Cellular response of antioxidant metalloproteins in Cu/Zn SOD transgenic mice exposed to hyperoxia

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Levy, Mark A., Yu-Hwai Tsai, Andrew Reaume, and Tammy M. Bray. Cellular response of antioxidant metalloproteins in Cu/Zn SOD transgenic mice exposed to hyperoxia. Am J Physiol Lung Cell Mol Physiol 281: L172–L182, 2001.—Ceruloplasmin, metallothionein, and ferritin are metal-binding proteins with potential antioxidant activity. Despite evidence that they are upregulated in pulmonary tissue after oxidative stress, little is known regarding their influence on trace metal homeostasis. In this study, we have used copper- and zinc-containing superoxide dismutase (Cu/Zn SOD) transgenic-overexpressing and gene knockout mice and hyperoxia to investigate the effects of chronic and acute oxidative stress on the expression of these metalloproteins and to identify their influence on copper, zinc, and iron homeostasis. We found that the oxidative stress-mediated induction of ceruloplasmin and metallothionein in the lung had no effect on tissue levels of copper, iron, or zinc. However, Cu/Zn SOD expression had a marked influence on hepatic copper and iron as well as circulating copper homeostasis. These results suggest that ceruloplasmin and metallothionein may function as antioxidants independent of their role in trace metal homeostasis and that Cu/Zn SOD functions in copper homeostasis via mechanisms distinct from its superoxide scavenging properties.

ceruloplasmin; metallothionein; ferritin; gene knockout mice; transgenic overexpressing mice; copper- and zinc-containing superoxide dismutase

THE BALANCE BETWEEN the antioxidant defenses and the rate of production of reactive oxygen species (ROS) is believed to be the critical factor that determines the extent of tissue injury in a number of pulmonary diseases (4, 23, 29, 42). For example, bronchopulmonary dysplasia (BPD) often develops in premature infants exposed to high levels of oxygen. Exposure to hyperoxia leads to increased production of ROS (19, 21) that can presumably overwhelm the immaturely developed antioxidant defense system and lead to oxidative tissue damage within the pulmonary tissue (8, 43, 44). The antioxidant defenses, which consist of a network of enzymes, proteins, and low molecular weight ROS scavengers, function to protect cellular structures against the damaging effects of ROS. Although antioxidant enzymes such as the superoxide dismutases [copper- and zinc-containing superoxide dismutase (Cu/Zn SOD) and manganese superoxide dismutase (Mn SOD)], glutathione peroxidases (GSH-Px), and catalase are generally considered to play the major role in antioxidant defense, this view is being expanded as our knowledge of the cellular antioxidant defense response to different forms of oxidative insult continues to advance.

Metalloproteins, such as ceruloplasmin, metallothionein, and ferritin, are well known for their critical role in metal homeostasis and function as storage reservoirs and/or chaperones for essential trace metals, such as copper, zinc, and iron. Evidence indicates that these proteins are induced during the acute-phase response (27, 38) and under oxidative stress (14, 41). It has been speculated that they ameliorate the deleterious effects of ROS. The antioxidant properties of these proteins have been attributed primarily to their binding of the redox active metals copper and iron, thus minimizing their capacity to catalyze ROS production via the Fenton reaction. Yet, the contribution of these metalloproteins to the antioxidant defense network in vivo is still not clearly defined. Although many investigators have focused on identifying the antioxidant properties of the induced metalloproteins, limited attention has been given to their tissue-specific expression or their influence on metal homeostasis after exposure to acute or chronic oxidative stress. Disruption in trace element homeostasis may further exacerbate the pathogenesis of pulmonary diseases associated with oxidative stress, such as BPD and adult respiratory distress syndrome (10, 22, 34).

The development of transgenic and knockout mice has provided researchers with powerful tools to manipulate the antioxidant defense status in vivo. We have used mice that express variable levels of the antioxidant enzyme Cu/Zn SOD and exposure to hyperoxia to systematically evaluate the in vivo consequences of chronic and acute oxidative stress on trace mineral metabolism. Cu/Zn SOD protects against oxidative stress by catalyzing the dismutation of superoxide generated under normal aerobic metabolism. Thus genetic deletion of this enzyme presumably leads to a chronic...
increase in endogenous levels of oxidative species, whereas overexpression of Cu/Zn SOD would have the opposite effect and would result in decreased levels of oxidative stress. In contrast, exposure to exogenous oxidative stresses, for example, exposure to a high level of oxygen, is known to generate an acute state of oxidative stress by elevating ROS production (19).

