Bone Marrow Peroxidases of Spontaneously Hypertensive Rats

Kimio KARIYA, Yasuhiro MIKI, Yukiko TSURUOKA, Satoko KITANO and Eibai LEE

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Ikawadani-cho, Nishi-ku, Kobe 673, Japan

Accepted January 8, 1990

Abstract—The activity of peroxidases and the level of myeloperoxidase in the bone marrow of spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHR-SP) were determined in comparison with normotensive Wistar Kyoto rats (WKY). In the cetyltrimethylammonium bromide extract of bone marrow, the peroxidase activities using guaiacol or KI as the electron donor of male and female WKY were different from those of SHR and SHR-SP. The peroxidase activity was also separately determined as myeloperoxidase and eosinophil peroxidase by the use of ion-exchange high pressure liquid chromatography. In males, SHR and SHR-SP contained a low activity of eosinophil peroxidase compared with WKY. Bone marrows of female SHR and SHR-SP contained a lower activity of myeloperoxidase, while SHR and SHR-SP possessed a higher activity of eosinophil peroxidase compared with WKY. No change of the level of myeloperoxidase in the bone marrow was observed among male animals. A significant decrease in the level of myeloperoxidase was observed in female SHR and SHR-SP. Therefore, these results indicate that the change in the activity of the peroxidases in the bone marrow is accompanied by the spontaneously hypertensive state.

Myeloperoxidase (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) is present in mammalian leukocytes (1–3). This enzyme is also observed in the bone marrow and is a marker enzyme in neutrophilic granulocytes (4). The physiological significance of myeloperoxidase in the mature leukocytes is thought to be a bactericidal function (5, 6). Regarding the peroxidase in the bone marrow, the treatment of rats with propylthiouracil decreased the enzyme activity with a concomitant loss of the leukocyte number (7). The inactivation of bone marrow peroxidase was accompanied by a change in the heme structure (8). In addition, it was demonstrated that rat bone marrow contains two peroxidases, namely myeloperoxidase and eosinophil peroxidase, and both peroxidases were homogeneously purified from rat bone marrow (9). The characteristics of myeloperoxidase including the catalytic activity, subunit composition and the structure surrounding the heme were different from those of eosinophil peroxidase (9). Although these evidences indicate that the two peroxidases play different roles in the bone marrow, little is known about the physiological and pathophysiological functions of the peroxidases in this tissue.

To clarify the pathophysiological significance of the peroxidases in the bone marrow, the alteration of the enzyme activity and the level of myeloperoxidase have been examined using SHR and SHR-SP in comparison with normotensive WKY.

Materials and Methods

Materials: Guaiacol, KI and hydrogen peroxide were purchased from Nacalai Tesque (Japan). Peroxidase anti-rabbit IgG (goat) was from BioMakor (Israel). Polyvinylidene difluoride (Durapore) filter was obtained from Millipore Corp. (U.S.A.). Water was
purified by the use of a NANOpure II-4P organic-free system (Barnstead, U.S.A.). All other reagents were of analytical grade. The concentration of hydrogen peroxide was determined as described previously (10).

Animals: WKY, SHR and SHR-SP were kindly provided by Dr. K. Okamoto (Kinki University). Both male and female animals were housed in a room with an automatic 12 hr alternating light and dark cycle (08:00–20:00), and food and water were available ad libitum. The mean blood pressures of the 18-week old animals used in this experiment were as follows: 140, 200 and 210 mmHg for male WKY, SHR and SHR-SP, respectively, and 135, 180 and 190 mmHg for female WKY, SHR and SHR-SP, respectively.

Assay of the peroxidase activity: Preparation of bone marrow cells of rats were carried out as described previously (9). Briefly, the 105,000×g pellet was solubilized with 10 mM potassium phosphate buffer (pH 7.0) containing 0.3% CETAB. The CETAB extract was used as the enzyme source. The peroxidase activity was determined using guaiacol or KI as the electron donor (9). The chlorinating activity was assayed by the use of monochlorodimedone as the substrate (10).

Separation of peroxidases: Separation of myeloperoxidase and eosinophil peroxidase from the CETAB extract was carried out on an SP-5PW (TOSO, 0.5x5 cm) column with a Waters M-600 multisolvent system. The solvents were 10 mM potassium phosphate buffer (pH 7.0, solvent A) and 10 mM potassium phosphate buffer (pH 7.0) containing 2 M NaCl (solvent B). The CETAB extract (500 µg protein) was injected at 10% (v/v) solvent B, and elution was carried out with a linear gradient from 10% to 70% (v/v) solvent B over 18 min with a flow rate of 0.8 ml/min.

Determination of myeloperoxidase level: Myeloperoxidase of the homogenate in the bone marrow was determined using immunostaining coupled with SDS-PAGE (11).

