Characterization of Clinical Tolerance to Inhaled Zinc Oxide in Naive Subjects and Sheet Metal Workers

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Clinical tolerance to the acute effects of zinc oxide inhalation develops in workers during periods of repeated exposure. The aims of this study were to determine whether clinical tolerance is accompanied by a reduction in the acute pulmonary inflammatory and cytokine responses to zinc oxide exposure and whether tolerance can be demonstrated in sheet metal workers who chronically inhale low levels of zinc oxide. Naive (never-exposed) subjects inhaled 5 mg/m^3 zinc oxide on 1 or 3 days and underwent bronchoalveolar lavage 20 hours after the final exposure. Sheet metal workers inhaled zinc oxide on 1 day and control furnace gas on another day. Among naive subjects in whom tolerance was induced, bronchoalveolar lavage fluid percent neutrophils and interleukin-6 (IL-6) levels were significantly decreased compared with subjects who underwent only a single exposure. Sheet metal workers were much less symptomatic, but they still experienced a significant increase in plasma IL-6. The results indicate that clinical tolerance to zinc oxide is accompanied by reduced pulmonary inflammation and that chronically exposed sheet metal workers are not clinically affected by exposure to zinc oxide fume at the Occupational Safety and Health Administration Permissible Exposure Limit. The increase in IL-6 levels observed in the clinically responsive, and to a lesser extent, tolerant, states following zinc oxide inhalation is consistent with the dual role of IL-6 as a pyrogen and anti-inflammatory agent. (J Occup Environ Med. *2000;42:1085–1091*)

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A major determinant of the toxicity of any exogenous noxious agent is whether a diminution in response (tolerance) to that agent occurs after repeated exposures. For inhaled environmental substances such as ozone and endotoxin, the importance of tolerance is accentuated by the ubiquity of the exposures and the difficulty in limiting them. Despite these issues, compared with the amount of research devoted to the study of sensitization to irritants and allergens, there is little on the phenomenon of tolerance; hence, not much is known about the mechanisms it entails.

Metal fume fever serves as an excellent model to study tolerance. The illness results from the inhalation of zinc oxide fume and is characterized by transient fever, multisystemic symptoms, pulmonary inflammation, and elevated plasma levels of the pyrogen, interleukin-6 (IL-6).[1–4] According to worker reports, repeated daily exposure to zinc oxide fume leads to diminution in symptomatic response. Drinker and coworkers corroborated these reports by inducing clinical tolerance to zinc oxide in the laboratory with experimental human exposures.[5] Workers have recounted that their acquired clinical tolerance to zinc oxide is lost in the absence of continuing exposures but can be regained after exposures resume.[6] In addition to the acquired form of tolerance to zinc oxide fume after repeated exposures, some individuals manifest little or no clinical response after their first ex-

posure to zinc oxide fume at levels that induce symptoms and fever in others.[4]

This variability in clinical response and the induction of clinical tolerance to zinc oxide fume complicate efforts to define protective occupational exposure limits. Further challenging these efforts is the concern that clinical tolerance or lack of symptomatic response does not preclude an unappreciated inflammatory response. Specifically, it is unknown whether clinical tolerance is accompanied by a decline in the pulmonary inflammatory response observed after single exposures.[3,7] Also, it is unclear whether workers who are chronically exposed in shops compliant with Occupational Safety and Health Administration standards manifest the same sensitivity to zinc oxide fume as do naive (never-exposed) persons.

We report here our experiments aimed at improving our understanding of the variability in response and the induction of tolerance. To better define the exposure parameters that confer clinical tolerance, and to determine whether clinical tolerance is accompanied by diminished pulmonary inflammatory response, we created a model of clinical tolerance in human subjects by using our zinc oxide fume generation system. We used this model to analyze the clinical and pulmonary inflammatory responses of naive subjects who were exposed singly versus repeatedly to zinc oxide fume and who underwent bronchoalveolar lavage (BAL). To determine whether chronically occupationally exposed sheet metal workers have the same sensitivity to zinc oxide fume as do naive subjects, we exposed workers to the 5-mg/m^3 zinc oxide concentration that had induced fever or symptoms in most of our naive subjects. We also measured levels of IL-6 because of its dual role as a pyrogen and anti-inflammatory cytokine, and tumor necrosis factor (TNF) because it has been cited as a key cytokine for initiation of zinc-induced effects,[8] to analyze their roles in the induction of the metal fume fever syndrome and the development of tolerance.

