief, isolated type II cells were homogenized in 150 mM NaCl/5 mM Tris/HCl, pH 7.4. Homogenization was done with 60 strokes of a tight-fitting pestle in a Dounce glass/glass homogenizer. The homogenate was centrifuged for 5 min at 10,000*g*, after which the resulting pellet was discarded. The supernatant was used for the enzyme activity assay.

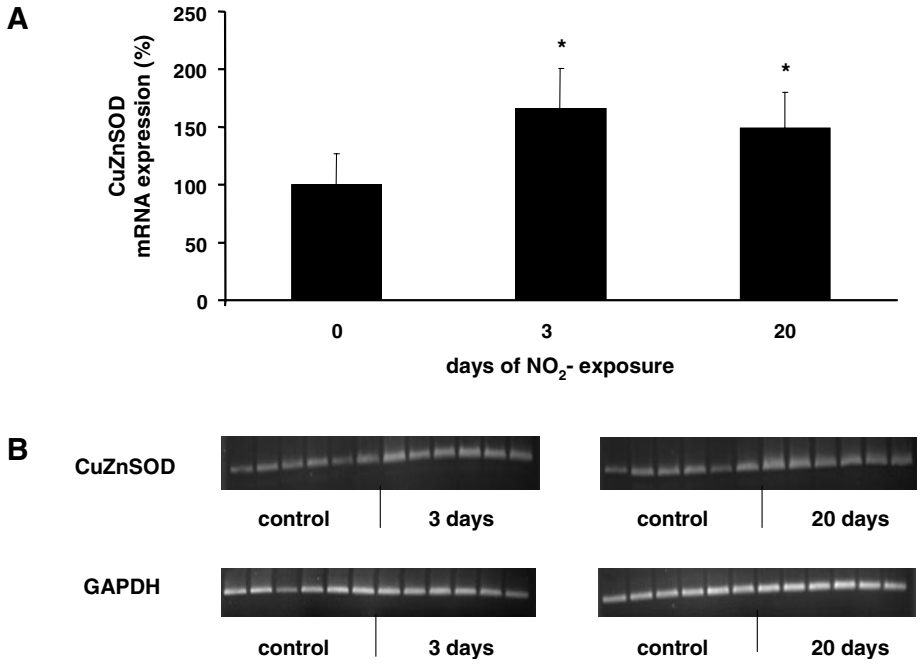

FIGURE 1 RT-PCR with cDNA amplification rate of CuZnSOD in type II pneumocytes of rats after exposure to NO_2 (3 and 20 days) or air (0 days). A: Amplification rate of CuZnSOD cDNA significantly increased for 66% (±35%; $P = 0.0042$) in type II pneumocytes after 3 days of NO_2-exposure compared to the control. After 20 days, the amplification rate was still increased for 50% (±30%; $P = 0.023$). B: Ethidium bromide-stained 1.8% agarose gels showing the CuZnSOD RT-PCR and GAPDH-PCR corresponding to their densitometrical evaluations.

RT-PCR

Four micrograms of total RNA was reverse transcribed with Superscript-RT (Gibco-BRL). Quantification of cDNA was tested with a GAPDH-specific primer pair (5′-primer: CGT CTT CAC CAC CAT GGA GA; 3′-primer: CGG CCA TCA CGC CAC AGT TT). Equal amounts of cDNA were used for RT-PCRs with a CuZnSOD-specific (5′-primer: TGT CAG GAC AGA TTA CAG GA; 3′-primer: AGC AGA TGC CGT AGA CT) and a MnSOD-specific (5′-primer: GCG CAG ATC ATG CAG CTG CA; 3′-primer: CAG ATA GTC AGG TCT GAC GT) primer pair. The RT-PCRs were done with 12 (CuZnSOD) or 10 (MnSOD) animals in the control and each exposure group and were repeated two times. For quantification of cDNA, RT-PCR products of ethidiumbromide-stained agarose gels were measured densitometrically (BIO 1D; Vilber Lourmat Biotechnology, France). The density of the control group was set 100% and the exposure groups were measured as percentage of control.