Statistical analysis: The statistical significance between the means were determined by Student's t-test.

Results

Peroxidase activity in WKY, SHR and SHR-SP: Since bone marrow contains two peroxidases, namely, myeloperoxidase and eosinophil peroxidase that preferentially oxidize guaiacol and KI, respectively, the enzyme activity was determined using these two compounds. As shown in Fig. 1, peroxidase activity toward guaiacol or KI was significantly lower in male SHR and SHR-SP compared with WKY. On the other hand, no significant changes in the activities toward guaiacol were observed in female animals (Fig. 2). Figure 2 also shows that the peroxidase activities using KI as the substrate of female SHR and SHR-SP were higher than those of WKY. The chlorinating activity, which is concerned with the antibacteriocidal function of neutrophils, was also determined using monochlorodimedone as the substrate. In both males and females, the chlorinating activity was not altered between the animals (Figs. 1 and 2).

The activities of myeloperoxidase and
Fig. 2. Peroxidase and chlorinating activities of bone marrow solubilized by CETAB in female WKY, SHR and SHR-SP. Peroxidase activities were determined using guaiacol or KI as an electron donor. Chlorinating activity was assayed by the use of monochlorodimedone. *P<0.05, compared with WKY.

Fig. 3. The activities of myeloperoxidase and eosinophil peroxidase separated by ion-exchange HPLC in male WKY, SHR and SHR-SP. Peroxidase activities were determined using guaiacol (□) and KI (□□). *P<0.05, compared with WKY.

Fig. 4. The activities of myeloperoxidase and eosinophil peroxidase separated by ion-exchange HPLC in female WKY, SHR and SHR-SP. Peroxidase activities were determined using guaiacol (□) and KI (□□). *P<0.05, compared with WKY.

eosinophil peroxidase separated from bone marrow: The activities of myeloperoxidase and eosinophil peroxidase from the CETAB extract of bone marrow were separately determined. The CETAB extract of WKY was separated into myeloperoxidase and eosinophil peroxidase by ion-exchange HPLC. The retention times of myeloperoxidase and eosinophil peroxidase were 12.5 and 25.0 min, respectively. The peroxidases from the CETAB extracts of SHR and SHR-SP were also separated into myeloperoxidase and eosinophil peroxidase by HPLC. Figure 3 shows the total activity of myeloperoxidase and eosinophil peroxidase separated by HPLC from the CETAB extracts of WKY, SHR and SHR-SP. No alteration of the activity of myeloperoxidase was observed between male animals. The activity of eosinophil peroxidase from male SHR and SHR-SP was lower than...
that of WKY. With respect to females, the activity of myeloperoxidase was significantly lower in female SHR and SHR-SP in comparison with WKY (Fig. 4). On the other hand, the activities of the eosinophil peroxidase of SHR and SHR-SP were higher than that of WKY.

The level of myeloperoxidase in the bone marrow: Table 1 shows the level of myeloperoxidase in the bone marrow of WKY, SHR and SHR-SP. No significant change in the level of the peroxidase was observed between male animals. On the contrary, female SHR and SHR-SP had a significantly lower level of myeloperoxidase compared with WKY.

Discussion

The present results show the catalytic activity of the bone marrow peroxidase of WKY was different from that of SHR and SHR-SP. As reported previously, rat bone marrow contains two peroxidases, namely, myeloperoxidase and eosinophil peroxidase (9). Because KI is a good substrate for eosinophil peroxidase and the activity of myeloperoxidase using KI as the substrate is very low, the activity toward KI is thought to be due to eosinophil peroxidase, but not myeloperoxidase (9). When KI was used as the electron donor, the peroxidase activity of male WKY was higher than that of SHR and SHR-SP, suggesting that the bone marrow of male SHR and SHR-SP contain a lower activity of eosinophil peroxidase compared with WKY. In addition, the peroxidase activities toward guaiacol of the CETAB extracts of SHR and SHR-SP were lower than that of WKY. These changes in the activity of peroxidase were not accompanied by the alteration of properties such as sensitivity to heme inhibitors because the peroxidase activities in the CETAB extract from WKY, SHR and SHR-SP were inhibited by azide and cyanide to the same degree (data not shown).

The peroxidases of rat bone marrow were resolved into myeloperoxidase and eosinophil peroxidase by ion-exchange HPLC. The eosinophil peroxidase activities of male SHR and SHR-SP were lower than that of WKY. On the other hand, no change in the activity of myeloperoxidase was detected between male animals. Thus, it is likely that the changes in the peroxidase activity of the bone marrow of male SHR and SHR-SP is due to a decrease in the level of eosinophil peroxidase, but not myeloperoxidase, in comparison with WKY.