Methods

Subjects

Naive subjects and sheet metal workers were informed of the risks of the experimental protocol, and they gave informed consent to participate in protocols approved by the institutional review boards of Norwalk Hospital, New York University Medical Center, and Yale University School of Medicine. The group of naive subjects initially consisted of 24 healthy, nonsmoking volunteers, among whom 4 were excluded because of their inability to comfortably undergo bronchoscopy, inability to reliably read a thermometer, or desire not to continue. Among the 20 subjects who completed the protocol, there were 17 men and 3 women with a mean ± SD age of 27 ± 5.8 years, none of whom had been previously exposed to zinc oxide fume or worked in professions in which they may have been exposed to zinc oxide fumes.

The group of sheet metal workers consisted of 10 men (no female sheet metal workers were identified) who were routinely exposed to low-level zinc oxide fume in fabrication shops. Their mean ± SD age was 47 ± 10.6 years, and two had smoked cigarettes (38 and 42 pack-years, respectively). All subjects were asked not to take nonsteroidal anti-inflammatory agents or acetaminophen for at least 24 hours before each exposure. No subject reported ever consuming high-dose oral zinc supplements.

Protocols

The sheet metal workers were exposed in a randomly ordered, single-blinded fashion to 5 mg/m^3 of zinc oxide fume and to control furnace gas alone. The two exposures were spaced by at least 1 week.

The naive subjects were randomly assigned to one of two unblinded subgroups. One subgroup (n = 11) inhaled zinc oxide fume on 3 successive days (one subject skipped 1 day between the first and second exposures) and underwent BAL the day after the third exposure. Although the intent of this protocol was to render tolerant by repeated exposures those who responded to the first exposure, the nonresponders also underwent the subsequent two exposures to maintain uniform participation and to assess their BAL fluid contents. To determine whether repeated zinc oxide exposures led to diminution in inflammatory BAL fluid indices, the results from this multiple exposure subgroup were compared with those of a second subgroup (n = 9), who inhaled zinc oxide fume on only 1 day and underwent BAL the next day.

Subjects inhaled zinc oxide or furnace gas while at rest for 2 hours through a snugly fitted mask (model #7910, Hans Rudolph, Inc, Kansas City, MO) vented to a hood. To standardize for diurnal variations, all exposures commenced between 8:30 and 9:30 AM.

The symptomatic effects of the exposures were assessed by having the subjects grade standard metal fume fever symptoms on a visual analog scale immediately before each exposure and 0, 3, 6, and 9 hours after exposure. The symptoms were cough, fever, chills, flushing, fatigue, muscle aches, sweating, sickness, irritability, headache, dyspnea, stomachache, and nausea. Itching was included as a negative control symptom because it has never been reported as part of the metal fume fever syndrome. The extent of each symptom was recorded by placing a slash mark through a 10-cm horizontal line. The headings of "slight," "mild," "moderate," and "severe" were centered at 2.5 cm, 5.0 cm, 7.5 cm, and 10 cm, respectively, along the line. A total symptom score was computed as the sum of all symptoms except itching at each time point.