Northern Blot

Six (for CuZnSOD) and two micrograms (for MnSOD) of total RNA was electrophoresed through a 1% agarose–1.9% formaldehyde gel and transferred onto nylon membranes (Roche, Mannheim, Germany).

The CuZnSOD cRNA, MnSOD cRNA and the control GAPDH cRNA probes were provided by H. Garn (Institute of Immunology, Marburg, Germany).

The CDP-Star® (Tropix, Bedford, Massachusetts, USA) was utilized for detection of alkaline phosphatase-conjugated probes on nylon membrane and the amounts of mRNA were measured densitometrically (BIO 1D).

For Northern blot, 10 rats were used per group. Every blot was repeated two times.

Protein Assay

Protein was measured in duplicates with the BCA protein assay kit (Pierce, Rockford, USA) according to the manufacturer's instructions.

Western Immunoblot

For protein immunodetection, 5 μg of protein was mixed with gel sample buffer, boiled for 3 min and separated by electrophoresis on a 12% SDS-PAGE gel under non-reducing conditions. The proteins were transferred to an Immobilon™-P membrane (Millipore, Eschborn, Germany). After transfer, the membranes were incubated overnight at 4°C in Tris-buffered saline (TBS: 10 mM Tris and 150 mM NaCl, pH 7.5) containing 5% skim milk to block unspecific proteins. The blots were incubated for 45 min with anti-human CuZnSOD (1:1000; OXIS) or anti-rat MnSOD (0.2 μg/ml; Stressgen, Victoria, Canada) antibody diluted in TBS and 0.5% skim milk. The membranes were washed five times in

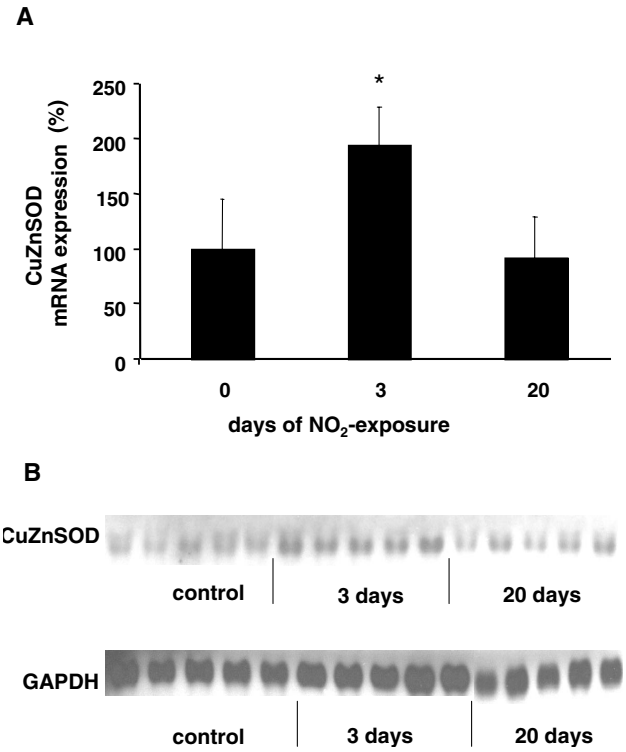

FIGURE 2 Northern blot showing increased mRNA expression of CuZnSOD in type II pneumocytes of rats after 3 days of NO_2-exposure. A: CuZnSOD mRNA expression significantly increased for 94% (±35%; $P = 0.026$) after 3 days of NO_2 exposure and returned to the control values after 20 days. B: 6 μg mRNA were blotted on nylon membrane and hybridized with a CuZnSOD cRNA probe. As control 2 μg mRNA were hybridized with a GAPDH cRNA probe.

washing buffer (TBS, 0.5% skim milk, 0.05% Tween 20). The proteins were detected using anti-sheep or anti-rabbit IgG alkaline phosphatase conjugate (Sigma, Taufkirchen, Germany) diluted 1:500 in TBS and 0.5% skim milk. The BCIP/NBT alkaline phosphatase substrate (Sigma Fast™, Sigma) was applied according to the manufacturer's instructions.