Hence, in this study, we have used mice with variable Cu/Zn SOD expression and hyperoxic exposure to investigate the effects of chronic and acute oxidative stress on the expression of the antioxidant metalloproteins ceruloplasmin, metallothionein, and ferritin and to identify the influence of these metalloproteins on copper, zinc, and iron homeostasis.

**MATERIALS AND METHODS**

**Cu/Zn SOD-overexpressing and knockout mice.** Cu/Zn SOD overexpressor [Sod1(+/+)] mice [TGN(SOD1)3Cje, Jackson Laboratories, Bar Harbor, ME] contain multiple inserts of the human Cu/Zn SOD gene and were derived from outbred substrains) and were provided as a generous gift from Cephalon, as previously described (36). In our laboratory, heterozygotes [Sod1(+/−)] were mated to produce knockouts, heterozygotes, and wild-type controls [Sod1(+/+)]. Mouse strain was similarly confirmed in the laboratory by SOD activity gel (5) and pedigree analysis. All procedures involving animals were approved by the Ohio State University Institutional Laboratory Animal Care Committee.

**SOD activity gel electrophoresis.** Lung homogenate (~5–10 μg of protein) was electrophoresed through a 10% polyacrylamide gel for 1–2 h at 100 volts. Staining for SOD activity was done as described (5), except that gels were soaked for 10 min in nitroblue tetrazolium followed by a 15-min soak in diamidine (PPD) as a substrate (35). Serum samples (2 mg of protein) were added to cuvettes containing 0.6 units. Catalase (0.1 mM), glutathione (1 mM), NADPH (0.11 mM), and glutathione reductase (0.6 units). The disappearance of NADPH was monitored at 340 nm for 5 min after the addition of 20 μl t-butyl hydroperoxide in methanol (0.25 mM). All concentrations were expressed as final molarity within the cuvette. Catalase activity was measured as the decomposition of H₂O₂ (9). Briefly, 333 μl of tissue homogenate were diluted in 1 volume of phosphate buffer (50 mM K₂HPO₄ and 50 mM KH₂PO₄, pH 7.0) and transferred immediately to a cuvette containing 1 volume of 30 mM H₂O₂, and the change in absorbance at 240 nm was recorded for 1 min. One unit of catalase activity is defined as 1 μmol of H₂O₂ consumed per minute per milligram of tissue protein.

Ceruloplasmin activity was measured using p-phenylene-diamine (PPD) as a substrate (35). Serum samples (2 mg of protein) were added to cuvettes containing 0.6 ml of acetate buffer (0.1 M, pH = 6.0) and 0.3 ml of 0.25% PPD in acetate buffer. Acetate buffer and PPD solution were equilibrated at 37°C for 5 min before sample addition. The change in absorbance at 530 nm was monitored for 30 min after allowing for a 10-min lag phase. Blank samples containing serum (2 mg protein), 0.3 ml of acetate buffer, 0.3 ml of 0.25% PPD buffer, and 0.3 ml of 0.1% NaOH in 0.1 M acetate buffer were prepared for each sample. Ceruloplasmin activity was determined using standard solutions of Bandrowski’s base, where one enzyme unit is equivalent to 1 μmol of Bandrowski’s base formed per minute per liter of serum (37).

**Western blot analysis.** Cu/Zn SOD, GSH-Px, and catalase as well as ceruloplasmin and ferritin proteins were separated by PAGE under standard conditions (26) using a Bio-Rad Mini Gel apparatus (Bio-Rad, Hercules, CA). For Cu/Zn SOD and catalase, samples containing 100 μg of protein were run on 10% polyacrylamide gels for 1.5–2 h at 100 volts. For GSH-Px, 100 μg of protein were separated over 2 h in a 12% polyacrylamide gel at 100 volts. Separation of ceruloplasmin and ferritin was accomplished by loading 100 μg of tissue sample protein on an 8% and 12% polyacrylamide gel, respectively, and running at 100 volts for 1.5–2 h. After proteins were separated, they were electrophoretically transferred onto a nitrocellulose membrane.
antioxidant metalloproteins in Cu/Zn SOD transgenic mice

Results

Cu/Zn SOD protein levels in the lung (Fig. 1A) and liver (Fig. 1B) of transgenic mice with variable levels of expression [Sod1(+++), Sod1(+/-), Sod1(+-), and Sod1(---)] are depicted. As anticipated, Cu/Zn SOD protein level was well correlated with the copy number of genes in the lung and liver of each Sod1 strain of mouse. Exposure to hyperoxia appears to induce Cu/Zn SOD protein expression in the lung and liver of Sod1(+++) and Sod1(+-) mice, although it did not reach statistical significance. Cu/Zn SOD enzyme activity was also measured and was found to mirror protein analysis (data not shown).

