ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 3 & 4, 2005

Forum Review

Essential Roles of Lipoxygenases in LDL Oxidation and Development of Atherosclerosis

YOSHITAKA TAKAHASHI,[1] HONG ZHU,[2] and TANIHIRO YOSHIMOTO[2]

ABSTRACT

Oxidative modification of low-density lipoprotein (LDL) is one of the critical steps for the development of atherosclerosis. Accumulating studies have indicated that 12/15-lipoxygenase highly expressed in macrophages plays an essential role in the oxidation of circulating LDL. It has been demonstrated that LDL needs to bind the LDL receptor-related protein (LRP), a cell-surface receptor, prior to its oxidation by 12/15-lipoxygenase expressed in macrophages. LRP is suggested to mediate the selective transfer of cholesteryl ester in LDL to the plasma membrane of macrophages without endocytosis and degradation of the LDL particle. At the same time, binding of LDL to LRP translocates the 12/15-lipoxygenase from the cytosol to the plasma membrane. It is also demonstrated that 5-lipoxygenase localized in macrophages generates leukotrienes, which exhibit strong proinflammatory activities in cardiovascular tissues and contribute to lesion development. Therefore, the inhibition of these lipoxygenases may be effective in the prevention and treatment of the inflammatory diseases. *Antioxid. Redox Signal.* 7, 425–431.

INTRODUCTION

LIPOXYGENASE is the enzyme incorporating one molecule of oxygen in a regiospecific and stereospecific manner into unsaturated fatty acids. In mammalian tissues, there are four lipoxygenases according to the position of oxygenation in arachidonic acid, namely, 5-, 8-, 12-, and 15-lipoxygenases (6, 15, 31, 59, 68). The oxygenation product is stereospecific with *S*-configuration in most mammalian lipoxygenases (Fig. 1) (6, 68). The produced hydroperoxy acid [hydroperoxyeicosatetraenoic acid (HPETE)] is immediately reduced to hydroxy acid [hydroxyeicosatetraenoic acid (HETE)] in the cells by peroxidases such as glutathione peroxidase (59, 71). The 5-lipoxygenase is known to initiate the reaction to produce strong proinflammatory leukotrienes from arachidonic acid (15, 51). On the other hand, a number of isozymes are found in 12- and 15-lipoxygenases as shown in Table 1, and they form a multigene family (14, 31, 71). Among the enzymes, leukocyte-type 12-lipoxygenase and 15-lipoxygenase-1 share enzymologically notable common features that are not observed in other lipoxygenases. First, both enzymes show a broad substrate specificity and oxygenate not only free fatty acids such as arachidonic and linoleic acids, but also esterified fatty acids such as phospholipid, cholesteryl ester, and cholesteryl ester in the low-density lipoprotein (LDL) particle (31, 68). Second, the tissue distribution of the two enzymes is similar and seems to depend on species difference (14, 71). For example, mouse, rat, porcine, and bovine macrophages have leukocyte 12-lipoxygenase, whereas human and rabbit macrophages express 15-lipoxygenase-1. Similarly, leukocyte-type 12-lipoxygenase is known to be expressed in bovine and porcine trachea, whereas the expression of 15-lipoxygenase-1 is observed in human and rabbit trachea. The amino acid sequence homology of the two enzymes is 80–90%, and phylogenetic tree analysis reveals that these enzymes are homologues of each other (14, 31). Therefore, the two enzymes are collectively referred to as 12/15-lipoxygenase (14).

Oxidative modification of LDL is the essential step for LDL to be atherogenic (54, 65). As demonstrated by Brown

[1]Department of Nutritional Science, Faculty of Health and Welfare Science, Okayama Prefectural University, Soja, Okayama, Japan.
[2]Department of Molecular Pharmacology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan.

