J. Biochem. 127, 971–976 (2000)

Role of Myeloperoxidase in the Neutrophil-Induced Oxidation of Low Density Lipoprotein as Studied by Myeloperoxidase-Knockout Mouse

Noriko Noguchi,*,[1] Kazumi Nakano,* Yasuaki Aratani,† Hideki Koyama,† Tatsuhiko Kodama,* and Etsuo Niki*

Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro, Tokyo 153-8904; and †Kihara Institute for Biological Research and Graduate School of Integrated Science, Yokohama City University, Totsuka, Yokohama 244-0813

Received February 9, 2000; accepted March 7, 2000

Low density lipoprotein was oxidized by neutrophils derived from either C57BL/6 mice or myeloperoxidase (MPO)-knockout mice. The generation of superoxide from neutrophils of MPO-knockout mice was about 70% of that from wild-type mice. The extent of the oxidation of human low density lipoprotein (LDL) by phorbol myristate acetate (PMA)–activated neutrophils of wild-type and MPO-knockout mice was assessed by measuring consumption of α-tocopherol and formation of phosphatidylcholine hydroperoxide (PCOOH) and cholesteryl ester hydroperoxide (CEOOH). Little consumption of α-tocopherol was observed in both oxidations. It was found, however, that lipid hydroperoxides were accumulated with time in both oxidations and that the rates of formation of PCOOH and CEOOH in the oxidation by MPO-knockout neutrophils were about 66 and 44% of those by wild-type neutrophils, respectively. The lipid peroxidation was completely inhibited by adding superoxide dismutase (SOD) in both cases. The addition of L-tyrosine and SOD enhanced lipid peroxidation of LDL induced by wild-type neutrophils but not by MPO-knockout ones. These results suggest that, regardless of their MPO activity, neutrophils induce lipid peroxidation of LDL by a superoxide-dependent pathway, and that MPO-catalyzed lipid peroxidation is enhanced by the presence of an appropriate amount of free tyrosine and further enhanced by SOD.

Key words: LDL, lipid peroxidation, MPO-knockout mouse, myeloperoxidase, neutrophil.

The oxidation of LDL has been accepted to play a key role in atherogenesis (*1*, *2*), but the mechanism involved has not been elucidated in detail. For example, the active species that initiates LDL oxidation *in vivo* has not been identified. Extensive *in vitro* studies have been carried out to identify the initiating species of the oxidation of LDL *in vivo* (*3–12*). Myeloperoxidase is considered to be involved in one of the pathways for oxidation *in vivo* (*13–17*). Myeloperoxidase is secreted by activated phagocytes and generates potent microbicidal oxidants in combination with hydrogen peroxide (H_2O_2). Immunohistochemical studies showed co-localization of the enzyme in part with lipid-laden macrophages, which are recognized in the early atherosclerotic lesion (*14*, *15*).

The stimulated phagocytes generate several kinds of reactive oxygen species. Oxygen is converted to the superoxide anion radical ($O_2^{\cdot-}$) by a membrane-associated NADPH oxidase, which dismutases to hydrogen peroxide (H_2O_2). H_2O_2 undergoes the Haber-Weiss reaction leading to generation of a hydroxyl radical (HO$^{\cdot}$). Nitric oxide (NO) is also released from these cells and reacts with $O_2^{\cdot-}$ to give peroxynitrite ($ONOO^-$) (*18*). These reactive oxygen species are capable of inducing the oxidation of LDL *in vitro*.

It has been difficult to evaluate the importance of MPO, partly because MPO-knockout mouse was not available. Recently it has been established (*19*), and the present study was carried out to investigate the importance of MPO in the oxidation of LDL induced by activated neutrophils obtained from wild-type and, for the first time, MPO-knockout mice.

MATERIALS AND METHODS

Materials—Superoxide dismutase (SOD), L-tyrosine, cytochrome *c*, and Hank's balanced salt solution (HBSS) (Ca^{2+}/Mg^{2+}-free), guaiacol, phorbol mirystate acetate (PMA), and *N*ω-nitro-L-arginine methyl ester were obtained from Sigma (St. Louis, MO, USA). Thioglycolate broth was obtained from Difco Lab. (Detroit, MI, USA). 2*R*,4*R*′,8*R*′-α-Tocopherol was a kind gift from Eisai (Tokyo). Mice with a homozygous mutant gene of MPO were obtained by use of gene targeting technique as described previously (*19*). Heterozygous F1 animals were obtained by mating of chimeric animals with C57BL/6. Male mice, 6–8