The effect of variable Sod1 expression and hyperoxia exposure on the antioxidant enzyme levels of GSH-Px and catalase in the lung and liver are presented in Fig.

Fig. 1. Copper- and zinc-containing superoxide dismutase (Cu/Zn SOD) protein expression in the lung (A) and liver (B) of Sod1 transgenic (+++), homozygous (+/+), heterozygous (+/-), and knockout (---) mice exposed to air (21% oxygen) or hyperoxia (85% oxygen) for 5 days. Representative Western blots in A and B show the presence of mouse and human Cu/Zn SOD in Sod1(+++) mice and the absence of Cu/Zn SOD in Sod1(---) mice. Treatment of animals, preparation of lung homogenates, and assays of Cu/Zn SOD protein and activity are described in MATERIALS AND METHODS. Data are presented as means ± SE. Bars with different letters are significantly different (P < 0.05). ND, not detected.
2. GSH-Px protein levels increased as Cu/Zn SOD protein levels decreased in the lung. Hyperoxia did not further induce GSH-Px protein levels, as expected (Fig. 2A). The inverse relationship between GSH-Px and Cu/Zn SOD protein level was not as apparent in the liver (Fig. 2C). Figure 2, B and D, demonstrates that Cu/Zn SOD protein levels did not affect catalase expression in either the lung or liver tissue. Unexpectedly, hyperoxia significantly reduced catalase protein levels in all Sod1 strains of mice in the lung (Fig. 2B) but not in the liver (Fig. 2D).

The activity of GSH-Px and catalase was then analyzed (Fig. 3) to determine if variable Cu/Zn SOD expression affected other antioxidant enzymes during hyperoxic exposure. Notably, GSH-Px activity in the lung (Fig. 3A) did not reflect protein levels (Fig. 2A). As a result, the previously noted inverse relationship between Sod1 gene expression and GSH-Px protein levels did not exist between Cu/Zn SOD gene expression (Fig. 1A) and GSH-Px activity (Fig. 3A). Moreover, GSH-Px activity in Sod1(+/-) and Sod1(-/-) mice was increased after exposure to hyperoxia without a corresponding increase in GSH-Px protein levels. In the liver, neither Sod1 genotype nor hyperoxic exposure influenced the activity of GSH-Px (Fig. 3C). In contrast to GSH-Px activity, the activity of catalase was significantly reduced after exposure to hyperoxia in the lung of all Sod1 genotypes (Fig. 3B), a result that mirrored the effects of hyperoxia on catalase protein expression (Fig. 2B). In the liver, catalase activity was not affected...

Fig. 2. Glutathione peroxidase (GSH-Px) and catalase protein levels in the lung (A and B) and liver (C and D) of Sod1(+++), Sod1(+/-), Sod1(-/-), and Sod1(-/-) mice exposed to 21% oxygen or 85% oxygen for 5 days. Data are presented as means ± SE. Bars with different letters are significantly different (P < 0.05). Representative Western blots of GSH-Px and catalase protein are presented for mice exposed to air (lanes 1, 3, 5, and 7) or hyperoxia (lanes 2, 4, 6, and 8). Each lane represents tissue homogenate prepared from a single animal.
by hyperoxic exposure. However, hepatic catalase activity in Sod1(+/+) mice was reduced by >50% (P < 0.05) compared with all Sod1 genotypes (Fig. 3D) despite similar levels of enzyme protein in all other strains (Fig. 2D).