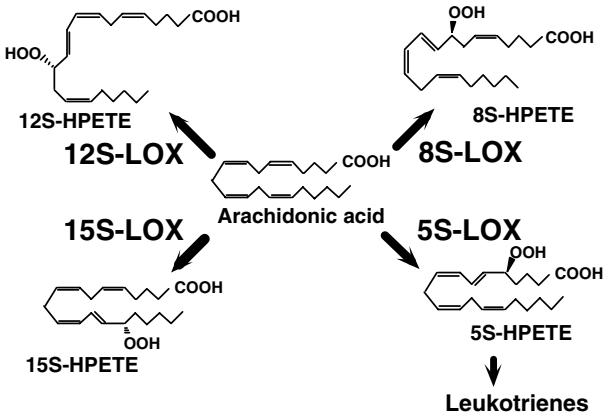

FIG. 1. Stereospecific oxygenation of arachidonic acid by lipoxygenases. The hydroperoxy products with *S*-configuration are generated by four lipoxygenases. LOX, lipoxygenase.

and Goldstein, denatured LDL, which is later shown to be oxidized LDL *in vivo*, is incorporated unlimitedly into macrophages via scavenger receptors, and the accumulation of lipids in macrophages leads to foam cell formation, which plays a key role in atherogenesis (7). LDL oxidation could occur by both cell-dependent and -independent processes (56). The former involves endothelial cells (23, 43), smooth muscle cells (22), fibroblasts (55), and monocytes/macrophages (36, 46). In the latter process, transient metals, hemin, thiols, ceruloplasmin, and many other catalysts are involved (4). As 12/15-lipoxygenase is shown to oxidize LDL, the enzyme has been one of the candidates for the initiation of the atherogenic process (16).

In this review, the detailed biochemical mechanism of the oxidation of circulating LDL by 12/15-lipoxygenase expressed in macrophages will be described. The inflammatory process is also discussed in the atherosclerotic lesion where 5- and 12/15-lipoxygenases in macrophages are implicated.

12/15-LIPOXYGENASE IS ESSENTIAL FOR LDL OXIDATION BY MACROPHAGES

There have been many studies suggesting involvement of 12/15-lipoxygenase in LDL oxidation. Purified 12/15-lipoxygenase oxygenates cholesteryl linoleate in the LDL particle when incubated with LDL (2, 32). An increase in electrophoretic mobility that is postulated to be due to the oxidation of lysine residues of apolipoprotein B-100 is observed in LDL incubated with purified 12/15-lipoxygenase, suggesting that enzymatic oxygenation initiates the formation of oxidized LDL (2, 32). Macrophages are cells expressing a high level of 12/15-lipoxygenase and are capable of *in vitro* LDL oxidation in culture medium free of metal ion additives (10). The 12/15-lipoxygenase expressed in monocytes/macrophages accumulating in atherosclerotic lesions is shown to colocalize with oxidized LDL (69, 70). Because 12/15-lipoxygenase produces stereospecifically oxygenated fatty acids, the occurrence of such products in the lesions strongly suggests that the enzyme plays a certain role in the progress of atherosclerosis *in vivo* (33). Several reports demonstrate that development of atherosclerosis in 12/15-lipoxygenase-knockout mice is diminished, indicating that the enzyme plays a critical role in the progress of the disease (11, 21, 57). A contradictory study suggests that atherogenesis is suppressed in 12/15-lipoxygenase transgenic rabbit (15, 52).

The essential role of 12/15-lipoxygenase in the generation of oxidized LDL by macrophages was finally established using a mouse macrophage-like cell line, J774A.1, in which endogenous enzyme activity was hardly detected (45). When incubated with LDL, J774A.1 cells overexpressing 12/15-lipoxygenase generated oxidized LDL; however, wild-type cells, as well as mock-transfected cells, did not oxidize LDL added into the culture medium. The oxidized LDL generated by 12/15-lipoxygenase-expressing cells was recognized by scavenger receptors as assessed by macrophage degradation assay (50). This means the enzyme-expressing cells generated

TABLE 1. ISOZYMES OF 12- AND 15-LIPOXYGENASES

	Reactivity as substrate		
Enzyme isoforms	*Free fatty acids*	*Esterified fatty acids*	*Tissue distribution (species)*
Leukocyte-type 12*S*-LOX	Many unsaturated fatty acids such as linoleic and arachidonic acids	Phosphatidylcholine, cholesteryl ester, cholesteryl ester in LDL	Leukocyte, trachea, macrophage, pineal gland, reticulocyte (mouse, pig, cow, rat, rabbit)
15*S*-LOX-1	Many unsaturated fatty acids such as linoleic and arachidonic acids	Phosphatidylcholine, cholesteryl ester, cholesteryl ester in LDL	Reticulocyte, trachea, leukocyte, macrophage, (human, rabbit)
Platelet-type 12*S*-LOX	Arachidonic acid >> linoleic acid	Almost inactive	Platelet, skin, cornea (human, mouse, cow)
Epidermis-type 12*S*-LOX	Arachidonic acid >> linoleic acid	Unknown	Skin (mouse)
15*S*-LOX-2	Arachidonic acid >> linoleic acid	Unknown	Skin, prostate, cornea (human, cow)
12*R*-LOX	Arachidonic acid >> linoleic acid	Methyl arachidonate	Skin, tonsil (human, mouse)