[1] To whom correspondence should be addressed. Tel: +81-35452-5204, Fax: +81-35452-5201, E-mail: nonoriko@oxygen.rcast.u-tokyo.ac.jp

Abbreviations: ApoB, apolipoprotein B 100; CEOOH, cholesteryl ester hydroperoxide; CTAB, cethyltrimethylammonium bromide; DTPA, diethylenetriamine pentaacetic acid; HBSS, Hank's balanced salt solution; LDL, low density lipoprotein; L-NAME, *N*ω-nitro-L-arginine methyl ester; MPO, myeloperoxidase; PMA, phorbol mirystate acetate; PCOOH, phosphatidylcholine hydroperoxide; SOD, superoxide dismutase.

© 2000 by The Japanese Biochemical Society.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

weeks, with a homozygous mutant of the MPO gene were used in the present study. Male C57BL/6 mice of the same age purchased from Oriental Kobo (Shizuoka) were used as a control wild type. Other chemicals were of the highest grade commercially available.

Preparation of Neutrophils—Mouse peritoneal neutrophils were obtained by following the literature (*19*). Briefly, mice were injected intraperitoneally with 1 ml of 3% fluid thioglycolate medium. After 5 h, peritoneal exudate cells were harvested by peritoneal lavage with 20 ml of phosphate-buffered saline (PBS). Cells were counted with a hemocytometer.

Measurement of MPO Activity and $O2^{-}$ Generation—The cells were washed with HBSS for three times. After centrifugation at 400 ×*g* for 5 m at 4°C, ice-cold 0.02% cethyltrimethylammonium bromide (CTAB) in 50 mM potassium phosphate buffer (KPB) (pH 6.0) was added. The cells were disrupted by sonification at 30 W for 30 s with a Branson Sonifier 250 and centrifuged at 4,000 ×*g* for 20 min at 4°C. The pellet was resuspended in 2 ml of CTAB solution, and its MPO activity was measured by using 14.3 mM guaiacol as a substrate in the presence of 330 μM H_2O_2 (*20*). $O_2^{\cdot-}$ generation was determined as the SOD-inhibitable reduction of cytochrome *c* (*21*). A suspension of 2×10^6 cells/ml HBSS (pH 7.4) was stimulated by the addition of 200 nM PMA in the presence of 50 μM cytochrome *c* at 37°C, and the increase of absorbance at 550 nm was followed.

Oxidation of LDL with Neutrophils—LDL was isolated from human plasma by ultracentrifugation (*22*) and its protein concentration was determined by using the BCA method in kit form as supplied by Pierce (Rockford, IL) with bovine serum albumin as a standard. LDL (0.2 mg protein/ml) was oxidized with neutrophils (2×10^6 cells/ml) in HBSS (Ca^{2+}, Mg^{2+}-free, pH 7.6) containing 100 μM diethylenetriamine pentaacetic acid (DTPA), 1 mg/ml D-glucose, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml soybean trypsin inhibitor at 37°C. Neutrophils were stimulated by adding 200 nM PMA. At an appropriate time, the reaction was stopped by cooling on ice. Cells were pelleted by centrifugation and the supernatant was assayed for lipid oxidation products.

Analysis of Lipid Peroxidation of LDL—The lipid fraction was extracted with 2 volumes of chloroform/methanol (2:1, v/v). α-Tocopherol was analyzed by HPLC on an LC-18 column (Supelco, 4 × 250 mm, 5 μm particle) with an electrochemical detector set at 600 mV. Methanol/*tert*-butyl alcohol (90:10, v/v) containing 50 mM $NaClO_4$ was used as an eluent at a flow rate of 0.8 ml per min. Phosphatidylcholine hydroperoxides (PCOOH) were analyzed by HPLC on an LC-Si column (Supelco, 4 × 250 mm, 5 μm particle) with chemiluminescence and UV detectors at 234 nm as reported previously (*23*). Methanol/40 mM phosphate buffer (90:10 v/v) was used as an eluent. For cholesterol ester hydroperoxides (CEOOH), an LC-8 column (Supelco, 4 × 250 mm, 5 μm particle) with the same detectors and methanol/*tert*-butyl alcohol (95:5 v/v) as an eluent were used.