Western immunoblots were done with 12 animals in the control and each exposure group and repeated two times with the same probes.

SOD Specific Enzyme Activity

The BIOXYTECH® SOD-525™ assay (OXIS Health Products, Inc., Portland, USA) was applied according to the manufacturer's instructions. The method[23] is based on SOD-mediated increase in the rate of autoxidation of a tetracyclic catechol.

The assay was repeated two times with four animals in the control and each exposure group.

Statistical Analysis

Densitometrical data received from BIO 1D software were applied for statistical analysis using Student's *t*-test with SigmaStat® (SPSS-Science, Erkrath, Germany). Values were expressed as mean ±SD. The results were considered significant with an error less than or equal to $P = 0.05$.

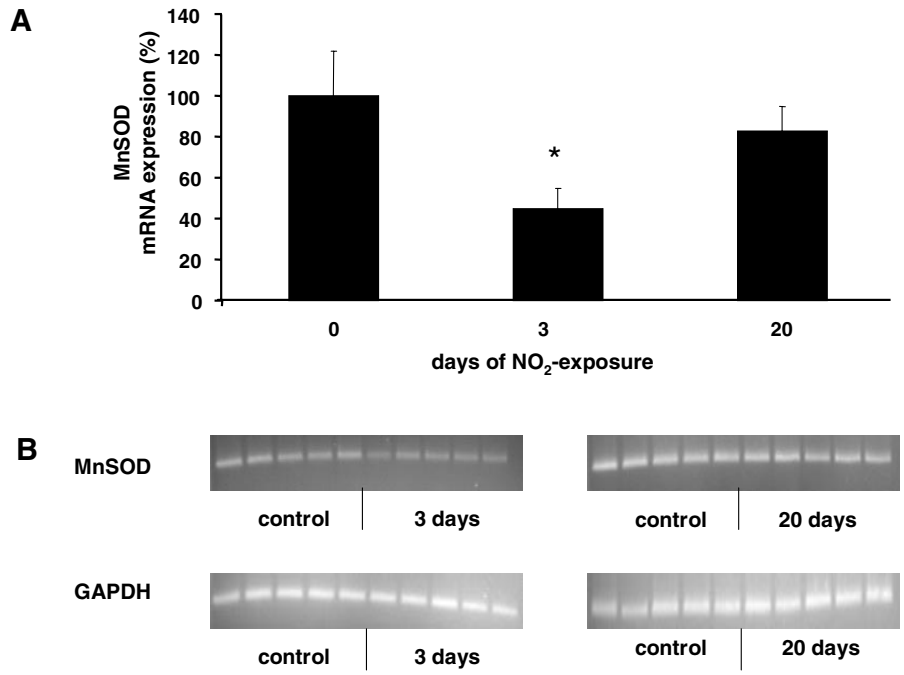

FIGURE 3 RT-PCR indicating reduced amplification rate of MnSOD cDNA in type II pneumocytes of rats during exposure to NO_2 for 3 days. A: After 3 days of NO_2-exposure the MnSOD cDNA amplification was significantly reduced for 55% (±10%; $P = 0.001$) and was comparable to the controls after 20 days of NO_2 inhalation. B: Ethidium bromide-stained 1.8% agarose gels showing the MnSOD RT-PCR and GAPDH PCR corresponding to their densitometrical evaluations.