Listed are isozymes of 12- and 15-lipoxygenases for which cDNA is cloned, the reactivity with unsaturated fatty acids, and the tissue distribution. LOX, lipoxygenase.

highly oxidized LDL (20). Detailed analysis of the structure of the oxygenated linoleate moiety esterified to cholesterol in the oxidized LDL particle revealed that the major product was 13*S*-hydroxyoctadeca-9*Z*,11*E*-dienoic acid, although a significant amount of 13*R*-derivatives was also detected (50). The maximum LDL oxidation was observed ~12 h after starting incubation of LDL with 12/15-lipoxygenase-expressing cells (50). However, the 12/15-lipoxygenase undergoes rapid suicidal inactivation, and the enzyme was actually shown to be inactivated during LDL interaction under physiological conditions (3). These results taken together strongly indicate that the 12/15-lipoxygenase in macrophage initiates the oxidation process of LDL by producing reactive hydroperoxides in the LDL particle, and induces subsequent nonenzymatic radical chain reactions to generate highly oxidized LDL, which is recognized by scavenger receptors.

BIOCHEMICAL ROLES OF LDL RECEPTOR-RELATED PROTEIN (LRP) IN 12/15-LIPOXYGENASE-MEDIATED LDL OXIDATION BY MACROPHAGES

The stereospecific oxygenation of the linoleate moiety of cholesteryl ester in LDL by the 12/15-lipoxygenase-expressing cells suggests the direct interaction of the enzyme with extracellular LDL (50). Secretion or leakage of the enzyme is ruled out, because no enzyme activity is detected in the culture medium (50). So far as is known, three receptors that can bind to native LDL are expressed on the surface of enzyme-expressing J774A.1 macrophage-like cells: the LDL receptor, LRP, and the scavenger receptor class B type I (SR-BI) (25, 67). The LDL receptor is not expressed in atherosclerotic lesions (25) and is not required in the cell-mediated LDL oxidation as shown by *in vitro* experiments (9) and LDL receptor-deficient mouse studies (21, 60). In contrast, LRP and SR-BI are expressed on the surface of macrophages in the lesions (25, 40).

It was established that LRP was required for 12/15-lipoxygenase-mediated LDL oxidation by macrophages (67). We demonstrated that cell-mediated LDL oxidation was reduced by pretreatment of the enzyme-expressing cells with antisense oligodeoxyribonucleotides against LRP, but not by those against SR-BI or the LDL receptor, which suppressed the expression of each receptor. An anti-LRP antibody inhibited the cell-mediated LDL oxidation by macrophages, as well as the enzyme-expressing cells (67).

The LDL is processed by the LDL receptor via receptor endocytosis, and cholesteryl ester in the LDL particle is delivered to lysozymes where it is degraded (7). In contrast, it is proposed that the binding of LDL to LRP selectively transfers the cholesteryl ester from LDL to the plasma membrane without endocytosis and degradation of the LDL particle (58; Takahashi *et al.* unpublished observations). For the efficient enzymatic oxygenation of the cholesteryl ester transferred to the plasma membrane via LRP, the 12/15-lipoxygenase itself should also be localized in the plasma membrane. However, the 12/15-lipoxygenase is predominantly localized in cytoplasm, but not in the membranes, in various cells including macrophages (39, 71).

The translocation of 12/15-lipoxygenase from cytoplasm to the plasma membrane was demonstrated in the enzyme-expressing cells and macrophages treated with LDL (73). Western blot using an anti-12/15-lipoxygenase antibody revealed that the enzyme associated with membranes reached a maximum in 15 min after LDL treatment of macrophages with a concomitant decrease in the cytosolic enzyme (Fig. 2). Immunocytochemical analysis showed that the plasma membrane was the major site of the enzyme translocation by the LDL treatment. The antibody against LRP blocked the translocation, indicating that LRP mediated this process. An enzyme translocation inhibitor suppressed the LDL oxidation caused by the 12/15-lipoxygenase-expressing cells (73). These results taken together strongly suggest that cholesteryl ester selectively transferred from the LDL particle to the plasma membrane is oxygenated by 12/15-lipoxygenase translocated to this membrane. The mechanism of translocation of 12/15-