Assay for Fragmentation of Apolipoprotein B—Intact apolipoprotein B 100 (apoB) before and after oxidation of LDL by PMA-stimulated neutrophils under the same conditions as were used for measurement of lipid peroxidation was assayed by SDS-PAGE as reported previously (*24*). In brief, the isolated apoB (2.5 μg) was applied to SDS-PAGE and the protein bands were stained with Coomassie Brilliant Blue. The bands were evaluated quantitatively by densitometry and the value was expressed as percentage of remaining intact apoB after oxidation against that of time 0 of each sample. Statistical significance was analyzed by student's *t*-test when indicated.

RESULTS

MPO Activity and Generation of $O_2^{\cdot-}$ of Neutrophils of Wild-Type and MPO-Knockout Mice—Measurement of MPO activity by the guaiacol oxidation method showed clearly that peritoneal neutrophils of MPO-knockout mice were completely deficient in MPO activity (Fig. 1). For the wild-type neutrophils, calculation using an extinction coefficient at 470 nm of 26.6 $mM^{-1}\,cm^{-1}$ (*20*) gave MPO activity of 0.41 ± 0.046 units/2×10^6 cells. The rate of generation of $O_2^{\cdot-}$ from neutrophils of wild-type and MPO-knockout mice after activation by PMA was measured as SOD-inhibitable cytochrome *c* reduction. The rate of generation of $O_2^{\cdot-}$ from wild-type neutrophils and MPO-deficient ones was 18 ± 2.5 nmol/m/2×10^6 cells and 12 ± 2.0 nmol/m/2×10^6 cells, respectively (Fig. 2). MPO-knockout neutrophils yielded $O_2^{\cdot-}$ at a lower rate than the wild type ($p < 0.005$).

Oxidation of LDL with Neutrophils of Wild Type and MPO Knockout Mice—Incubation of human LDL with

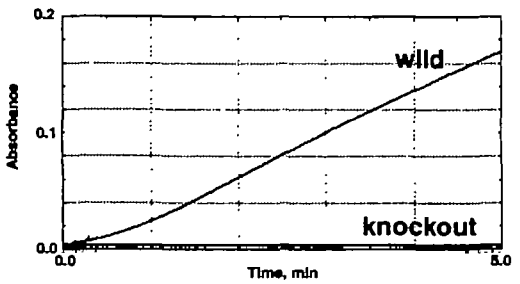


Fig. 1. **Myeloperoxidase activity of neutrophils of wild-type mice and MPO-knockout mice.** Myeloperoxidase activity in cell pellets (2×10^6) was assessed at 25°C by measuring the increase in absorbance of tetraguaiacol at 470 nm due to the catalysis of guaiacol by myeloperoxidase in the presence of 330 μM H_2O_2. Results are representative of more than six independent experiments.

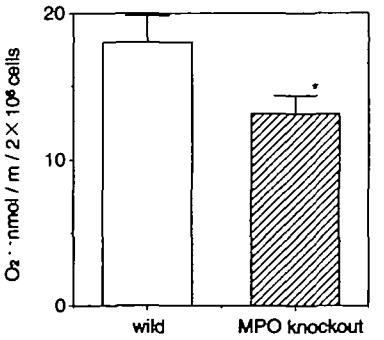


Fig. 2. **Generation of $O_2^{\cdot-}$ from neutrophils of wild type and MPO-knockout mice.** The rate of $O_2^{\cdot-}$ generation from neutrophils was measured from the initial rate of reduction of cytochrome c after the addition of 200 nM PMA at 37°C. Results are means ± SD for six samples obtained from different mice. The difference in $O_2^{\cdot-}$ generation between wild-type and knockout neutrophils was significant ($p < 0.005$) as calculated with an unpaired *t* test.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