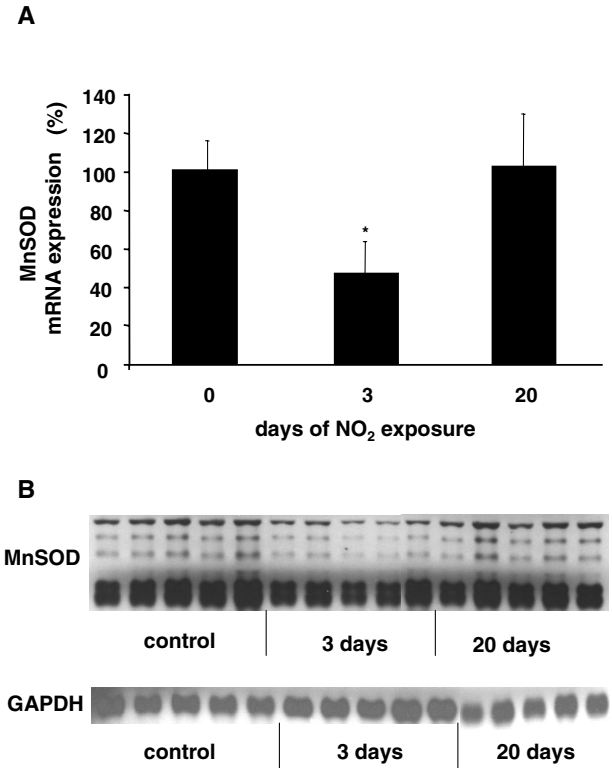

FIGURE 4 Northern blot showing decreased MnSOD mRNA expression in rat type II pneumocytes after 3 days of NO_2 inhalation. A: Expression of the 4-kb MnSOD species was significantly reduced for 53% (±17%; $P = 0.0009$) after 3 days of NO_2-exposure. After 20 days, MnSOD expression was comparable to the controls. B: 2 μg mRNA were blotted on nylon membrane and hybridized with a MnSOD cRNA probe. 6 different MnSOD species between 1.3 and 4 kb could be detected and they all showed diminished expression after 3 days of NO_2 inhalation. As control 2 μg mRNA were hybridized with GAPDH cRNA probe.

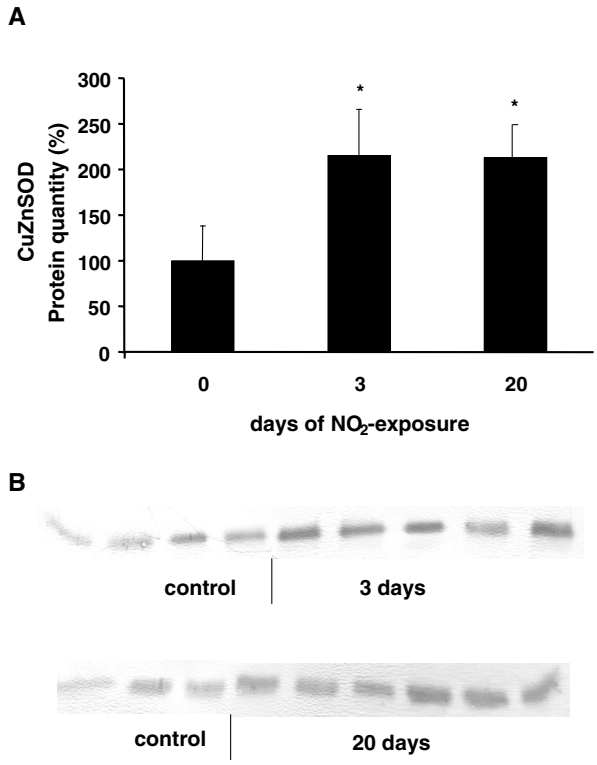

FIGURE 5 Western immunoblot of CuZnSOD protein from type II pneumocytes of rats exposed to NO_2 (3 or 20 days) or air (0 days). A: Quantity of CuZnSOD protein. After 3 and 20 days of NO_2 exposure, the protein amount elevated for 115% (±51%; $P = 0.0082$ and $P = 0.0064$) compared to the controls. The corresponding immunoblot is shown in B. B: 5 μg type II pneumocytes protein were separated on 12% SDS-polyacrylamide gel and blotted to Immobilon-P membrane. The membrane was incubated with an anti-human CuZnSOD-specific antiserum, which was detected by alkaline phosphatase conjugated secondary anti sheep IgG antibody.