Subjects were instructed to record their oral temperatures with a stan-

dard mercury thermometer prior to each exposure and a minimum of every 2 hours afterward until they went to sleep that night. Temperatures were recorded in Fahrenheit to facilitate accurate readings. Each subject was assigned the same thermometer for use throughout the study. Because the circadian temperature variation is about 0.6° C, temperature increases from the morning baseline greater than that were regarded as indicating fever.[9]

Venous blood samples were drawn before and 3 and 6 hours after each exposure. The plasma portions were immediately separated and stored at −70° C for later analysis. Measurement of plasma and BAL fluid IL-6 and TNF levels was performed using high-sensitivity enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN; IL-6 sensitivity 0.156 pg/mL and TNF sensitivity 0.5 pg/mL) and a microtiter plate reader (Anthos 2000, Fallsburg, Austria). One naive subject did not have plasma IL-6 levels measured because of a technical error.

All of the naive subjects underwent BAL by fiberoptic bronchoscopy approximately 20 hours after finishing their previous-day inhalational exposure. All bronchoscopies were performed by one investigator (J.M.F.). Subjects were mildly sedated with intermittent intravenous doses of midazolam. The pharynx and airways were anesthetized with 2 mL of nebulized 4% lidocaine, topical application of 2% lidocaine, and instillation of 4 mL of 1% lidocaine at the vocal cords and right middle lobe orifice. The bronchoscope (Olympus model 1T20, New Hyde Park, NY) was wedged into the right middle lobe, which was lavaged with five 50-mL aliquots of sterile 0.9% NaCl solution.

The retrieved BAL fluid was pooled and filtered through gauze, and an aliquot was drawn off for cell count using a standard hemocytometer. The remaining BAL fluid was cytocentrifuged at 500 g for 10 minutes. Supernatant was preserved at −70° C for cytokine and protein analyses. Cell differential counts were performed after Diff-Quik staining.

Zinc Oxide Generation

Zinc oxide fume was generated by the method described by McCarthy and coworkers.[10] Briefly, zinc shavings were heated to approximately 550° C in a furnace ventilated with inert argon gas. The zinc vapors released were carried downstream to react with oxygen, yielding a supersaturated atmosphere of zinc oxide vapor that condenses to ultrafine particles. These primary particles aggregate in chains to form secondary particles. Measurements performed with a differential mobility analyzer (model 3021, TSI, St. Paul, MN) have demonstrated that these secondary particles have a 0.3-μm mass median diameter and 1.5 geometric standard deviation. The zinc oxide fume was diluted with metered room air filtered through a high-efficiency particulate air filter and an activated charcoal filter and was humidified to 30% to 50% relative humidity using a cascade humidifier. Zinc oxide was collected during successive 20-minute intervals (6 times total) from the manifold of the exposure system onto a polytetrafluoroethylene filter (TX40HI20-WW, Pallflex Products Corp, Putnam, CT). Each filter was immediately weighed on a microbalance (Cahn C-30, Cerritos, CA), and based on each filter weight, the furnace system was immediately adjusted to yield the desired output. The control exposure consisted of furnace gas composed of 97% filtered room air and 3% argon. The mean (± SD) zinc oxide output of the furnace was 5.15 (± 0.37) mg/m^3.

Workplace Exposure to Zinc Oxide Fume

To verify that metal fabrication shops from which the workers were recruited contained low levels of zinc oxide in the ambient air, an industrial hygienist collected air filter samples at two of the shops. Personal samples were collected in the breathing zone of the plasma cutter operators during active galvanized sheet metal cutting. The personal air sampling pumps were calibrated to draw approximately 2 L per minute. Area samples were collected at interval distances of up to 9 meters away from the operator. The samples were collected on mixed cellulose ester fiber filters and were analyzed by an American International Health Alliance–accredited laboratory using flame absorption spectrophotometry (National Institute for Occupational Safety and Health method 7030). The lower limit of detection was 0.03 mg/m^3. Also, prior to laboratory exposure, each sheet metal worker performed a 24-hour urine collection, which was analyzed for zinc content (SmithKline Beecham Clinical Laboratories, Waltham, MA). These measurements were performed to further quantify ongoing low-level exposure.

