Relationship between Leukopenia and Bone Marrow Myeloperoxidase in the Rat Treated with Propylthiouracil

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Abstract—The relationship between the toxic effect of propylthiouracil (PTU) and myeloperoxidase activity of rat bone marrow was examined. The administration of PTU for 1 or 2 weeks caused a decrease in leukocyte count with the concomitant inhibition of the activity of myeloperoxidase in the bone marrow. The decreases in both leukocyte counts and myeloperoxidase activity were restored to control levels at 2 weeks after the discontinuation of the administration. PTU treatment did not affect the affinity of H₂O₂ for the enzyme; however, an increase in the Kₘ value for guaiacol was seen. PTU, incubated with bone marrow peroxidase in vitro increased the Kₘ value of the enzyme for guaiacol, but had no effect on the Kₘ value for H₂O₂. The results suggest that the mechanism of inhibition of myeloperoxidase activity by PTU given in vivo or incubated with the enzyme in vitro may be the same. The activity of bone marrow glutathione peroxidase was not influenced by PTU treatment.

Myeloperoxidase (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) catalyzes the oxidation of some organic substrates in the presence of hydrogen peroxide (1). This enzyme appears in the leukocyte during maturation (2). The activity of myeloperoxidase in human leukemia line HL-60 cells has been found to change markedly during in vitro cell differentiation (3). Thus, the activity of myeloperoxidase in the bone marrow appears to be related to granulocyte differentiation.

PTU, one of the thioureylene antithyroid drugs, inhibits the synthesis of thyroid hormones by preventing thyroid peroxidase-catalyzed iodination of thyroglobulin (4–7). It is recognized that thioureylene antithyroid drugs such as PTU and methimazole caused bone marrow depression during the treatment of hyperthyroidism (8, 9). However, the mechanism of agranulocytosis and leukopenia induced by PTU is uncertain.

Previously, it was found that the administration of 0.5–1.5 mmol/kg PTU to rats for 1 or 2 weeks caused a dose-dependent decrease in leukocyte counts (10). Thus, the rats treated with toxic doses of PTU serve as a useful model for an adverse reaction of PTU, namely granulocytopenia. In PTU treated rats, the decrease in leukocyte count was accompanied by a reduction in the spleen weight (11) and by an increase in the activity of GSH S-transferase of the liver (12). Little is known about the relationship between the effect of PTU on the leukocyte counts and bone marrow function. In this experiment, we have studied further the effect of PTU on bone marrow. The results indicate that the activity of bone marrow myeloperoxidase is reduced by repeated administration of PTU.

Materials and Methods

Chemicals: PTU, guaiacol and GSH were purchased from Sigma Chemical Co. All other reagents were of analytical grade and available in our laboratory.

PTU treatment: PTU suspended in propylene glycol was administered intraperitoneally to male Sprague-Dawley rats, weighing about 80 g, once a day for 1 or 2 weeks. Control animals received only pro-
Pylene glycol. Rats were sacrificed at 24 hr after the final administration of PTU.

Preparation of enzymes: Bone marrow was obtained from femurs and tibias. Hemoglobin-free bone marrow cells were isolated according to the method of Himmelhoch et al. (2) except for the use of 0.9% NaCl containing 2 mM EGTA instead of calcium and magnesium-free Krebs-Ringer buffer. Isolated bone marrow cells were homogenized with 0.25 M sucrose using a Polytron apparatus, and the homogenate was centrifuged at 105,000 x g for 60 min. The resulting pellet was resuspended and the centrifugation repeated. The final pellet was suspended in 0.25 M sucrose and used as the enzyme source.

Assay of enzyme activity: Myeloperoxidase activity was measured using guaiacol as the electron donor. The assay system for the enzyme activity consists of 100 mM potassium phosphate buffer (pH 7.0), 33 mM guaiacol, 0.5 mM H2O2 and the enzyme (2.5 μg protein) in a total volume of 1.0 ml. The reaction was started by adding H2O2 and was followed at 470 nm using a recording spectrophotometer (Hitachi, model 100-21) at 25°C. A guaiacol unit (GU) was defined as the oxidation of 1 μmole of guaiacol per minute based on the absorption coefficient for tetraguaiacoquinone of 5.57 cm⁻¹ mM⁻¹ (13). In some experiments, 5 mM potassium iodide was used as the electron donor instead of guaiacol. The rate of the oxidation of iodide was followed spectrometrically at 350 nm. A iodide unit (IU) was defined as the oxidation of 1 μmole iodide per minute based on the absorption coefficient for iodide of 26.0 cm⁻¹ mM⁻¹ (13). The activity of GSH peroxidase was determined as previously described (14). Protein was measured by the method of Lowry et al. (15).

The statistical significance of differences between the means were determined using the unpaired t-test.

Results

As reported previously (10, 11), PTU (1.5 mmol/kg) repeatedly administered to rats for 1 or 2 weeks significantly decreased the leukocyte counts (Table 1). The leukocyte counts in rats treated with PTU for 2 weeks returned to the control value by 2 weeks after the discontinuation of the administration of PTU. In contrast, the erythrocyte counts were unchanged by PTU administration for even 2 weeks (Table 1). Table 1 also shows that the PTU treatment for 1 or 2 weeks caused a decrease in myeloperoxidase activity of the bone marrow. The decrease in enzyme activity was restored at 2 weeks after discontinuation of the administration of PTU. Thus, the decrease in both leukocyte number and myeloperoxidase activity by PTU treatment was reversible. The decrease in the myeloperoxidase activity caused by PTU administration for 2 weeks was also observed when potassium iodide was used instead of.