RESULTS

mRNA Expression of CuZnSOD and MnSOD in Type II Pneumocytes

Rat type II pneumocytes from controls and the 3 and 20 day NO_2 inhalation experiments were isolated, in order to compare the mRNA expression of CuZn-SOD and MnSOD in different stages of lung injury. Expression pattern was investigated by RT-PCR and Northern blot. For RT-PCR, the amount of cDNA was determined by densitometrical evaluation of GAPDH RT-PCR. Northern blots were compared with GAPDH hybridization of 2 μg total RNA. The expression of CuZnSOD was significantly increased after 3 days of NO_2 inhalation for 66% (±35% vs. 100 ± 27%) by RT-PCR (Fig. 1) and for 94% (±35% vs. 100 ± 45%) by Northern blot (Fig. 2). After 20 days, an increase of 50% (±30%) was found by RT-PCR, while expression in Northern blot analysis was unchanged compared to the control. For MnSOD expression, RT-PCR showed a 45% (±10% vs. 100 ± 22%) reduction in type II cells of acute injured lungs that returned to control values in cells of chronic lung injury (Fig. 3). Hybridization with the MnSOD cRNA probe is shown in Fig. 4. There are six species of mRNA ranging from about 1.3 to 4 kb that demonstrate reduction of MnSOD expression after 3 days of NO_2 inhalation. We quantified the area of the largest mRNA species for comparison (Fig. 4A) and the results confirmed with those of RT-PCR (47 ± 17% vs. 100 ± 16%).

Protein Quantity of CuZnSOD and MnSOD in Type II Pneumocytes

The amount of CuZnSOD and MnSOD protein was evaluated by Western immunoblot. Immunodetection of CuZnSOD showed a signal of approximately 19 kDa in all probes that was significantly increased in type II pneumocytes isolated from rats with acute and chronic bronchitis (215 ± 51% vs. 100 ± 38% for controls) (Fig. 5). With a specific antisera for MnSOD, two bands between 20 and 30 kDa were detectable in all samples (Fig. 6). The same phenomenon was observed with human MnSOD-specific antisera that

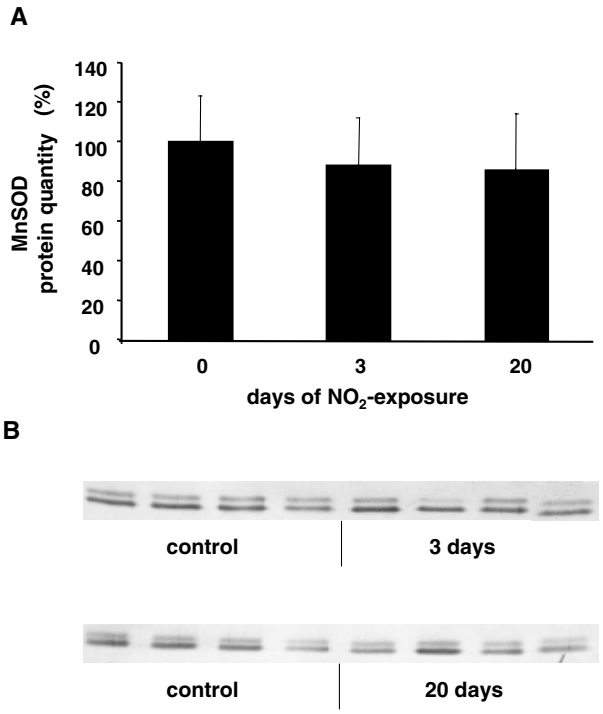

FIGURE 6 Western immunoblot of MnSOD protein from rat type II pneumocytes after NO_2 inhalation. A: Quantification of the entire area of both bands did not show any differences of protein levels between the intergroups. B: 5 μg protein of type II pneumocytes were separated on 12% SDS-polyacrylamide gel and blotted to Immobilon-P membrane. The membrane was incubated with an anti-rat MnSOD-specific antibody, which was detected by an alkaline phosphatase conjugated secondary anti-rabbit IgG antibody. Two bands between 20 and 25 kDa were detected; the smaller one showed a stronger signal intensity in all samples.

