Induction of Myeloperoxidase Deficiency in Granulocytes in Lead-Intoxicated Dogs

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Lead interferes with heme synthesis in erythrocytes and has a deleterious effect on red cell membranes. We measured myeloperoxidase (MPO) enzyme activity in the granulocytes of dogs fed increasing quantities of lead. Concurrently, iodination capability and in vitro bactericidal activity were measured. Blood lead levels were monitored. Three of 4 dogs poisoned with lead developed significant decreases in MPO enzyme activity in their granulocytes. The decline in MPO activity correlated with cumulative lead toxicity as judged by blood lead levels and clinical signs of lead poisoning. Iodination ability in all 4 dogs decreased with cumulative lead toxicity. After discontinuation of lead administration, recovery of granulocyte MPO activity preceded recovery of iodination ability. This observation suggests the possibility of separate effects of lead on iodination ability and MPO activity. Moderate impairment of bactericidal capacity developed in 3 of 4 dogs with severe lead poisoning. Clinical infections were not observed during the course of the study.

MYELOPEROXIDASE (MPO) is an enzyme containing two heme groups that is important in bactericidal and candidacidal mechanisms in granulocytes and monocytes. MPO activity varies from species to species, but MPO activities appear to be generally similar in dog and human granulocytes.

Patients with congenital deficiency of MPO have been described whose granulocytes showed impaired in vitro bactericidal and candidacidal activity. One of the patients suffered chronic Candida infections. A patient with myelomonocytic leukemia, deficient MPO activity, and impaired in vitro bactericidal capacity has also been described.

Lead has been shown to interfere with heme synthesis in erythrocytes, and it may have a deleterious effect on red cell membranes.

The purpose of our study was to investigate the effect of lead poisoning on granulocyte function in dogs, with particular reference to MPO enzyme activity. We also studied the iodination and bactericidal capabilities of the dog granulocytes. Concurrent blood lead levels were monitored. We found that MPO activity decreased in 3 of 4 dogs poisoned with lead, and it correlated best with cumulative lead toxicity. Iodination ability decreased in all 4 dogs. Moderate impairment of bactericidal capability developed in 3 of 4 dogs, but no clinical signs of infection occurred.
LEAD-INDUCED MYELOPEROKIDASE DEFICIENCY

MATERIALS AND METHODS

Lead-Poisoned Dogs

Four adult mongrel dogs were fed lead acetate at an initial dosage 2.5 mg/kg/day. The lead acetate was gradually increased over a period of 6 mo to 50 mg/kg/day. Blood samples from each dog were obtained at approximately 2-wk intervals for 6 mo.

Granulocyte Preparation

Blood withdrawn from the jugular vein of each dog was anticoagulated with heparin (40 units/ml) in plastic containers (30 × 115 mm, 50-ml Falcon tubes). The granulocytes were separated by 6% dextran sedimentation. The cells were then washed twice with 5 ml of Hanks balanced salt solution (HBSS) for 5 min at 1200 rpm (HBSS, calcium- and phenol-red-free, GIBCO, Grand Island, N.Y.) and resuspended in HBSS. For the MPO assay the red cells were lysed with distilled water, the granulocytes were rescued with hypertonic saline and washed three times with HBSS, and the intact granulocytes were counted. They were then frozen at 70°C until used for the MPO assay. After thawing, the granulocytes were lysed by employing sonication. For the iodination procedure the residual erythrocytes were lysed using two volumes of 0.87% NH4Cl and one volume of plasma. Residual red cells were not removed from the bactericidal assays.

MPO Assay

MPO enzyme activity was measured by use of a double oxidation reaction. Glucose was combined with H2O and O2 in the presence of glucose oxidase to generate hydrogen peroxide. Catalyzed by MPO, the H2O2 then oxidized the hydrogen donor O-dianisidine to brown yellow dye in amounts proportionate to peroxidase activity. Results are expressed as ΔO.D./100,000 granulocytes/min.

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\beta-D-glucose + H_2O + O_2 \xrightarrow{\text{glucose oxidase}} H_2O_2 + D-gluconic acid
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\[
H_2O_2 + DH_2 \xrightarrow{\text{MPO}} 2H_2O + D
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Iodination

Iodination was measured by a modification of the method of Pincus and Klebanoff. Granulocytes (0.2 ml, 50 × 10^6 cells/ml), 0.05 ml of homogenized (Schwarz Mann) zymosan (10 mg zymosan/1 ml calcium-free Krebs solution), 0.05 ml of frozen pooled undiluted human serum, 0.1 ml of carrier-free Na\(^{125}\)I (0.1 μCi of \(^{125}\)I), 0.1 ml of glucose (7.2 mg/10 ml of calcium-free Krebs), and 0.09 ml of calcium-free Krebs-Ringer phosphate solution (pH 7.4), for a total incubation mixture of 0.5 ml, were incubated in a shaking water bath at 37°C. After 1 hr, 0.1 ml of 0.1-M sodium thiosulfate and 1 ml of cold 10% trichloracetic acid (TCA) were added to each plastic tube (12 × 75 mm) and spun at 2000 rpm for 5 min. The mixtures were washed and vortexed three times in 1 ml of 10% TCA, and the pellet was counted for 1 min in a gamma counter (Picker). A reaction blank without granulocytes was run simultaneously, and experimental samples were tested in duplicates. Results are expressed as percentage uptake of \(^{125}\)I.