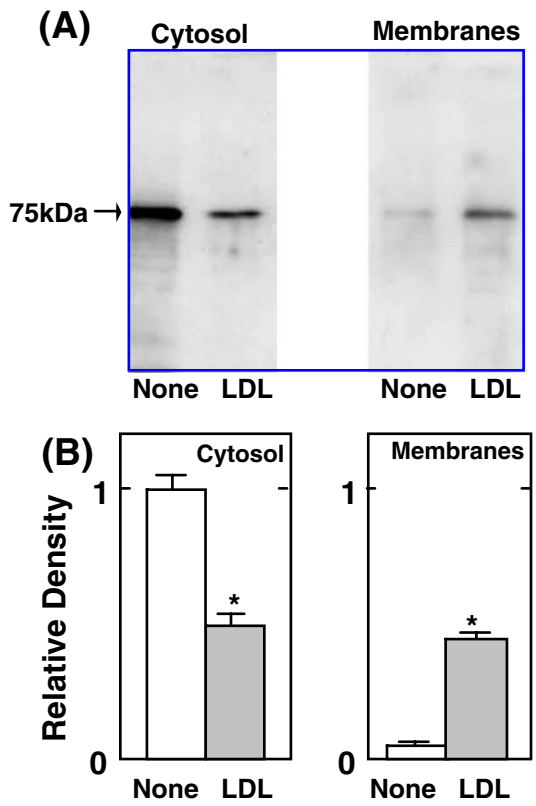

FIG. 2. LDL treatment causes membrane translocation of 12/15-lipoxygenase in peritoneal macrophages. (**A**) Peritoneal macrophages were treated with or without LDL for 15 min, disrupted by sonication, and ultracentrifuged to prepare cytosol and membrane fractions. Each fraction at 10 μg of protein was analyzed by western blot using an anti-12/15-lipoxygenase antibody. (**B**) The density of the bands was quantified by densitometric analysis. *$p < 0.001$.

lipoxygenase in macrophages is not known (39), although the N-terminal C2-like domain is proposed to be responsible for the enzyme binding to the membrane phospholipids in a calcium-dependent way (61). Site-directed mutagenesis of surface-exposed hydrophobic amino acids to negatively charged residues impaired calcium-dependent membrane binding of the enzyme, suggesting that the enzyme associates to biomembranes via hydrophobic interaction between these amino acid side chains and membrane lipids supported by calcium (62). Further investigation is necessary to elucidate the signal transduction pathway for calcium release by LDL binding to LRP. The mechanism of the efflux of oxygenated cholesteryl ester to the LDL particle is not yet known. The selective uptake of cholesteryl ester from high-density lipoprotein in the plasma membrane is mediated by SR-BI (1). This receptor is shown to mediate cholesterol efflux to high-density lipoprotein (26, 27). It is possible that LRP also mediates the reuptake of oxygenated cholesteryl ester from plasma membrane to the LDL particle. Figure 3 shows the postulated mechanism of LRP-mediated LDL oxidation by 12/15-lipoxygenase in macrophages.

LRP is a cell-surface multifunctional receptor capable of binding a variety of ligands and is postulated to participate in a number of diverse pathophysiological processes, including atherosclerosis, fibrinolysis, infection, neuronal degeneration, and apoptosis (17, 24, 64). LRP may play a role in lipoprotein homeostasis, as LRP expressed in liver is reported as a remnant clearance receptor using LRP-disrupted mice in a liver-specific manner (48). Furthermore, a role of LRP in protecting vascular wall integrity and preventing atherosclerosis by suppressing platelet-derived growth factor receptor signaling is proposed using mice in which LRP in vascular smooth muscle cells is inactivated (5). The *in vivo* overall effect of LRP on the progress of atherosclerosis needs further investigation.