were crossreactive with rat (data not shown). The signal intensity of the smaller bands was stronger in all groups. We quantified the entire area of both bands and did not find any differences of protein amount between the intergroups.

Enzyme Activity of Total SOD in Type II Pneumocytes

Enzyme activity was investigated using a photometrical assay that measured the autoxidation of a tetracyclic catechol. The enzyme activity in control cells was 4 U/mg protein. After 3 days of NO_2 inhalation, the SOD enzyme activity was reduced significantly (2.5 ± 0.5 U/mg protein vs. 4.1 ± 1.13 U/mg protein for controls). After 20 days, the activity was comparable to the control (3.7 ± 0.73 U/mg protein) (Fig. 7).

DISCUSSION

The lung oxidative stress results in an oxidant–antioxidant imbalance that leads to different lung diseases like adult respiratory distress syndrome, various pneumoconioses, COPD and lung cancer.[4] During normal breathing, the lung is protected from oxidant-derived radicals by antioxidative and defense mechanisms.[1,2] In the alveoli besides their role in surfactant metabolism, type II pneumocytes seem to be an important cell population in antioxidant defense. Evans *et al.*[24,25] could show a significant increase of type II pneumocyte proliferation after NO_2 inhalation. They also observed that type II cells transform into type I cells. These facts underline their importance in antioxidative defense. Until today there are only few data on the role of type II cells in antioxidative reactions. To elucidate adaptive mechanisms in type II cells, we investigated the role of CuZnSOD and MnSOD for antioxidative reactions after oxidant stress.

For this purpose, an NO_2-inhalation model was used[11] exposing Sprague–Dawley rats to 10 ppm NO_2 for 3 (acute) or 20 days (chronic) to induce acute and chronic injured lungs. This NO_2-inhalation

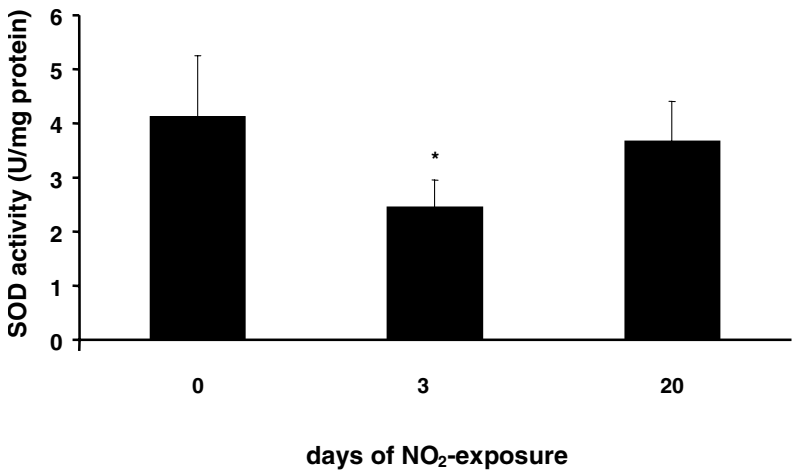

FIGURE 7 Total SOD enzyme activity. The assay is based on the SOD-mediated increase in the rate of autoxidation of a tetracyclic catechol. After 3 days of NO_2 inhalation, the SOD enzyme activity was reduced significantly (2.5 ± 0.5 U/mg protein vs. 4.1 ± 1.13 U/mg protein for controls; $P = 0.017$); while after 20 days the enzyme activity was comparable to the control.

model represents a well-defined and reproducible model of oxidative stress, and the destructive effects have been described morphologically and biochemically.[7]

