Interleukin 1 Slowly Increases Lung Fibroblast Cu-Zn Superoxide Dismutase Activity Levels (43246)

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Abstract. **Certain pulmonary stress raises rat lung Cu-Zn superoxide dismutase (SOD) activity levels, but cytokines released during stress are reported to exert no regulatory effects on Cu-Zn SOD levels in cultured cells. In contrast, our study found that interleukin 1 (IL-1) can increase Cu-Zn SOD activities in human WI38 lung fibroblasts. The difference in results could be explained by differences in experimental conditions. The increases seen here did not occur during the first 24 hr, but Cu-Zn SOD activities more than doubled by 3 days. In addition, little increase occurred unless the medium was changed at 24-hr intervals. On the other hand, some other potential experimental variables showed little or no effects on IL-1-induced increases in Cu-Zn SOD activities. These variables included IL-1 isoform (α, β, or both), IL-1 concentration (0.5, 2, 5, or 7 units IL-1α/ml medium), and the presence or absence of exogenously added copper as CuO or ceruloplasmin. In addition, combining IL-1 with dexamethasone, a synthetic glucocorticoid that enhances some IL-1 actions, produced only additive, not synergistic, increases in Cu-Zn SOD activities. In conclusion, IL-1, in several different experimental protocols, raised lung fibroblast Cu-Zn SOD activity levels, but only after a 1 day lag time. Stress-induced increases in Cu-Zn SOD activity levels *in vivo* also tend to occur only after lag times.** [P.S.E.B.M. 1991, Vol 197]

Several types of pulmonary stress increase rat lung Cu-Zn superoxide dismutase (SOD) activity levels after a lag of at least 2 days. For instance, 85% O_2 hyperoxia elevates these values, but not during the first 3 days of exposure (1). The combination of >95% O_2 hyperoxia plus endotoxemia also increases rat lung Cu-Zn SOD, but not during the first 48 hr (2). In addition, intratracheal bleomycin administration elevates Cu-Zn SOD activities, but only after a lag time of over 2 days (3). These adaptations have been proposed to work against stress-induced injury in the lung (1, 2, 4).

Cytokine hormones, which are secreted during stress states (5), could mediate the stress-induced increases in rat lung Cu-Zn SOD activity concentrations. Endotoxin injection is known to increase serum levels of these hormones (5), and bleomycin can induce secretion of cytokines by phagocytes *in vitro* (6). Chronic hyperoxia may also increase serum cytokine levels since this treatment elevates serum ceruloplasmin levels (7), an effect duplicated by cytokine injection (8).

White *et al.* (4) found that injection of a cytokine mixture increases lung SOD activity levels in rats exposed to near 100% O_2 hyperoxia. This study did not distinguish cytosolic Cu-Zn SOD from the mitochondrial Mn SOD. However, the time course for the elevation in SOD values (4) is very similar to that obtained for Cu-Zn SOD in rats exposed to endotoxin plus hyperoxia (2). In contrast to the findings of White *et al.* (4), Berg *et al.* (9) reported no change in lung SOD activities in rats treated with hyperoxia plus cytokine-rich serum from endotoxin-treated rats. Possibly, the dose and administration route of the cytokines in the latter case did not sustain elevated serum cytokine levels long enough to produce the delayed increase of SOD values.

Studies with cultured cells have not supported the notion that cytokines mediate increases in lung cell Cu-Zn SOD activities. Cu-Zn SOD mRNA levels, unlike Mn SOD mRNA levels, are unchanged in several cell types after exposure to cytokines such as interleukin 1

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(IL-1) (10–13). Only two of these studies (12, 13) actually assessed SOD activities. However, in both cases, effects on the two types of SOD activities paralleled the effects on message levels.

One drawback of these studies on cytokines and SOD is that none examine cytokine effects beyond 24 hr. Conceivably, elevations of Cu-Zn SOD activities in cell cultures could occur after long lag times, as is the case *in vivo*. The present study examined possible effects of IL-1 on Cu-Zn SOD activity levels over a 3-day period. In addition, this study examined other factors that could potentially limit cytokine effects on Cu-Zn SOD activity levels.

Materials and Methods

Materials. Human WI38 lung fibroblast line was purchased from American Type Culture Collection (Bethesda, MD). IL-1 used for most experiments was graciously provided by Dr. P. T. Lomedico of Hoffmann-LaRoche, Nutley, NJ. For the experiment comparing IL-1α and IL-1β, the cytokines were purchased from Collaborative Research Inc., Bedford, MA. Cell culture grade dexamethasone and human ceruloplasmin were purchased from Sigma Chemical Co., St. Louis, MO. Nu-Serum, a partial serum replacement, was obtained from Collaborative Research, Inc.

Cell Cultures. Cells were grown to confluency in basal medium Eagle plus 10% fetal calf serum that was not heat inactivated. The gas environment was 5% CO_2 plus air. For experimental manipulations, approximately 4.7×10^6 cells were cultured per 100-mm plate in basal medium Eagle plus 10% Nu-Serum, with or without IL-1 and other variable additions. This medium was replaced after 24 hr for 2-day experiments, and replaced at both 24 and 48 hr for 3-day experiments.