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<th>Table 1. Effect of the treatment of rats with propylthiouracil on blood cell counts and the activities of myeloperoxidase and glutathione peroxidase of bone marrow</th>
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<td><strong>Treatment</strong></td>
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<td>Erythrocytes (×10⁶ cells/mm³)</td>
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<td>Myeloperoxidase activity (GU/mg protein)</td>
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Administration of propylthiouracil (1.5 mmol/kg) and assay of the enzyme activities were carried out as described in Materials and Methods. Values are the mean±S.E.M. of 5 to 7 experiments. PTU, Propylthiouracil; N.D., Not determined. *P<0.05, **P<0.01, compared with the control.
guaiacol for the assay of the enzyme activity (control, 3.92±0.59; PTU, 2.11±0.23 IU/mg protein).

No change in myeloperoxidase activity was seen at 3, 6 and 24 hr after administration of a single dose of 1.5 mmol/kg PTU. In addition, leukocyte counts were not influenced by the acute administration (data not shown). The activity of GSH peroxidase was not affected by the administration of PTU for 2 weeks (Table 1).

As shown in Fig. 1, PTU treatment decreased the $V_{\text{max}}$ of myeloperoxidase without affecting the $K_m$ of the enzyme for $H_2O_2$. Thus, the affinity of the enzyme for $H_2O_2$ was not changed by PTU treatment. In contrast, the $K_m$ value for guaiacol was increased by repeated-administration of PTU (Fig. 1).

Azide and cyanide inhibit myeloperoxidase (16) through the interaction with the heme iron of the enzyme (17). Figure 2 shows that these compounds inhibited the bone marrow myeloperoxidase activity in a concentration-dependent manner. The concentration-response curves for azide and cyanide in the control were the same as in PTU-treated rats. The IC50 values for azide and cyanide were...
22 and 23 μM, respectively.

Figure 3 shows the effect of PTU on myeloperoxidase activity in vitro. PTU markedly inhibited the activity of myeloperoxidase, with an IC50 value of 21 μM. PTU, in vitro, caused a decrease in the $V_{\text{max}}$ of the enzyme without affecting the $K_m$ value for $\text{H}_2\text{O}_2$. However, an increased $K_m$ value for guaiacol was observed (Fig. 4).

Discussion

PTU, which is used as an antithyroid drug, exhibits toxic effects in mammals, including the induction of leukopenia (8). It is well established that thioureylen antithyroid drugs inhibit thyroid peroxidase (4-7). However, the inhibition of myeloperoxidase in bone marrow by PTU in vivo has not been demonstrated. The present study revealed that PTU treatment caused the inhibition of myeloperoxidase in rat bone marrow. Leukopenia was also observed when PTU was administered to rats for 1 or 2 weeks. Thus, the decrease in leukocyte count caused by repeated administration of PTU parallels the inhibition of myeloperoxidase activity. A single administration of 1.5 mmol/kg PTU had no effect on the leukocyte counts or the activity of the enzyme. Although the role of myeloperoxidase in the bone marrow is unknown, the enzyme activity changes during cell differentiation (3).

PTU inhibits thyroid functions by inhibiting thyroid peroxidase (4-7). A decrease in thyroid function causes a lowering of hematopoiesis (18), decreasing not only the number of leukocytes but also the number of erythrocytes. As shown in Table 1, PTU treatment decreased only leukocyte number. These findings indicate that the leukopenia induced by the repeated administration of PTU is not likely due to a decrease in thyroid function.
The inhibition of the enzyme activity in bone marrow was selectively observed in myeloperoxidase. GSH peroxidase also decomposes hydrogen peroxide (19). However, the activity of GSH peroxidase was not influenced by PTU treatment for 2 weeks.

PTU also inhibits the activity of myeloperoxidase of the bone marrow in vitro. In vitro, PTU decreases the myeloperoxidase activity noncompetitively with respect to H₂O₂ and increased the Kₘ value for guaiacol. The same results were also obtained in vivo. Therefore, inhibition of myeloperoxidase activity by PTU treatment in vivo may be due to the same mechanism observed in vitro.

In another experiment, the 105,000 x g pellet of the bone marrow was solubilized by cetyltrimethylammonium bromide and chromatographed on Sephacryl S-200 to partially purify the myeloperoxidase. The specific activities of the chromatographic peaks containing enzyme activity were 32.3 and 14.6 GU/mg protein in the control and PTU-treated rats, respectively. Thus, the decreased activity of myeloperoxidase following PTU treatment was also observed in the partially purified preparation of the enzyme. These results indicate that PTU irreversibly inactivated myeloperoxidase in the bone marrow.

Azide and cyanide, which were typical peroxidase inhibitors, depressed myeloperoxidase activity in the control as well as in PTU-treated rats. In addition, the degree of inhibition by azide and cyanide in the control was not different from that in PTU-treated animals, suggesting that PTU treatment does not interfere with the heme group of myeloperoxidase in the bone marrow.

The above evidences indicate that leukopenia may be related to the inhibition of bone marrow myeloperoxidase in the rats repeatedly administered with PTU.

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References