INFLAMMATORY PROCESS OF ATHEROGENIC LESIONS INVOLVES LIPOXYGENASES

It is postulated that atherosclerosis is a chronic inflammatory process of the arterial wall (35, 49). Among lipoxygenases, 5-lipoxygenase is a rate-limiting enzyme producing leukotrienes, which are chemical mediators in inflammation (51). Bioactivities of leukotrienes in the progress of atherosclerosis might include activation of polymorphonuclear leukocytes and monocytes/macrophages (12), stimulation of arterial wall smooth muscle cell contraction (8) and chemotaxis (28), and induction of intimal thickening (19, 30) and inflammatory cytokines (38). Transcripts of 5-lipoxygenase, leukotriene A_4 hydrolase, leukotriene C_4 synthase, and four leukotriene receptors are expressed in human atherosclerotic lesions (53). 5-Lipoxygenase is abundantly expressed in human monocytes/macrophages (66), and the number of the 5-lipoxygenase-expressing cells increases in atherosclerotic lesions during transition from early to advanced stages (53). A mutation in 5-lipoxygenase that results in reduced enzyme activity is found in atherosclerosis-resistant CAST/CON6 mice (34). A knockout study identifies 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice (37). These results indicate that 5-lipoxygenase also plays a key role in the development of atherosclerosis.

It is reported that 12/15-lipoxygenase products mediate inflammatory cytokine gene expression in vascular smooth muscle cells (41, 47). In 12/15-lipoxygenase-deficient mouse macrophages, production of interleukin-12 is remarkably decreased (72). These findings suggest the contribution of 12/15-lipoxygenase to atherogenesis not only by initiating LDL oxidation, but also by modulating inflammatory or immunological processes.

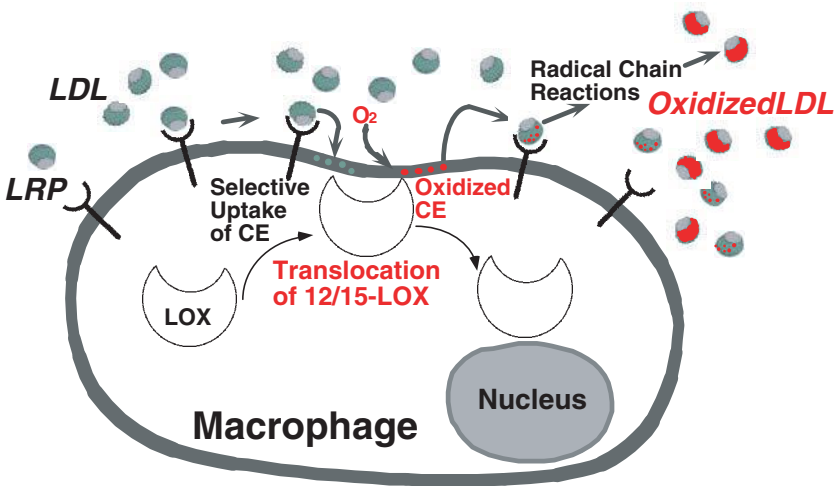

FIG. 3. Possible mechanism of LRP-mediated LDL oxidation by 12/15-lipoxygenase in macrophages. Binding of LDL to LRP induces selective uptake of cholesteryl linoleate into plasma membranes. At the same time, cytosolic 12/15-lipoxygenase translocates to the membranes and oxygenates the linoleate moiety of cholesteryl ester. The oxygenated cholesteryl ester would be returned to the LDL particle, followed by subsequent radical chain reaction to generate highly oxidized LDL recognized by scavenger receptors. CE, cholesteryl ester; LOX, lipoxygenase.

CONCLUSIONS

Despite the significant contribution of lipoxygenases to the initiation and progress of atherosclerosis, the enzymes did not seem to attract world attention for a long time, in particular, attention by clinicians treating the patient of this disease (18, 29, 42) For example, it was very recent that the medicines for asthma blocking the leukotriene receptor could be considered as a choice for the patient of cardiovascular diseases (13, 44, 63). The development of novel lipoxygenase inhibitors may be useful for the prevention and treatment of this very common disease in the future.

ACKNOWLEDGMENTS

This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the Honjin Foundation, and the Ono Medical Research Foundation.

ABBREVIATIONS

H(P)ETE, hydro(pero)xyeicosatetraenoic acid; LDL, low-density lipoprotein; LRP, low-density lipoprotein receptor-related protein; SR-BI, scavenger receptor class B type I.