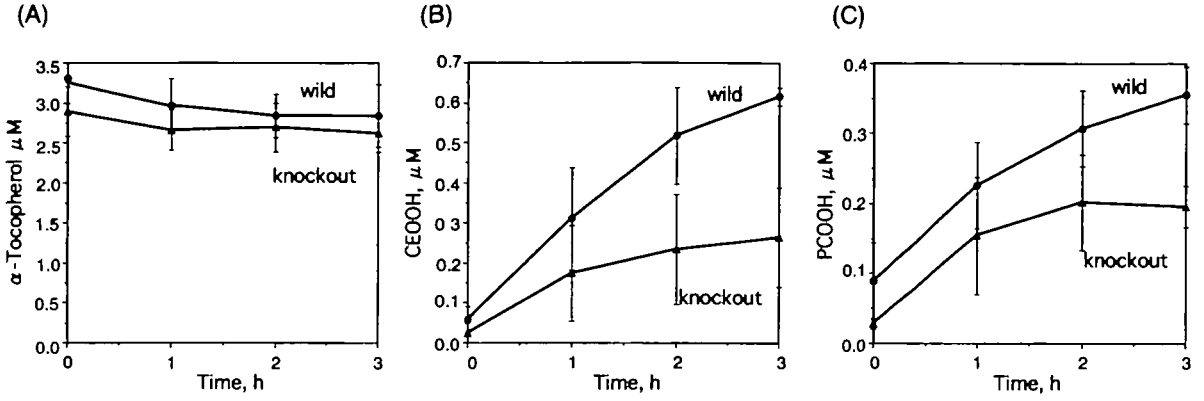


Fig. 3. **Oxidation of LDL by PMA-stimulated neutrophils of wild-type and MPO-knockout mice.** Human LDL (0.2 mg protein/ml) was incubated with neutrophils of either wild-type or MPO-knockout mice in HBSS (Ca^{2+}, Mg^{2+}-free, pH 7.6) at 37°C. Cells were stimulated by 200 nM PMA. The consumption of α-tocopherol (A) and formation of hydroperoxide of cholesteryl ester (CEOOH) (B) and phosphatidylcholine (PCOOH) (C) were measured as described in "MATERIALS AND METHODS". Results are means ± SD for independent triplicate experiments.

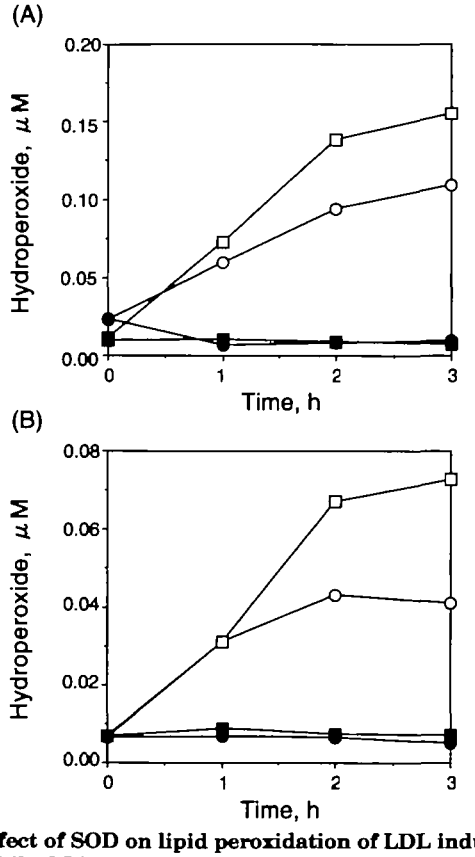


Fig. 4. **Effect of SOD on lipid peroxidation of LDL induced by neutrophils.** LDL was oxidized with PMA-stimulated neutrophils of (A) wild-type and (B) MPO-knockout mice as shown in Fig. 3 in the absence (open mark) or presence (closed mark) of 0.5 μM SOD. The formation of PCOOH (circle) and CEOOH (square) was measured. Results are representative of more than three independent experiments.

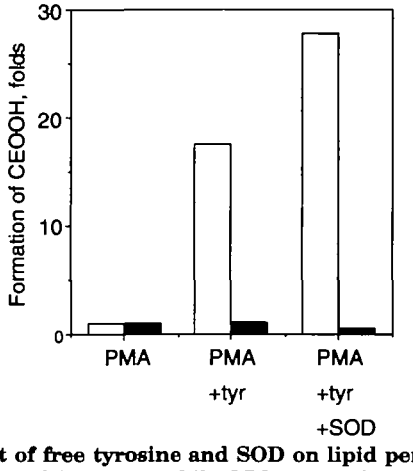


Fig. 5. **Effect of free tyrosine and SOD on lipid peroxidation of LDL induced by neutrophils.** LDL was oxidized with PMA-stimulated neutrophils of wild-type (open bar) and MPO-knockout (slash bar) mice as shown in Fig. 3 for 3 h in the absence or presence of 0.5 mM free L-tyrosine and/or 0.5 μM SOD. The absolute values of 1-fold for wild-type and knockout mice were 0.62 and 0.35 μM, respectively.