Figure 4 shows an increase in ceruloplasmin protein in the lung (Fig. 4A) but not the liver (Fig. 4C) of Sod1 mice after exposure to hyperoxia. Notably, ceruloplasmin protein level was not influenced by Cu/Zn SOD gene expression, as there were no observable differences in protein level based on Sod1 genotype in the lung or liver. However, ceruloplasmin protein levels were found to be responsive to the oxidative challenge of hyperoxia in the target tissue, as they were elevated in the lung but remained unchanged in the liver after 5 days of hyperoxia exposure. The significant increases in ceruloplasmin protein levels in the lung did not, however, affect copper concentrations (Fig. 4B). In contrast to the lung, liver copper concentrations exhibited substantial variability (Fig. 4D), yet ceruloplasmin levels remained constant across all treatments (Fig. 4B). Notably, copper levels were found to correlate with Cu/Zn SOD expression rather than ceruloplasmin protein levels, i.e., copper levels were highest in Sod1(++) mice, intermediate in Sod1(+/+) and Sod1(+/-) mice, and lowest in Sod1(−/−) mice.

In contrast to ceruloplasmin, metallothionein synthesis was found to be responsive to the oxidative challenge of hyperoxia in both the target and nontarget tissue of all Sod1 strains, with statistically significant increases observed in the lungs of Sod1(++) and Sod1(++−) and the liver of Sod1(−/−) mice (Fig. 5, A and C). The increases in metallothionein protein levels in the lung and liver were not, however, correlated with increases in zinc concentrations (Fig. 5, B and D). In the lung, zinc concentrations were slightly reduced by hyperoxia in all Sod1 genotypes, whereas, in the liver, zinc levels were increased by hyperoxia in Sod1(+/+) and Sod1(−/−) mice. Liver zinc concentrations were also affected by Cu/Zn SOD expression, i.e.,
zinc levels were highest in Sod1(+/+) mice, intermediate in Sod1(+/−) and Sod1(+/−) mice, and lowest in Sod1(−/−) mice (Fig. 5D).

Ferritin protein levels and iron concentrations in the lung and liver of each strain of Sod1 mice exposed to hyperoxia are depicted in Fig. 6. Lung and liver ferritin protein levels (Fig. 6, A and C) exhibited little fluctuation in response to either Cu/Zn SOD gene expression or hyperoxic exposure. In the lung, there is a trend toward a decrease in iron levels after exposure to hyperoxia, with significant reductions observed in Sod1(+/−) and Sod1(−/−) mice (Fig. 6C). Although liver iron concentrations were not affected by exposure to hyperoxia, iron concentration was influenced by Sod1 genotype, as Sod1 overexpressors had significantly lower iron levels (Fig. 6D). Significantly lower iron levels were also observed in the lung of Sod1(+/+) mice (Fig. 6B).

Figure 7 depicts circulating trace mineral levels and serum ceruloplasmin activity in each strain of Sod1 mice after exposure to hyperoxia. Circulating iron levels were significantly elevated in Sod1(+++), Sod1(+/−), and Sod1(−/−) mice after hyperoxia (Fig. 7A). Hyperoxia did not, however, have any effect on circulating copper (Fig. 7B) or zinc (Fig. 7C) levels, nor did it influence serum ceruloplasmin activity (Fig. 7D). Notably, both circulating copper levels and serum ceruloplasmin activity were markedly influenced by overexpression of Cu/Zn SOD, as each was significantly reduced in Sod1(+++) mice compared with all other genotypes.

DISCUSSION

In this study, we have used genetically altered transgenic mice and exposure to a high but nonlethal level of
oxygen as an animal model that is relevant clinically to the precarious situation experienced by premature infants. By using Cu/Zn SOD overexpressor and knockout transgenic mice, we have incorporated animals that disproportionately express a component of the endogenous antioxidant defense system. These animals are therefore chronically exposed to graded levels of oxidative stress in a manner analogous to premature infants that invariably inherit a compromised antioxidant defense system. We have also employed hyperoxic exposure, an exogenous oxidative stress that is relatively acute but nonlethal, to parallel its clinical use as a therapeutic treatment for preterm infants. Hence, by employing both Cu/Zn SOD transgenic mice and hyperoxia, our laboratory has been provided with a unique opportunity to examine the effects of both chronic and acute oxidative stress within a physiologically relevant condition. In particular, this model has allowed us to systematically examine the impact of oxidative stress on tissue-specific responses of metalloproteins as part of the cellular defense system and to identify the role of these metalloproteins in trace element homeostasis.