In our study, we found a significant increase of CuZnSOD mRNA expression in type II pneumocytes of acute and chronic lung injury. The induction was reflected on protein level, demonstrating a tow-fold higher amount of CuZnSOD protein after 3 and 20 days of NO_2 inhalation compared to that of control. Analyses on total lung homogenates[16,17] and isolated bronchial epithelial cells[18] have not shown any intergroup differences of CuZnSOD mRNA expression. This could be due to the use of different types and concentrations of oxidants. It is questionable if the different stages of lung injury induced by cigarette smoke or O_2 inhalation ($>95\%$) correspond to those of lung injury that were obtained by the NO_2 inhalation. Such differences might reflect the degree of CuZnSOD mRNA expression. On the other hand, CuZnSOD expression in injured lung might be a type II cell-specific reaction and cannot be detected in whole lung because of the different proportion of cell population in the different injured stages. The observed unchanged mRNA expression of CuZnSOD in total lung and the increased expression in type II cells suggest a decreased expression of this enzyme in the other non-type II cells of the lung. In rat lung tissue, Hass *et al.*[20] observed an increase of CuZnSOD synthesis only after short-time hyperoxia (24 h 95% O_2) and a mortality rate of 80% after 74 h of exposure. Their notion of intolerance to $>95\%$ O_2 as a result of inability to increase synthesis of CuZnSOD corresponds with our observation of increased CuZnSOD synthesis even after 20 days and a mortality rate under 10% at that time.

As found in this study, continuous NO_2 exposure for 3 days significantly reduced the expression of MnSOD mRNA, while a longer exposure (20 days) returned the mRNA level to control values. There were no differences in quantity of MnSOD protein between different stages of lung injury and the control.

Until today only little is known about MnSOD expression in type II cells of injured lungs. So far studies have been done only on total lungs from cigarette smoke or O_2-exposed animals or pulmonary cell lines and the results are in part contradictory and not in accordance with our data. Our observation of six different mRNA species correlates with results from Ho *et al.*[17] who found mRNA ranging from 1.3 to 4.2 kb. Hurt *et al.*[26] could show that these mRNAs are derived by utilization of different transcription sites.

In situ hybridization studies in rat lungs after exposure to cigarette smoke indicates a transient increase of MnSOD expression particularly in bronchial epithelial cells, which normally express MnSOD at low levels.[16] In contrast Erzurum *et al.*[18] observed no change of MnSOD mRNA expression of bronchial epithelial cells after inhalation of 100% O_2 inducing tracheobronchitis. In lungs from 85% oxygen exposed rats expression of MnSOD mRNA was dramatically increased after 3 days and progressively decreased at later exposure times.[17] Total lung from rats exposed to $>95\%$ O_2 for 48 h also showed an elevation of MnSOD mRNA.[19,21] In acute injured total lungs the induced mRNA expression of MnSOD seems to be a transient event and does not reflect the expression pattern of type II cells. It is not out of question that nature and concentration of oxidant inducing lung injury may influence the expression pattern. However, so far the regulation of the MnSOD gene in type II cells is not known. In this context Garn (personal communication) could demonstrate a reduction in the proinflammatory cytokine TNF-α in BAL of rat lungs after short-time exposure to NO_2. It has been shown that MnSOD could be induced by TNF-α, IL-1β and H_2O_2 via distinct signaling pathways. Warner *et al.*[27] showed in the human pulmonary adenocarcinoma cell line H441 that ROS mediate the induction of MnSOD by TNF-α. It is also described that the time of induction of MnSOD by H_2O_2 differ from TNF-α induced MnSOD expression. Treatment with TNF-α rapidly increased NF_kB DNA-binding while H_2O_2 did not influence this DNA-binding activity. In contrast Rogers *et al.*[28] could show in pulmonary epithelial and endothelial cells treated with inflammatory mediators and various inhibitors that NF_kB modulates IL-1β signaling, but not the TNF-α pathway. If induction of MnSOD in type II cells after NO_2 exposure is also mediated by one of these cytokines and if it depends on NF_kB induction so far has to be investigated.