PMA-activated mouse neutrophils induced lipid peroxidation of LDL giving CEOOH and PCOOH, but little consumption of α-tocopherol was observed (Fig. 3). As in free radical-mediated oxidation, the oxidation of LDL induced by neutrophils gave more CEOOH than PCOOH. When LDL was incubated with neutrophils without PMA, formation of lipid hydroperoxide was not observed (data not shown). The neutrophils derived from MPO-knockout mice did induce the oxidation of LDL in the presence of PMA, but the extent of lipid peroxidation was lower than that of the wild type (Fig. 3). The formation of lipid hydroperoxides in the oxidation of LDL with MPO-deficient neutrophils reached the plateau faster. As clearly shown in Fig. 4, both oxidations of LDL were completely inhibited by the addition of SOD, which suggests that the oxidation of LDL by neutrophils, regardless of the MPO activity, is dependent on $O_2^{\cdot-}$. It has been reported (*25*) that the tyrosyl radical is important in MPO-catalyzed oxidation of LDL. The addition of L-tyrosine to the oxidation reaction medium containing wild-type neutrophils caused a marked increase in lipid peroxidation (Fig. 5). Furthermore, SOD increased the tyro-

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

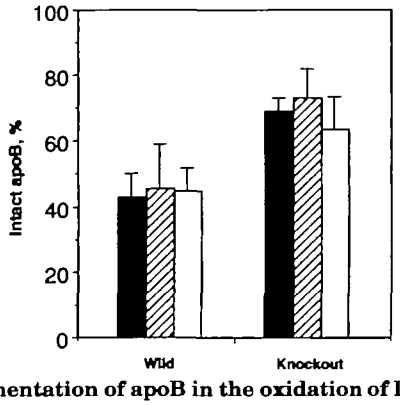


Fig. 6. **Fragmentation of apoB in the oxidation of LDL by neutrophils assayed by SDS-PAGE.** LDL was oxidized with PMA-stimulated neutrophils of wild-type and MPO-knockout mice as shown in Fig. 3 for 3 h in the absence (solid) or presence of 0.5 μM SOD (slash) or 200 μM L-NAME (open). The extent of fragmentation was expressed by percentage of remaining intact apoB to native LDL (100%).

sine-dependent oxidation of LDL. In contrast, lipid peroxidation of LDL induced by MPO-deficient neutorophils was enhanced by the addition of neither tyrosine nor both tyrosine and SOD (Fig. 5).

To investigate the contribution of peroxynitrite to lipid peroxidation of LDL, L-NAME, which is an inhibitor of inducible nitric oxide synthase (i-NOS), was added into the oxidation system. It was found that L-NAME did not affect the consumption of α-tocopherol and formation of lipid hydroperoxides in the oxidation induced by both types of neutrophils (data not shown).

Fragmentation of apoB by Neutrophils from Wild-Type and MPO-Knockout Mice—The loss of intact apoB by incubation of LDL with neutrophils derived from wild-type and MPO-knockout mice was examined by SDS-PAGE. The fragmentation of apoB did not occur in LDL by incubation without neutrophils or non-stimulated neutrophils. After 3 h of incubation, the loss of intact apoB due to fragmentation induced by wild-type neutrophils was larger than that by MPO-knockout neutrophils (Fig. 6). However, no significant difference in the pattern of SDS-PAGE, such as the appearance of new bands with smaller molecular weight, was observed between them. In contrast to lipid peroxidation, neither SOD nor L-NAME attenuated the extent of fragmentation of apoB. The addition of L-tyrosine and L-tyrosine/SOD did not enhance the fragmentation of apoB induced by even wild-type neutrophils (data not shown).