Results from this study provide in vivo evidence that, under acute oxidative stress, the metalloproteins ceruloplasmin and metallothionein may function as antioxidants without affecting tissue concentrations of copper or zinc. Specifically, our results demonstrate that ceruloplasmin may function as an important antioxidant in vivo, as lung tissue levels were markedly induced after acute exposure to hyperoxia (Fig. 4A), without significant changes in tissue copper levels (Fig. 4B). In the liver, the nontarget organ of hyperoxic...
exposure, ceruloplasmin levels were not affected by hyperoxia (Fig. 4C). Our results extend earlier observations of others that showed tissue-specific induction of ceruloplasmin mRNA in rat lung tissue during acute inflammation and hyperoxia (17). Furthermore, it is noted that tissue ceruloplasmin expression was induced by acute stress only and was not responsive to exposure to chronic oxidative stress imposed by Cu/Zn SOD knockout.

Similarly, metallothionein protein was induced in the lung (Fig. 5A) after hyperoxia without significantly affecting zinc concentrations (Fig. 5B). Previous studies have shown that metallothionein mRNA levels (metallothionein I and metallothionein II) are increased 10- to 12-fold in the liver of Sod1(−−) mice. This induction was specific to the liver, as no significant induction was observed in the kidney, spleen, heart, or lung of these mice (20). Our study extends these findings by demonstrating that, under hyperoxic exposure, metallothionein protein levels are significantly induced not only in the liver but also in the lung of each Sod1 strain of mice. In particular, induction of metallothionein expression was highest in the lung and liver of Sod1(−−) mice compared with the other Sod1 strains (Fig. 5, A and C). Furthermore, metallothionein appears to be more responsive to oxidative stress than ceruloplasmin, as metallothionein was elevated in both the lung and liver under hyperoxic conditions, whereas ceruloplasmin was elevated in the lung only. In addition, liver metallothionein was also induced in response to chronic oxidative stress imposed by diminished Cu/Zn SOD expression (Fig. 5C). Together, these results demonstrate that, under acute oxidative stress, ceruloplasmin and metallothionein may function as antioxidant metalloproteins independent of their role in trace metal homeostasis.
The mechanism by which the induction of these metalloproteins protects against acute oxidative stress is still unknown. It is interesting to speculate on the functional role of ceruloplasmin in lung tissue. Notably, the lung has been recently identified as a major site of extrahepatic ceruloplasmin synthesis (46). Ceruloplasmin protein has been shown in vitro to exhibit GSH-Px-like activity by removing H₂O₂ and lipid hydroperoxides (32). Moreover, Atanasiu et al. (2) have demonstrated in vitro that ceruloplasmin was an effective peroxyl radical scavenger and an effective chain-breaking antioxidant independent of its iron regulatory ferroxidase activity. Collectively, the characterization of GSH-Px-like activity and radical scavenging abilities of ceruloplasmin, as well as the hyperoxia-induced decline in pulmonary catalase protein (Fig. 2B) and activity (Fig. 3B) observed in our experiments, clearly suggest a critical role for ceruloplasmin as a compensatory antioxidant protein that is induced in the lung during acute oxidative stress. Indeed, the absence of hyperoxia-induced increases in ceruloplasmin in the liver or circulation may point to the tissue specificity of ceruloplasmin synthesis during oxidative stress. This proposed antioxidant function of ceruloplasmin under acute oxidative stress occurs independent of its function in binding copper and preventing Fenton-catalyzed ROS generation. In fact, our experiments demonstrate that the hyperoxia-induced increase in ceruloplasmin protein levels did not significantly alter lung tissue copper levels.

Similar to ceruloplasmin, the mechanism by which metallothionein exerts its antioxidant function is not clear. In vitro evidence reveals that cysteine-rich metallothionein reacts directly with ROS (1, 45). Metallothionein may also function as an antioxidant indirectly by releasing zinc, which may then inhibit metabolic activity, protect free thiols from oxidation, and stabilize biomembranes (7). Unfortunately, we were not able to quantify the release of zinc from metallothionein in vivo. Nevertheless, because the significant induction in metallothionein protein levels in the lung and liver under acute oxidative stress were not accompanied by an increase in zinc levels, our results are consistent with an antioxidant function of metallothio-
nein distinct from its role in trace metal homeostasis. It is well documented that metallothionein is a redox-sensitive gene that is elevated during various forms of oxidative stress, including the administration of endotoxin, heavy metals, cytokines, and glucocorticoids (20). Metallothionein antioxidant properties may be indirectly executed through redox signaling, but the precise mechanisms remain unknown. Nevertheless, metallothionein protection against oxidative injury has been explored extensively using metallothionein-over-expressing transgenic mouse models. For example, under both acute and chronic conditions of oxidative stress, cardiac tissue of metallothionein-overexpressing mice displayed a marked resistance to oxidative injury compared with wild-type control animals (24).