Statistical Analysis

For each outcome measured at multiple time points, the difference was computed between the response at each time point following exposure and the value before exposure. Because subjects varied in the time during the 9-hour post-exposure observation period at which they manifested their peak symptomatic and temperature responses, the maximum post-exposure responses within that 9-hour period were selected. Differences in mean maximum responses for paired data were tested using the 2-tailed paired *t* test. Responses of the single exposure naive group were compared with those of the multiple exposure group by using the 2-tailed *t* test for independent samples. BAL fluid results from the single and multiple exposure groups were also compared with those from a group (n = 7) of normal, nonsmoking laboratory control subjects whose BAL fluids were collected and analyzed by the same methods as for the exposed subjects. Comparisons involving

these three groups were performed by using one-factor analysis of variance with the post-hoc Student-Newman Kuels test. Correlations between cytokine levels and symptomatic and temperature responses were analyzed using Spearman rank correlation.

Results

Following the first exposure to zinc oxide fume, 16 of the 20 naive subjects (80%) recorded an increase in symptoms from pre-exposure baseline, ranging from very slight to moderate. Ten of the 20 naive subjects (50%) had increases in temperature >0.6° C from pre-exposure baseline. Mean ± SD increase in temperature among responders was 1.14° ± 0.45 °C. The most prominent symptoms recorded were fatigue, headache, and chills.

Plasma IL-6 levels of the naive subjects drawn 6 hours after exposure increased significantly from pre-exposure values (mean increase 4.89 ± 5.28 pg/mL, $P = 0.008$), but the levels drawn 3 hours after exposure did not (mean increase 0.35 ± 0.83 pg/mL, $P = 0.08$). Fifteen (75%) of the subjects developed an increase in plasma IL-6 levels at both 3 and 6 hours after exposure. One subject had no clinical response to zinc oxide fume but a substantial increase in plasma IL-6 level (from 0.95 pg/mL pre-exposure to 8.16 pg/mL at 6 hours). Mean plasma TNF levels for all 20 subjects following exposure did not significantly change from pre-exposure baseline levels (mean change −0.06 ± 0.71 pg/mL at 3 hours and −0.05 ± 1.16 pg/mL at 6 hours).

Among the 11 naive subjects randomized to the group who received zinc oxide fume on 3 successive days, nine subjects displayed symptomatic responses, and five of these nine subjects recorded maximal temperature increases >0.6° C after the first exposure. For the nine symptom responders, the mean ± SD maximal total symptom score declined following the three exposures from 19.6 ± 12.0 to 5.6 ± 6.8 to 0.8 ± 4.1, $P <$ 0.05 for the difference between values for the second and third exposures compared with the first exposure (Fig.1). All five temperature responders in the group experienced a decline in peak temperature following each successive exposure: first exposure mean ± SD 1.19° ± 0.42° C, second 0.62° ± 0.28°, and third 0.44° ± 0.38° (Fig. 2). Plasma IL-6 levels were elevated 6 hours after the first exposure compared with the pre-exposure baseline levels in 8 of the 11 naive subjects who received three zinc oxide exposures. In seven of these eight subjects, this IL-6 response was reduced following the third zinc oxide exposure; however, the mean decline among these eight subjects was not significant (3.28 ± 2.23 pg/mL after first exposure to 1.31 ± 3.8 pg/mL after third exposure, $P = 0.25$).