In the literature on MnSOD protein level in injured lung data are contradictory. Clerch *et al.*[21] observed diminished MnSOD concentration during an initial exposure to $>95\%$ O_2. Chang *et al.*[29] showed an increase of MnSOD content in type II cells after 7 days of 85% O_2 exposure.

Our data of type II pneumocytes after 3 days NO_2 exposure are in good accordance with Ho *et al.*,[17] who observed an unchanged protein level after short-term exposure to 85% O_2. In contrast after long-term exposure, they showed an increased amount of MnSOD protein, while we did not find any changes in MnSOD protein content. With an MnSOD-specific antisera, we detected two bands between 20 and 30 kDa in Western immunoblots. In the literature, this phenomenon is not described until today. Whether the larger band is a glycosylated form or the protein is digested by proteinases, has to be studied.

In spite of the increased CuZnSOD mRNA and protein levels and an unchanged MnSOD protein content, we observed a remarkable drop in total SOD enzyme activity during acute lung injury. However, in the chronic stage the activity was comparable to that of control. The fall in activity may be caused, at least in part, by oxidant damage to the protein. This is suggested by Clerch *et al.*,[19] who observed increasing MnSOD activity in extracts of lungs from O_2-exposed rats after addition of reducing agents. In addition, they did not find any influence of reducing agents in air-breathing rats. Because MnSOD is active only in the mitochondria, the reduced enzyme activity may also be caused by impairment of the transport to or entry into mitochondria. Reduction of MnSOD mRNA followed by an unchanged protein expression and a reduced enzyme activity could be the result of a posttranscriptional event similar to that observed by Clerch *et al.*[21] in O_2-exposed lungs. Ho *et al.*[17] observed an increase of MnSOD mRNA expression, which is paralleled by an unchanged MnSOD protein content and MnSOD activity. In addition to transcriptional activation, they suggest translational and/or posttranslational regulations to be involved in MnSOD gene expression. CuZnSOD activity did not exhibit intergroup differences at any exposure time.[17,19].

Until today nothing is known about CuZnSOD or MnSOD activity in type II cells of injured lungs. Because of limited cell yield, we first investigated total SOD activity. Our observation of reduced enzyme activity after short-time NO_2 exposure does not confirm studies on adult isolated type II cells exposed to 95% O_2 that show a three-fold increase of total SOD activity.[30] These contradicting results may reflect the different experimental design like use of exposure gases, different cell-isolation techniques of type II pneumocytes or use of different rat strains.

The fact of increased mRNA expression and protein amount of CuZnSOD, but reduced total SOD enzyme activity may indicate an inactivated form of the CuZnSOD enzyme in type II cells. An enzymatically active form of CuZnSOD has been found in BAL.[31] This suggests that during secretion from type II cells into the alveolar space this enzyme is transformed into its active form.

If CuZnSOD is inactivated in type II cells, the reduced total SOD activity may reflect the MnSOD activity. A hyperoxia-induced enzymatically inactive form of MnSOD has been described by Chang *et al.*[29]

In this study, we have shown that type II pneumocytes of acute and chronic lung injury induced by NO_2 inhalation respond with different mRNA expression and protein level of CuZnSOD and MnSOD. Only the induced mRNA expression of CuZnSOD of both injury stages is reflected in protein content. On the other hand, total SOD enzyme activity is reduced significantly in acute injured lung.

Dissociation of mRNA levels, protein content, and enzyme activity of CuZnSOD and MnSOD in type II pneumocytes of NO_2-exposed rats indicate that expression of both genes is controlled at the transcriptional, translational and/or posttranslational levels. To elucidate acute and chronic cellular defense responses, molecular mechanisms that regulate expression of these genes have to be investigated.

Acknowledgements

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