DISCUSSION

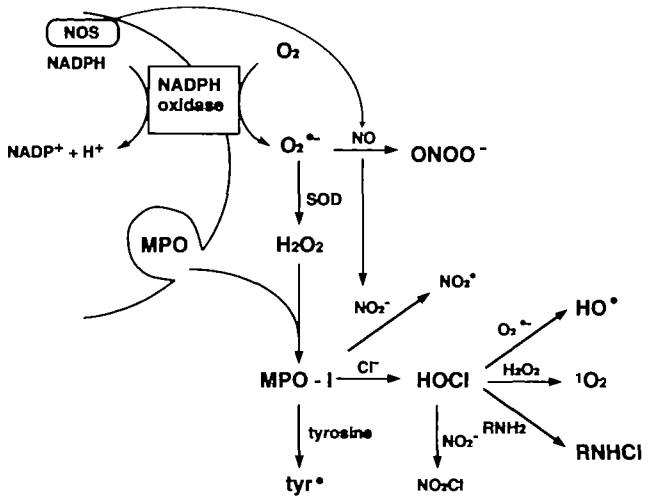
Myeloperoxidase is a strong candidate for an initiating species of LDL oxidation *in vivo*. The contribution of MPO to the oxidation of LDL caused by phagocytes and its mechanism have been studied. Immunohistochemical studies showing co-localization of the enzyme with lipid-laden macrophages in atherosclerotic lesion (*14*, *15*) support such a contribution. Moreover, elevation of 3-chlorotyrosine, which is a specific marker of MPO-catalyzed oxidation, was recognized in LDL isolated from human atherosclerotic intima (*16*). Hiramatsu *et al.* (*26*) reported that PMA-stimulated human monocytes induced lipid peroxidation of LDL, which was inhibited by SOD. Furthermore, they showed that monocytes from a patient with MPO deficiency caused oxidation of the lipoprotein equivalent to that observed with normal cells. Similar results were obtained in the present study, in which lipid peroxidation of LDL was induced by PMA-stimulated neutrophils from MPO-knockout mice. However, the extent of lipid peroxidation of LDL induced by MPO-knockout neutrophils was less than that by wild-type neutrophils.

It was speculated that the difference in the extent of the oxidation might be due to a difference in MPO activity. According to the mechanisms for the oxidation by neutrophils summarized in Scheme 1, it was expected that SOD would enhance LDL oxidation by wild-type neutrophils due to generation of H_2O_2, which is required for MPO-catalyzed oxidation. In contrast to our expectation, SOD inhibited both oxidations induced by wild-type and MPO-knockout neutrophils almost completely. We confirmed that SOD did not affect lipid peroxidation of LDL induced by oxidation reagent containing isolated MPO, H_2O_2, and chloride (data not shown). These results are different from that observed in the oxidation of LDL with human circulating neutrophils (*25*), in which SOD did not inhibit lipid peroxidation but rather enhanced it slightly. MPO activity of neutrophils from wild mice used in the present study was 0.41 ± 0.046 unit/2 × 10^6 cells , which was one-tenth of that of human neutrophils. Therefore, the contribution of MPO to the oxidation of LDL induced by mouse neutrophils might be small. Thus the discrepancy in the extent of oxidation between wild and MPO-knockout was not explained by MPO-catalyzed oxidation. These results clearly show that lipid peroxidation of LDL induced by PMA-stimulated neutrophils of mouse is dependent on O_2^- generation rather than MPO.

The next candidate for induction of lipid peroxidation of LDL by mouse neutrophils is reactive nitrogen species such as peroxynitrite, which is formed by a diffusion-limited fast reaction between O_2^- and NO (*27*). It has recently been reported that MPO can generate reactive nitrogen intermediates from NO_2^-, a major decomposition product of NO (*27–30*). As shown in Scheme 1, the one-electron oxidation of NO_2^- by MPO compound I (MPO-I) gives NO_2. Another pathway for the generation of a nitrogen intermediate is the reaction of NO_2^- with HOCl to yield NO_2Cl. However, the fact that L-NAME, an inhibitor of i-NOS, showed no effect on the formation of lipid hydroperoxides suggests that these reactive nitrogen species make little or no contribution to neutrophil-induced lipid peroxidation of LDL.

The addition of L-tyrosine to the reaction medium resulted in a marked increase of lipid peroxidation of LDL by wild-type neutrophils but not by MPO-knockout neutrophils. These results showed that MPO in wild-type neutrophils was still active but was able to induce marked oxidation of LDL in the presence of an appropriate amount of free tyrosine. Formation of tyrosyl radicals should have a key role in MPO-catalyzed lipid peroxidation of LDL (*8*, *25*, *31*, *32*). The enhancement of lipid peroxidation by the addition of SOD in the presence of tyrosine observed in the oxidation by wild-type neutrophils may be explained by increased formation of H_2O_2 by dismutation of O_2^- by SOD and subsequent formation of MPO-I, which converts tyrosine to tyrosyl radicals to induce lipid peroxidation (Scheme 1).

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012



Scheme 1. **Proposed mechanism for generation of reactive oxygen and nitrogen species from activated neutrophils.** MPO compound I (MPO-I), a complex of MPO, and H_2O_2, generates HOCl in the presence of Cl^-. MPO-I converts tyrosine to tyrosyl radical (tyr). NO and NO_2^- provide other pathways for generation of reactive oxidizing and nitrating species.