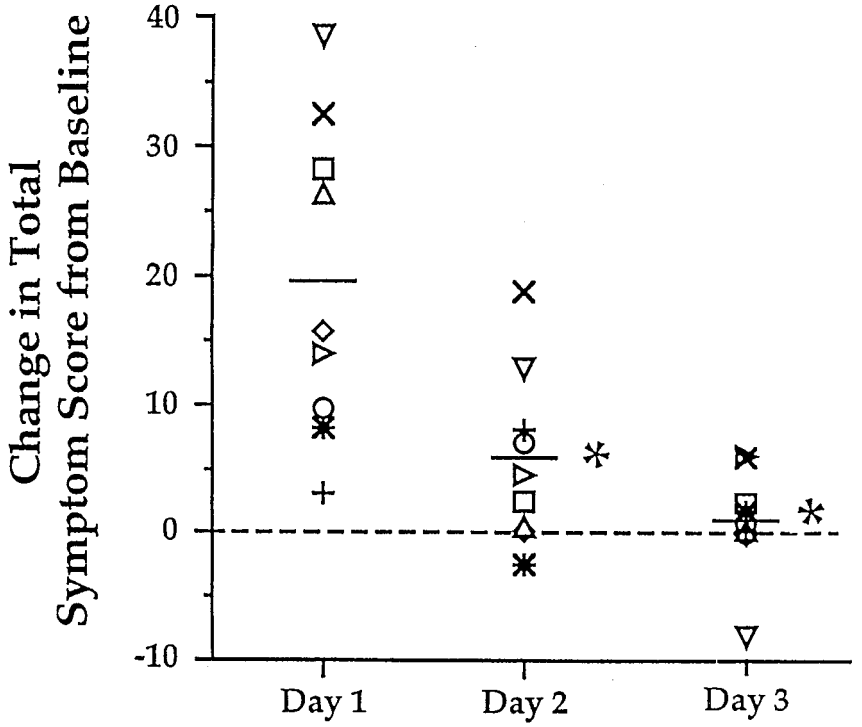

Fig 1. Maximum changes in total symptom score from pre-exposure baseline following 2-hour inhalational exposure to 5 mg/m^3 zinc oxide on 3 successive days for individual naive subjects ($n = 9$) who experienced symptoms after the first exposure. Bars mark mean values. * denotes significant difference ($P < 0.05$) from mean first exposure score.

The BAL fluid of naive subjects who underwent a single exposure contained a higher percentage of neutrophils and more IL-6 than that of our normal laboratory reference subjects (30 ± 14% vs 2.2 ± 1.1% neutrophils and 15.9 ± 17.9 pg/mL vs 0.9 ± 0.4 pg/mL IL-6) and that of the naive subjects who underwent three exposures to zinc oxide (Table 1). IL-6 was detectable in the BAL fluid of all subjects who received three zinc oxide exposures except one. There was no significant difference ($P < 0.57$) in mean cell counts between the subjects receiving one and three exposures. Mean BAL fluid protein levels in both exposure groups were not statistically different and were only slightly higher than the mean BAL fluid protein level (71 ± 31 μg/mL) in the normal laboratory reference subjects. TNF, which was undetected in the BAL fluid of normal laboratory reference subjects, was detectable in the BAL fluid of all zinc oxide–exposed subjects but one; that subject underwent lavage after three exposures and experienced neither fever nor symptoms.