Regarding the females, the peroxidase activities toward KI of SHR and SHR-SP were higher than those of WKY, suggesting that SHR and SHR-SP contain a higher activity of eosinophil peroxidase. On the other hand, the enzyme activity toward guaiacol was not changed among the animals. In addition, no difference of the properties of the peroxidase such as sensitivity to heme inhibitors was observed among the females as well as the males (data not shown). The myeloperoxidase activities of SHR and SHR-SP were lower than that of WKY, whereas the bone marrows of SHR and SHR-SP contained higher activities of eosinophil peroxidase compared with WKY. In addition, since the myeloperoxidase levels of SHR and SHR-SP were lower than that of WKY, the bone marrows of female SHR and SHR-SP contain a lower concentration of myeloperoxidase and a higher level of eosinophil peroxidase in comparison with WKY. Therefore, these changes in the level of peroxidases in the bone marrow may result in the alteration of the peroxidase activities of SHR and SHR-SP.

To date, it is thought that myeloperoxidase in the bone marrow is a marker for neutrophilic
granulocytes (4) and for the differentiation of a leukemia cell line (12). Previously, we have found that leukopenia induced by propylthiouracil is accompanied by a decrease in the peroxidase activity in the bone marrow (7). In addition, the peroxidase system in bone marrow may be involved in drug metabolism (10). It has been demonstrated for the first time that the activities of peroxidases in the bone marrow were changed in pathophysiological conditions such as hypertension. Thus, the hypertensive state was accompanied by changes in the activity and the level of peroxidases in the bone marrow. Although the role of peroxidases in the bone marrow in the hypertensive state has not been ascertained, SHR and SHR-SP will be useful for the elucidation of the pathophysiological significance of the peroxidases.

It should be noted that there is a marked sex-related difference of the activity of peroxidases in rat bone marrow (Fig. 1). A difference in the level of myeloperoxidase was also observed (Table 1). The degree of the difference was increased in SHR and SHR-SP. The difference in SHR-SP was the most remarkable among the animals. Therefore, the sex-related difference of the peroxidase may become remarkable in the severe pathophysiological condition of SHR-SP. Further studies are needed on the sex-related difference of the peroxidases in the bone marrow.

Acknowledgments: This study was supported in part by the Science Research Promotion Fund from the Japan Private School Promotion Foundation and by a Grant-in-Aid (No. 63770144) from the Ministry of Education, Science and Culture of Japan.

References

1. Agner, K.: Verdoperoxidase. Acta Physiol. Scand. 2, 5-62 (1941)
2. Schultz, J.R., Oddi, F., Kaminker, J. and Jones, W.: Myeloperoxidase of the leucocyte of normal human blood. Arch. Biochem. Biophys. 111, 73-79 (1965)
3. Bos, A., Wever, R. and Roos, D.: Characterization and quantification of the peroxidase in human monocytes. Biochim. Biophys. Acta 528, 37-44 (1978)
4. Bainton, D.F., Ulliot, J.L. and Farquhar, M.G.: The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. J. Exp. Med. 134, 907-934 (1971)
5. Klebanoff, S.J.: Antimicrobial mechanism in neutrophilic polymorphonuclear leukocytes. Semin. Hematol. 12, 117-142 (1975)
6. Rosen, H. and Klebanoff, S.J.: Formation of singlet oxygen by the myeloperoxidase-mediated antimicrobial system. J. Biol. Chem. 252, 4803-4810 (1977)
7. Kariya, K., Lee, E. and Hirouchi, M.: Relationship between leukopenia and bone marrow myeloperoxidase in the rat treated with propylthiouracil. Japan. J. Pharmacol. 36, 217-222 (1984)
8. Lee, E., Hirouchi, M., Hosokawa, M., Sayo, H., Kohno, M. and Kariya, K.: Inactivation of peroxidases of rat bone marrow by repeated administration of propylthiouracil is accompanied by a change in the heme structure. Biochem. Pharmacol. 37, 2151-2153 (1988)
9. Kariya, K., Lee, E., Hirouchi, M., Hosokawa, M. and Sayo, H.: Purification and some properties of peroxidases from rat bone marrow. Biochim. Biophys. Acta 911, 95-101 (1987)
10. Lee, E., Miki, Y., Hosokawa, M., Sayo, H. and Kariya, K.: Oxidative metabolism of propylthiouracil by peroxidases from rat bone marrow. Xenobiotica 18, 1135-1142 (1988)
11. Lee, E., Miki, Y., Yoshida, T., Tsuruoka, Y. and Kariya, K.: Bone marrow peroxidase in mice: Determination of the level and the catalytic activity. Res. Commun. Chem. Pathol. Pharmacol. 66, 135-143 (1989)
12. Rovera, G., Santoli, D. and Damsky, C.: Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. Proc. Natl. Acad. Sci. U.S.A. 76, 2779-2783 (1979)