Hazell *et al.* (*33*) reported that lysine residues were a major target of OCl^- rather than lipids in LDL. It is also reported that HOCl damaged proteins by reaction with amino acid side-chains or backbone cleavage (*34*). The reaction of OCl^- with amino acid proceeds independently of tyrosine concentration (Scheme 1), and chloramines are formed through the reaction of HOCl with protein amine groups (*35*). It is interesting to compare the extent of apoB modification by wild-type and MPO-knockout neutrophils. Fragmentation of apoB was observed in LDL incubated with PMA-stimulated neutrophils regardless of MPO activity. The extent of the fragmentation by wild-type neutrophils was larger than that by MPO-knockout ones. Little effect of L-NAME on the fragmentation suggests little contribution of reactive nitrogen species to the fragmentation of apoB in both cases. The fact that SOD did not affect the fragmentation might explain in part the difference between wild-type and knockout neutrophils by MPO activity. Furthermore, the addition of L-tyrosine did not enhance the fragmentation by wild-type neutrophils, suggesting that the fragmentation of apoB does not proceed by tyrosine-dependent pathway (*35*). Further investigation into the mechanism of the fragmentation of apoB induced by PMA-stimulated mouse neutrophils is needed.

Together, these findings indicate that, except in the presence of tyrosine, MPO does not have a major role in oxidative modification of LDL induced by mouse neutrophils.

REFERENCES

1. Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L. (1989) Beyond cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320**, 915–924
2. Parthasarathy, S. and Rankin, S.M. (1992) The role of oxidized LDL in atherogenesis. *Prog. Lipid Res.* **31**, 127–143
3. Heinecke, J.W. (1987) Free radical modification of low-density lipoprotein: mechanisms and biological consequences. *Free Radical Biol. Med.* **3**, 65–73
4. Jurgens, G., Hoff, H.F., Chisolm, G.M., and Esterbauer, H. (1987) Modification of human serum low density lipoprotein by oxidation-characterization and pathophysiological implications. *Chem. Phys. Lipid.* **45**, 315–336
5. Steinbrecher, U.P., Zhang, H.F., and Lougheed, M. (1990) Role of oxidatively modified LDL in atherosclerosis. *Free Radical Biol Med.* **9**, 155–168
6. Witztum, J.L. and Steinberg, D. (1991) Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* **88**, 1785–1792
7. Penn, M.S. and Chisolm, G.M. (1994) Oxidized lipoproteins, altered cell function and atherosclerosis. *Atherosclerosis* **108**, suppl. S21–S29
8. Berliner, J.A. and Heinecke, J.W. (1996) The role of oxidized lipoproteins in atherogenesis. *Free Radical Biol. Med.* **20**, 707–727
9. Ames, B.N., Shigenaga, M.K., and Hagan, T.M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* **90**, 7915–7922
10. Stadtman, E.R. (1992) Protein oxidation and aging. *Science* **257**, 1220–1224
11. Baynes, J.W. (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes* **40**, 405–412
12. Kuhn, H., Belkner, J., Zaiss, S., Fahrenklemper, T., and Wohlfeil, S. (1994) Involvement of 15-lipoxygenase in early stages of atherogenesis *J. Exp. Med.* **179**, 1903–1911
13. Gordon, A.F., Armand, J.M., Edwin, L.B., and Heinecke, J.W. (1993) Oxidative tyrosylation of high density lipoprotein by peroxidase enhances cholesterol removal from cultured fobroblasts and macrophages foam cells. *Proc. Natl. Acad. Sci. USA* **90**, 6631–6635
14. Daugherty, A., Dunn, J.L., Rateri, D.L., and Heinecke, J.W. (1994) Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J. Clin. Invest.* **94**, 437–444
15. Hazell, L.J., Armol, L., Flfoers, L., Waeg, G., Malle, E., and Stocker, R. (1996) Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *J. Clin. Invest.* **97**, 1535–1544
16. Hazen, S. and Heinecke, J.W. (1997) 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J. Clin. Invest.* **99**, 2075–2081
17. Leewenburg, C., Rasmussen, J.E., Hsu, F.F., Mueller, D.M., Pennathur, S., and Heinecke, J.W. (1997) Mass spectrometric quantification of markers for protein oxidation by tyrosyl radical, copper, and hydroxyl radical in low density lipoprotein isolated from human atherosclerotic plaques. *J. Biol. Chem.* **272**, 3520–3526
18. Beckman, J. and Crow, J.P. (1993) Pathological implications of nitric oxide, superoxide and peroxynitrite formation. *Biochem. Soc. Trans.* **21**, 330–334
19. Aratani, Y., Koyama, H., Nyui, S., Suzuki, K., Kura, F., and Maeda, N. (1999) Severe impiarment in early host defence against Canadida albica in mice deficient in myeloperoxidase. *Infect. Immun.* **67**, 1828–1836
20. Chance, B. and Maehly, A.C. (1955) Assay of catalases and peroxidases in *Methods in Enzymology* (Sidney, P.C. and Nathan, O.K., eds.) Vol. 2, pp. 764–775, Academic Press, New York
21. Fridovich, I. (1985) Cytochrome *c* in *Hand Book of Methods for Oxygen Radical Research* (Greemwald, R.A., ed.) pp. 121–122, CRC Press, Boca Raton, FL
22. Ramos, P., Gieseg, S.P., Schuster, B., and Esterbauer H. (1995) Effect of temperature and phase transition on oxidation resistance of low density lipoprotein. *J. Lipid Res.* **36**, 2113–2128
23. Yamamoto, Y. (1994) Chemiluminescence-based high-performance liquid chromatography assay of lipid hydroperoxides in *Methods in Enzymology* (Packer, L., ed.) Vol. 233, pp. 319–324, Academic Press, New York
24. Noguchi, N. and Niki E. (1994) Apolipoprotein B protein oxidation in low-density lipoproteins in *Methods in Enzymology* (Packer, L., ed.) Vol. 233, pp. 490–494, Academic Press, New York