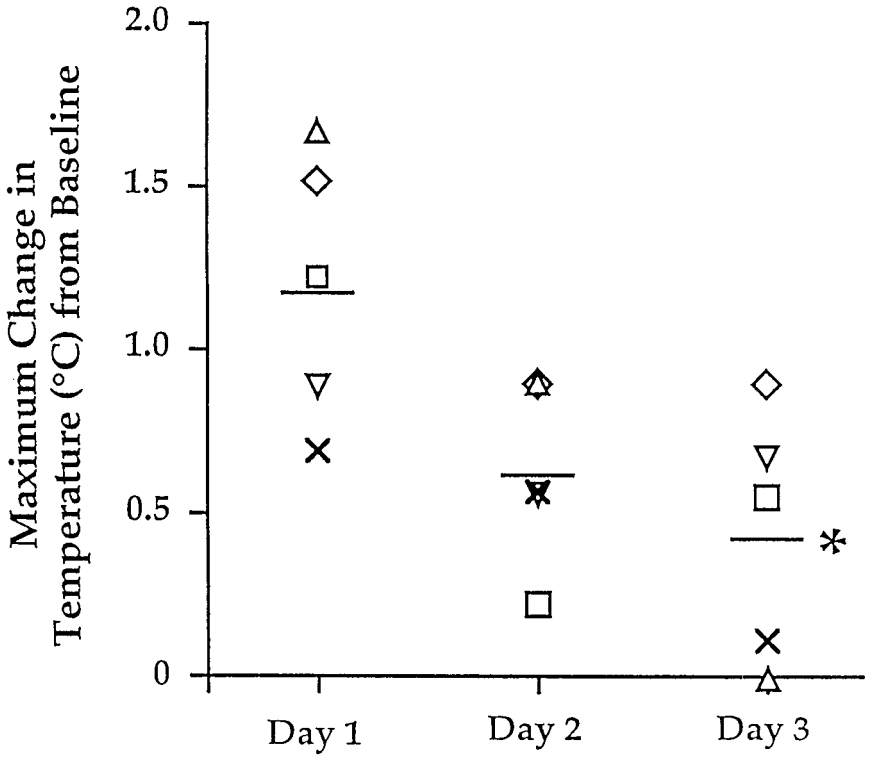

Fig 2. Maximum changes in oral temperature from the pre-exposure baseline following 2-hour inhalational exposure to 5 mg/m^3 zinc oxide on 3 successive days for individual naive subjects ($n = 5$) who experienced temperature increases after the first exposure. Bars mark mean values. * denotes significant difference ($P < 0.05$) from mean first exposure maximum temperature change.

Mean BAL fluid TNF levels were not significantly lower in subjects who received three exposures compared with those who received a single exposure ($P = 0.13$).

A significant correlation was seen between maximum total symptom scores and plasma IL-6 levels among the 20 naive subjects following their first exposure ($r = 0.49$, $P = 0.03$). Likewise, the IL-6 levels in the BAL fluids of the 20 naive subjects were significantly correlated with maximum total symptom scores reported after the exposure preceding the bronchoscopy ($r = 0.45$, $P = 0.049$). No significant correlations were observed between BAL fluid TNF levels and symptom response or between IL-6 plasma and BAL fluid levels.

In contrast to the symptomatic response of the naive subjects to zinc oxide fume, the sheet metal workers were much less affected by the exposure. The mean maximum total symptom score was 4.8 ± 4.3 following zinc oxide fume exposure, which is 29% of that observed in all of the naive subjects in this study after their first exposure and not significantly different from the mean maximum total symptom score after control air exposure (3.3 ± 3.3, $P = 0.21$). The temperature changes for the sheet metal workers after zinc oxide fume exposure were also not different from those after control air exposure. Despite the minimal clinical responses, however, mean plasma IL-6 levels increased significantly among the workers 3 hours after zinc oxide fume exposure compared with after control air exposure (Fig. 3). Plasma TNF was not detected after the exposures.

Nine of the 10 sheet metal workers tested brought in 24-hour urine collections prior to undergoing exposure. Three had mildly elevated 24-hour urinary zinc excretion, 1394, 1278, and 1259 μg/24 hours, compared with the laboratory reference range of 150 to 1250 μg/24 hours. The mean urinary zinc level for all nine sheet metal workers who supplied urine samples was 977 μg/24 hours, well within the normal range for unexposed persons. Their median level of 1036 μg/24 hours, however, was above the median level of 550 μg/24 hours for this laboratory.

Air samples from two sheet metal shops revealed generation of very low levels of zinc oxide during active plasma cutting of galvanized sheet metal. Personal samples from a plasma cutter operator in each of the two shops contained 0.12 and 0.04 mg/m^3. Area samples from 1 to 9 meters away from the plasma cutters contained from 0.10 to < 0.03 mg/m^3 of zinc oxide.