The third metalloprotein we examined, ferritin, is characterized primarily as an iron storage protein (3). There is a growing body of evidence that the role of this protein may extend beyond metal storage and include, among other functions, a direct role in free radical defense. For example, mRNA encoding the ferritin light chain, which is involved in long-term iron storage, was increased severalfold in the lung tissue of rats exposed to hyperoxia (39). Ferritin protein levels were also found to increase in mice after exposure to 95% oxygen for 3 days (13). Our results, however, do not support these findings, since we did not detect any increase in lung or liver ferritin levels either in response to hyperoxia or Sod1 gene dosage (Fig. 6, A and C).

It is generally recognized that SOD, GSH-Px, and catalase form a network that provides the first line of cellular defense against oxidative stress, and there is evidence that manipulating the expression of one enzyme may lead to an imbalance in antioxidant defense (15, 31). Therefore, we measured the effects of Cu/Zn SOD on the expression and the activity of GSH-Px and catalase in both lung and liver tissue. First, we observed that Cu/Zn SOD protein levels were not responsive to acute oxidative stress, since neither lung nor liver Cu/Zn SOD levels were significantly affected by exposure to hyperoxia (Fig. 1, A and B). Others have reported similar observations (18, 25). Subsequently, we observed that, in the lung, GSH-Px protein levels were increased as Cu/Zn SOD expression decreased, yet enzyme analysis revealed that GSH-Px activity did not reflect GSH-Px protein levels (Fig. 3A). However, we did observe increased GSH-Px activity after hyperoxia in Sod1(+/-) and Sod1(-/-) animals, findings that are consistent with previous research (25).

Finally, we measured the activity and expression of catalase, an enzyme located primarily within peroxisomes. The results demonstrated, quite unexpectedly, that, in the lung, both the protein level and the activity of this enzyme were suppressed by >50% under the acute oxidative stress of hyperoxia (Figs. 2B and 3B). To our knowledge, this is the first time that this phenomenon has been reported. In the liver, the nontarget organ, catalase activity was reduced by 50% in mice that overexpress Cu/Zn SOD. The reason for this decline is not readily apparent and requires further study. However, it is interesting to note that the activity of catalase, a heme-containing enzyme, is lowest in the liver of Sod1(+/+) mice, a result that coincides with significantly lower tissue iron levels in this same group (Fig. 6D). Nevertheless, our studies did not demonstrate a linkage between the expression and activity of the three primary antioxidant enzymes Cu/Zn SOD, GSH-Px, and catalase.

Copper is an integral component of Cu/Zn SOD and ceruloplasmin. It is generally recognized that ceruloplasmin is the primary extracellular copper transport protein, particularly when excess copper is present. Our study demonstrated that liver Cu/Zn SOD plays a critical role in copper homeostasis. This is indicated by the parallel increase in hepatic copper levels and hepatic Cu/Zn SOD expression (Figs. 4D and 1B). Moreover, the increased expression of Cu/Zn SOD in Sod1(+/+) mice coincides with a significant reduction in circulating ceruloplasmin and copper levels (Fig. 6, D and B). Hence, we speculate that the demand for increased liver copper brought about by elevated Cu/Zn SOD protein levels was met, at least in part, by the supply of copper from circulating ceruloplasmin. Hence, these findings are consistent with previous work demonstrating that, in vitro, ceruloplasmin donates copper to Cu/Zn SOD (12) and may provide in vivo evidence that ceruloplasmin functions as a reservoir that supplies copper for Cu/Zn SOD synthesis.

In this study, we have employed transgenic animals in an in vivo model to describe the homeostatic relationship between the induction of antioxidant metalloproteins and trace mineral metabolism under chronic and acute oxidative stress. Understanding the regulatory link between the antioxidant defense system and trace mineral homeostasis under normal and pathophysiological conditions may provide insights for future therapeutic strategies, for example, in premature infants exposed to hyperoxia and other forms of respiratory diseases associated with excessive oxidative stress.

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