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

25. Savenkova, M.L., Mueller DM., and Heinecke JW. (1994) Tyrosyl radical generated by myeloperoxidase is a physiological catalyst for the initiation of lipid peroxidation in low density lipoprotein. *J. Biol. Chem.* **269**, 20394–20400
26. Hiramatsu, K., Rosen, H., Heinecke, J.W., Wolfbauer., G., and Chait, A. (1987) Superoxide initiates oxidation of low density lipoprotein by human monocytes. *Arteriosclerosis* **7**, 55–60
27. Beckman, J. (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* **87**, 1620–1624
28. Byun, J., Mueller D.M., Fabjan, J.S., and Heinecke, J.W. (1999) Nitrogen dioxide radical generated by the myeloperoxidase-hydrogen peroxide-nitrite system promotes lipid peroxidation of low density lipoprotein. *FEBS Lett.* **455**, 243–246
29. Hazen, S.L., Zhang, R., Shen, Z., Wu, W., Podrez, E.A., MacPherson, J.C., Schmitt, D., Mitra, S.N., Mukhopadhyay, C., Chen, Y., Cohen, P.A., Hoff, H.F., and Abu-Soud, H.M. (1999) Formation of nitric oxide-derived oxidants by myeloperoxidase in monocytes. Pathways for monocyte-mediated protein nitration and lipid peroxidation *in vivo*. *Circ. Res.* **85**, 950–958
30. Chisolm, G.M., Hazen, S.L., Fox, P.L., and Cathcart, M.K. (1999) The oxidation of lipoproteins by monocytes-macrophages. *J. Biol. Chem.* **274**, 25959–25962
31. Kapiotis, S., Sengoelge, G., Hermann, M., Held, I., Seelos, C., and Gmeiner, B.M. (1997) Paracetamol catalyzes myeloperoxidase-initiated lipid oxidation in LDL. *Arterioscl. Thromb. Vasc. Biol.* **17**, 2855–2860
32. Hazen, S.L., Heller, J., Hsu, F.F., d'Avignon, A., and Heinecke, J.W. (1999) Synthesis, isolation, and characterization of the adduct formed in the reaction of *p*-hydroxyphenylacetaldehyde with the amino headgroup of phosphatidylethanolamine and phosphatidylserine. *Chem. Res. Toxicol.* **12**, 19–27
33. Hazell, L.J., van den Berg, J.M., and Stocker, R. (1994) Oxidation of low density lipoprotein by hypochlorite causes aggregation that is mediated by modification of lysine residues rather than lipid oxidation. *Biochem. J.* **302**, 297–304
34. Hawkins, C.L. and Davis, M.J. (1998) Hypochlorite-induced damage to proteins: formation of nitrogen-centred radicals from lysine residues and their role in protein fragmentation. *Biochem. J.* **332**, 617–625
35. Hawkins, C.L. and Davis, M.J. (1999) Hypochlorite-induced oxidation of proteins in plasma: formation of chloramines and nitrogen-centred radicals and their role in protein fragmentation. *Biochem. J.* **340**, 539–548

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012