Discussion

The results of these experiments agree with our earlier finding that the clinical features of metal fume fever can be induced in approximately 80% of naive subjects exposed for 2 hours to the 8-hour, time-weighted average American Conference of Governmental Industrial Hygienists Threshold Limit Value and the Occupational Safety and Health Administration Permissible Exposure Limit of 5 mg/m^3.[11,12] Further, these experiments extend our controlled human model for induction of metal fume fever to the induction of significant tolerance in nearly 90% of those who developed metal fume fever. As reported by Drinker in his classic works on metal fume fever, reduction of clinical response to zinc oxide fume exposure can be observed after the second consecutive daily exposure, and as noted here, the decline continues after the third daily exposure.[5,13]

Little clinical response to zinc oxide was observed in our group of sheet metal workers. This finding suggests that workers chronically exposed to low levels of zinc oxide are less sensitive to 5 mg/m^3 of zinc oxide fume than are naive persons. One explanation for this finding is

TABLE 1
Bronchoalveolar Fluid Cellular, Protein, and Cytokine Content Following One and Three Exposures to Zinc Oxide Fume*

No. of Exposures	*n*	Cells/mL	% Alveolar Macrophages	% Lymphocytes	% PMNs	% Eosinophils	Protein (μg/mL)	IL-6 (pg/mL)	TNF (pg/mL)
1	9	$5.5 \times 10^5 \pm 2.4 \times 10^5$	61 ± 15	8 ± 2	30 ± 14	1 ± 1	118 ± 16	15.9 ± 17.9	3.0 ± 1.7
3	11	$4.9 \times 10^5 \pm 1.3 \times 10^5$	81 ± 6	12 ± 6	6 ± 3†	1 ± 2	107 ± 42	4.7 ± 2.6†	2.2 ± 1.4

* Values are expressed as mean ± standard deviation. PMN, polymorphonuclear leukocytes; IL-6, interleukin-6; TNF, tumor necrosis factor.
† $P < 0.001$ for comparison between single- and multiple-exposure groups.

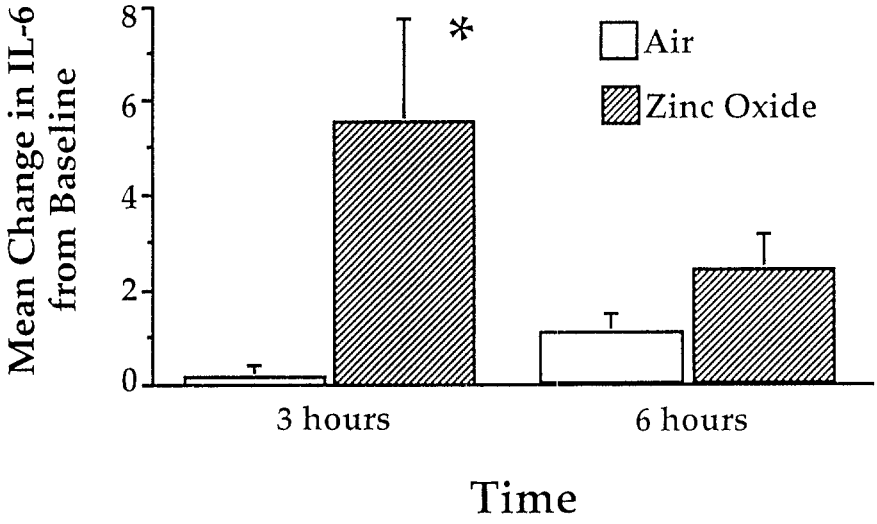

Fig 3. Mean (±SE) changes in plasma IL-6 levels score from pre-exposure baseline following air and 5 mg/m^3 zinc oxide exposure of sheet metal workers. * denotes significant difference ($P = 0.04$) from baseline.

that the workers are self-selected for relative unresponsiveness to zinc oxide, the so-called "healthy worker effect." Another explanation is that tolerance may be a function of age, given that the sheet metal workers were, on average, older than the naive subjects (47 vs 27 years old). However, we found no such observation relating to age in the metal fume fever literature or in anecdotal reports, and we observed no clear age-related responses in our experiments within the groups of naive subjects or workers.