Bacterial Killing

Granulocytes (0.5 ml, 10 × 10^6 cells/ml HBSS) and 0.1 ml of Staphylococcus albus (1 × 10^6 bacteria in 0.1 ml saline by turbidimetry) were incubated with 0.1 ml of pooled human AB serum and 0.3 ml of HBSS and rotated end over end at 37°C. Phagocytic mixtures were sampled at 0 and 180 min and incubated overnight in nutrient agar at 37°C. The colonies were then counted. Simultaneous viability (cell-free) and sterility controls were run. The results are expressed as percentage colonies counted at 180 min compared with 0 min incubation time.

RESULTS

Iodination in 3 Dogs

Three of 4 dogs showed marked decreases in iodination ability with cumulative lead toxicity (Fig. 1). The maximal depression of iodination ability occurred at the
time that the blood lead levels were the highest. After discontinuation of lead, 2 dogs showed some recovery of iodination ability. The third dog had died.

**MPO in 3 Dogs**

When MPO activity, blood lead levels, and iodination ability were plotted for 3 dogs (Fig. 2), it was apparent that with cumulative lead toxicity both MPO and...
iodination ability decreased markedly. Each line represented the moving average of the data for 3 dogs. The maximal depression occurred at the time that blood lead levels were the highest. With cessation of lead administration, recovery of iodination ability appeared to lag behind recovery of MPO activity. These data suggest separate effects of lead on MPO and iodination.

**MPO and Iodination in 1 Dog**

One dog showed depression of iodination ability with cumulative lead toxicity that was similar to that in the other dogs, but his MPO activity remained essentially unchanged (Fig. 3).

**Bactericidal Activity in 4 Dogs**

The in vitro bactericidal capabilities of the granulocytes of all 4 dogs are shown in Fig. 4. The lines represent numerous points for each dog that were subjected to linear regression analysis and plotted versus time. There is a suggestion of some impairment of bactericidal capability with cumulative lead toxicity in 3 dogs. Despite discontinuation of lead, bactericidal capability did not appear to improve even though MPO activity had returned to normal. One dog showed essentially no change in bacterial killing. This dog also showed no decrease in MPO activity.

**DISCUSSION**

The participation of MPO, H$_2$O$_2$, and a halide in bacterial killing in granulocytes has been well shown. The iodination reaction is also dependent on phagocytosis, MPO, and H$_2$O$_2$, and it has been used as a marker of the MPO-H$_2$O$_2$ antimicrobial system in intact granulocytes. Although iodination ability is not strictly proportional to in vitro bactericidal capacity, Klebanoff has reported a strong association in cases of hereditary MPO deficiency.

Our data show that MPO activity and iodination ability decrease markedly with cumulative lead toxicity. Since MPO is a heme-containing enzyme, we believe that lead may have a deleterious effect on its function or formation. Klebanoff has
shown that azide and cyanide, which inhibit heme-containing enzymes, may also lead to loss of MPO activity. It should be noted that MPO activity never reached zero in our studies, and this may explain in part why only moderate impairment in bacterial killing was found. Moreover, measurement of bactericidal function at times earlier than 3 hr might have demonstrated a more pronounced defect. Other authors have shown early deficiency in bacterial killing by MPO-deficient neutrophils that approaches normal at 3–4 hr of incubation of cells and bacteria. Moreover, Klebanoff has suggested that the granulocytes of patients with genetic absence of MPO may compensate for lack of MPO by other nonperoxidase microbicidal mechanisms. This observation would help to account for the general lack of infections seen in patients with genetic absence of MPO. Our dogs may have shown similar compensation over the period of time that MPO was being poisoned with lead. It remains to be determined if acute loss of MPO activity with lead administration can lead to a more marked bactericidal defect.

After cessation of lead administration, we noted a lag in recovery of iodination ability, as compared with recovery of MPO activity. This suggests that lead may have separate effects on MPO activity and iodination ability. From our data it is not clear where this possible deleterious effect of lead on iodination ability may have occurred. It is known, however, that iodination ability is inhibited by agents that decrease phagocytosis or degrade H₂O₂ and is stimulated by superoxide dismutase. We cannot rule out a damaging effect of lead on the granulocyte cell membrane.

Our dogs showed no clinical evidence of infection, despite markedly depressed levels of MPO activity and iodination ability and moderately impaired in vitro bactericidal capacity.

Laboratory animals poisoned with small amounts of lead have demonstrated increased mortality when simultaneously exposed to endotoxin. Moreover, chronic subclinical lead poisoning in mice increases their susceptibility to infections when challenged with Salmonella typhimurium. As far as we know, MPO activity and iodination ability have not been measured in lead-poisoned patients. Similarly, it is not known if patients with clinical lead poisoning demonstrate in vivo or in vitro bactericidal defects. In the future, we hope to study the granulocyte function of lead-intoxicated patients with particular attention to MPO activity and in vitro bactericidal and iodination capabilities. In addition, the possibility that
infection may occur in patients with clinical lead poisoning should be considered when the diagnosis of lead poisoning is made.

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REFERENCES

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