SOD and Protein Measurements. Cells were washed twice with cold phosphate-buffered saline and harvested in 1.5 ml of phosphate-buffered saline plus 0.5% Tween 20. The harvested cells were stored at −20°C until analysis. At that time, the cell preparations were thawed, homogenized briefly with a probe sonicator, and then mixed with 0.4 volume of cold ethanol:chloroform (25:15, v:v) to inactivate the Mn SOD (14). Precipitated material was pelleted by microcentrifugation (15,600*g*). The cell extract supernatant was used to determine total extract protein and Cu-Zn SOD activity. Protein was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Rockville Centre, NY).

Cu-Zn SOD was assessed by the pyrogallol assay of Marklund and Marklund (15) as modified by Prohaska (14) to improve sensitivity. Pyrogallol was added at concentrations that, without sample addition, gave a change in absorbance at 320 nm of 0.008/30 sec. Samples were assayed at various dilutions until 50% inhibition of the blank rate was obtained (absorbance change of 0.004/30 sec). The amount of sample producing 50% inhibition was defined as 1 unit. Cu-Zn SOD activity was expressed as units/mg extract protein.

Statistical Analysis. Most results were evaluated by analysis of variance followed by Fisher's least significant difference test. Data from Table I were evaluated using Student's *t* test.

Results

Time, Dose, and Isoform Dependence of IL-1 Effects. Figure 1 illustrates that IL-1 (2 units/ml) increased cell Cu-Zn SOD activity concentrations, but the increase occurred slowly. No increase was obtained at the 1 day time point. About a 50% increase was observed by 2 days, and the increase rose to over 100% by 3 days. The increase for the 3-day values was very small if cells were incubated in the same medium for 3 days, rather than receiving fresh medium every 24 hr (data not shown). For the latter case, the increase was not restricted to a very narrow range of IL-1 concentrations (Fig. 2). In fact, the effects were very similar for four concentrations spanning a 14-fold range. The 5

Table I. Comparison of IL-1α and β Effects on Cu-Zn SOD Activities[a]

Treatment	Cu-Zn SOD (units/mg protein)
None	15.9 ± 0.7
IL-1α	27.1 ± 3.5
IL-1β	27.1 ± 1.8
IL-1α + β-IL-1β	27.1 ± 1.8

[a] Values are means from three plates ± SD. Each IL-1 value is significantly different from the control value, but not from the other IL-1 values ($P < 0.01$, ANOVA + LSD). Protein is that in the cellular extracts used for enzyme assay. Cells were incubated with or without IL-1 (2 units/ml) for 3 days with medium changed at 0, 24, and 48 hr.

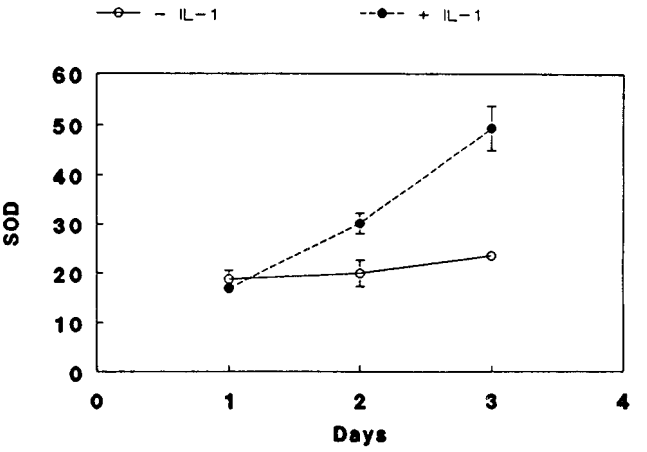

Figure 1. Time course for IL-1-induced increases in Cu-Zn SOD activity levels. Values (units/mg protein) are the means of three plates ± SD. IL-1 values are statistically different from controls at days 2 and 3 ($P < 0.01$, Student's *t* test). Medium (basal medium Eagle plus Nu-Serum with or without IL-1 at 2 units/ml) was replaced at 0, 1, and 2 days.

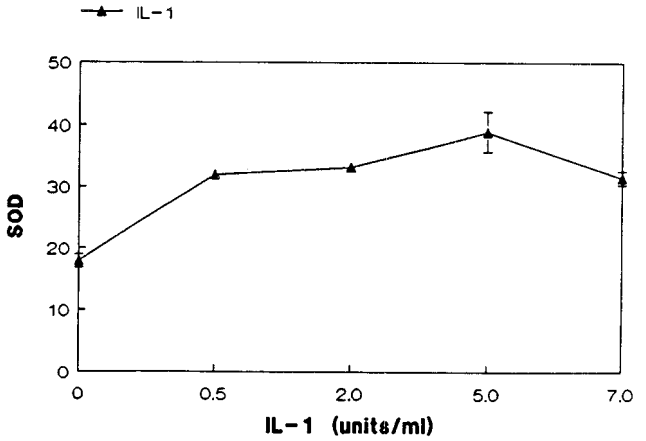

Figure 2. Response of Cu-Zn SOD activity levels to various concentrations of IL-1. Values (units/mg protein) are the means from three plates ± SD. All values for the IL-1 groups were significantly different from the no IL-1 group value ($P < 0.01$ ANOVA followed by LSD). The 5 units/ml group value was significantly different from the other IL-1 group values.