Although our measurements of the zinc oxide concentrations in the atmosphere of the sheet metal shops suggested that exposures were at a low level, exposures at a higher level may have occurred. Studies of brass foundry workers from Egypt and China report mean 24-hour urinary zinc levels between 422 and 1035 μg[14,15] compared with 977 μg/24 hours for our workers, so the urinary zinc levels do not exclude this possibility. Nonetheless, our sheet metal workers' urinary zinc levels are at the upper end of normal for the reference laboratory and may reflect merely dietary practices.

Among the naive subjects, clinical tolerance was accompanied by diminished pulmonary inflammatory response after multiple exposures to zinc oxide fume. Decreases in cellular and biochemical indices of pulmonary inflammation in conjunction with diminished clinical response have been also observed with repeated ozone exposure in humans.[16] Similar to our study, the subjects who received multiday ozone exposure had significantly less IL-6 and percentage of neutrophils and no significant decrease in protein in their BAL fluid compared with subjects who received a single-day ozone exposure.

In our naive subject group, plasma IL-6 levels increased after zinc oxide exposure, as was observed in our earlier study,[4] and trended down (albeit to a non-statistically significant extent) after multiple treatments. A more clear-cut decline occurred with BAL fluid IL-6 when comparing the one- and three-exposure groups. These declines in IL-6 levels correlated with the decline in symptoms as tolerance was evoked by multiple exposures. Even with this decline, however, IL-6 remained detectable in the BAL fluid of 10 of the 11 subjects who underwent multiple exposures. This pattern may be consistent with the role of IL-6 as a pyrogenic and an anti-inflammatory cytokine.[17,18] In a rat model of ozone-induced pulmonary injury, for example, pretreatment with IL-6 decreased BAL fluid neutrophilia and pretreatment with anti-IL-6 receptor antibody increased BAL fluid neutrophilia following ozone exposure.[19] The IL-6 manipulations exerted no effect on ozone-induced pulmonary edema, as signified by BAL fluid protein levels. Given that the BAL fluid IL-6 levels and neutrophil counts tracked together in our study, and that the BAL fluid protein did not significantly vary between the single– and multiple–zinc oxide exposure groups, an analogous role for IL-6 as a suppressor of neutrophil-mediated injury may be hypothesized. This anti-inflammatory role may further account for why plasma

IL-6 levels increased in both naive subjects and workers who did not manifest much clinical response to zinc oxide fume.

The assignment of IL-6 to the role of pyrogen in this human model of metal fume fever remains largely conjectural. There are numerous endogenous pyrogens, the vast majority of which were not sampled for in this study. Furthermore, the circulating levels of IL-6 detected may be insufficient to produce the adverse responses observed. Tsigos et al reported that subcutaneous injections of recombinant human IL-6 into human subjects yielded plasma IL-6 levels that were approximately 10- to 20-fold higher than those observed in these experiments but produced mild fever without other symptoms in only two of six subjects.[20] Higher doses of IL-6 that led to plasma IL-6 levels of approximately 200-fold or greater resulted in fevers of up to 39.0 °C and a constellation of symptoms similar in time course and nature to those seen in metal fume fever. These dose-response findings, nonetheless, do not preclude the possibility that the inhalation of zinc oxide fume results in a different pattern of IL-6 formation and release into the circulation than does the subcutaneous injection of recombinant IL-6.

In summary, this study indicates that the pulmonary inflammatory response to zinc oxide fume diminishes with multiple inhalational exposures. Also, this study reproduces our earlier findings that inhalation of 5 mg/m^3 for 2 hours induces the syndrome of metal fume fever and that, therefore, the permissible exposure limits and total lung volume of 5 mg/m^3 for 8 hours, time-weighted average, is unlikely to fully protect never-exposed workers. For previously exposed workers, in contrast, this exposure level does not appear to induce clinical signs or symptoms. The increase in IL-6 level that occurs in the peripheral circulation and in the lung after zinc oxide inhalation and that is attenuated by multiple, consecutive inhalations may play a part in both the febrile and inflammatory responses. Further experimentation, likely best accomplished in an animal model, is required to better explicate the systemic response to zinc oxide fume.

Acknowledgments

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