Table II. Effects of IL-1 Plus or Minus Dexamethasone on Cu-Zn SOD Activity Levels[a]

Treatment	Cu-Zn SOD	
	Units/mg protein	% increase
Control	20.1 ± 0.5	0
IL-1	44.8 ± 0.9	123
Dexamethasone	25.1 ± 1.0	25
IL-1, dexamethasone	52.6 ± 0.3	162

[a] Values are the means from six plates ± SD. All values are significantly different from the others ($P < 0.01$, ANOVA + LSD). Dexamethasone was present at 55 n*M*. Other experimental conditions were the same as those in Table I.

units/ml treatment gave the highest response, but the value for this group was only about 17% higher than 2 units/ml. The latter concentration was used for the rest of the study since the 5 units/ml group showed the highest SD. The 3-day increase for 2 units/ml was the same for IL-1α and β, as well as for a 1:1, unit:unit, mixture of the two isoforms (Table I).

Combined Effects of IL-1 and Dexamethasone. Some IL-1-induced effects are enhanced by the synthetic glucocorticoid dexamethasone (i.e., Ref. 16). Therefore, the effects of this hormone on IL-1 induced increases in Cu-Zn SOD activities were examined (Table II). Dexamethasone by itself increased Cu-Zn SOD activity levels, though not to as great an extent as IL-1. Combining the two hormones produced additive, but not synergistic increases. This is illustrated by the percent increases over control values shown in Table II.

One experiment was done to determine whether cellular Cu-Zn SOD activities were limited by medium copper, particularly when activities were increased by IL-1 plus dexamethasone. Medium copper concentration was measured as 0.2 ppm by atomic absorption spectrometry. This value is well below serum copper levels typical of healthy human adults (17). However, neither 0.3 ppm of copper as CuO (Copper Standard, Fisher Scientific, Pittsburgh, PA), nor 1.0 ppm of copper in the serum protein ceruloplasmin, increased Cu-Zn SOD activity levels in cells with or without IL-1 plus dexamethasone (data not shown). The CuO preparation was previously shown to be suitable for activating Cu-Zn SOD in intact erythrocytes *in vitro* (18). The level of ceruloplasmin bound copper used was typical of control human serum (17).

Discussion

This study demonstrated that a cytokine, IL-1, can increase Cu-Zn SOD activity levels in pulmonary fibroblasts *in vitro*, but only after a lag time of 1 day. This result does not contradict previous studies, which showed that cytokines do not regulate cellular Cu-Zn SOD levels. Rather, the present work demonstrates that an IL-1-induced increase in Cu-Zn SOD activities requires 2- to 3-day exposures with daily changes of medium. Conceivably, other experimental variables could also influence whether a cytokine effect is exerted on Cu-Zn SOD activities. However, this study found that IL-1 effects on Cu-Zn SOD activities were not drastically affected by varying the isoform of IL-1 used, or by adding copper or glucocorticoids to the medium. The increase was also fairly consistent for several concentrations of IL-1. Possibly, IL-1 effects on Cu-Zn SOD activities could have been increased or decreased by varying culture conditions to a greater extent than done here (i.e., include much higher and lower IL-1 concentrations).

A time lag preceded the IL-1 elevation of Cu-Zn SOD values in culture (Fig. 1), just as a time lag proceeds stress- or cytokine-induced increases in rat lung SOD activities *in vivo* (1–4). This observation is consistent with the hypothesis that cytokines act directly on lung cells to increase lung Cu-Zn SOD activities *in vivo*. Nonetheless, there are some differences in the results of the culture experiments compared with those from the studies *in vivo*. For example, the lag time before increased SOD activities is shorter for the cell culture studies (Fig. 1). In addition, the endotoxin and cytokine injections *in vivo* required hyperoxia cotreatment to elevate SOD activity levels (2, 4). Perhaps these differences result from differences in overall hormone composition of lung cell environment when in culture rather than in an intact rat.

The results of this present study were obtained in cells that had not been transformed. Different results might be obtained with transformed cells. IL-1-induced changes in Cu-Zn SOD levels could resemble those produced by thyroid hormones. The latter elevate Cu-

Zn SOD values in normal, but not in transformed WI38 cells (19).

IL-1 raised Cu-Zn SOD activity levels in cultured lung fibroblasts after a lag time. This effect resembles the increase in lung Cu-Zn SOD activity levels observed after exposure of rats to cytokines and certain stress states.

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