REFERENCES

1. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, and Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 271: 518–520, 1996.
2. Belkner J, Wiesner R, Rathman J, Barnett J, Sigal E, and Kuhn H. Oxygenation of lipoproteins by mammalian lipoxygenases. *Eur J Biochem* 213: 251–261, 1993.
3. Belkner J, Stender H, and Kuhn H. The rabbit 15-lipoxygenase preferentially oxygenates LDL cholesteryl esters, and this reaction does not require vitamin E. *J Biol Chem* 273: 23225–23232, 1998.
4. Berliner JA and Heinecke JW. The role of oxidized lipoproteins in atherogenesis. *Free Radic Biol Med* 20: 707–727, 1996.
5. Boucher P, Gotthardt M, Li WP, Anderson RG, and Herz J. LRP: role in vascular wall integrity and protection from atherosclerosis. *Science* 300: 329–332, 2003.
6. Brash AR. Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. *J Biol Chem* 274: 23679–23682, 1999.
7. Brown MS and Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232: 34–47, 1986.
8. Burke JA, Levi R, Guo ZG, and Corey EJ. Leukotrienes C4, D4 and E4: effects on human and guinea-pig cardiac preparations in vitro. *J Pharmacol Exp Ther* 221: 235–241, 1982.
9. Cathcart MK, Li Q, and Chisolm GM 3rd. Lipoprotein receptor interactions are not required for monocyte oxidation of LDL. *J Lipid Res* 36: 1857–1865, 1995.
10. Chisolm GM, Hazen SL, Fox PL, and Cathcart MK. The oxidation of lipoproteins by monocytes-macrophages. Biochemical and biological mechanisms. *J Biol Chem* 274: 25959–25962, 1999.
11. Cyrus T, Witztum JL, Rader DJ, Tangirala R, Fazio S, Linton MF, and Funk CD. Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. *J Clin Invest* 103: 1597–1604, 1999.
12. Dahlen SE, Bjork J, Hedqvist P, Arfors KE, Hammarstrom S, Lindgren JA, and Samuelsson B. Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. *Proc Natl Acad Sci U S A* 78: 3887–3891, 1981.
13. De Caterina R and Zampolli A. From asthma to atherosclerosis—5-lipoxygenase, leukotrienes, and inflammation. *N Engl J Med* 350: 4–7, 2004.
14. Funk CD. The molecular biology of mammalian lipoxygenases and the quest for eicosanoid functions using lipoxygenase-deficient mice. *Biochim Biophys Acta* 1304: 65–84, 1996.
15. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 294: 1871–1875, 2001.
16. Funk CD and Cyrus T. 12/15-Lipoxygenase, oxidative modification of LDL and atherogenesis. *Trends Cardiovasc Med* 11: 116–124, 2001.
17. Gardai SJ, Xiao YQ, Dickinson M, Nick JA, Voelker DR, Greene KE, and Henson PM. By binding SIRPalpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* 115: 13–23, 2003.
18. Goldstein JL and Brown MS. Lipoprotein receptors and the control of plasma LDL cholesterol levels. *Eur Heart J* 13 Suppl B: 34–36, 1992.
19. Hagihara H, Nomoto A, Mutoh S, Yamaguchi I, and Ono T. Role of inflammatory responses in initiation of atherosclerosis: effects of anti-inflammatory drugs on cuff-induced leukocyte accumulation and intimal thickening of rabbit carotid artery. *Atherosclerosis* 91: 107–116, 1991.
20. Hajjar DP and Haberland ME. Lipoprotein trafficking in vascular cells. Molecular Trojan horses and cellular saboteurs. *J Biol Chem* 272: 22975–22978, 1997.
21. Harats D, Shaish A, George J, Mulkins M, Kurihara H, Levkovitz H, and Sigal E. Overexpression of 15-lipoxygenase in vascular endothelium accelerates early atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 20: 2100–2105, 2000.
22. Heinecke JW, Rosen H, and Chait A. Iron and copper promote modification of low density lipoprotein by human arterial smooth muscle cells in culture. *J Clin Invest* 74: 1890–1894, 1984.
23. Henriksen T, Mahoney EM, and Steinberg D. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptors for acetylated low density lipoproteins. *Proc Natl Acad Sci U S A* 78: 6499–6503, 1981.

24. Herz J and Strickland DK. LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest* 108: 779–784, 2001.
25. Hiltunen TP and Yla-Herttuala S. Expression of lipoprotein receptors in atherosclerotic lesions. *Atherosclerosis 137* Suppl: S81–S88, 1998.
26. Ji Y, Jian B, Wang N, Sun Y, Moya ML, Phillips MC, Rothblat GH, Swaney JB, and Tall AR. Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. *J Biol Chem* 272: 20982–20985, 1997.
27. Jian B, de la Llera-Moya M, Ji Y, Wang N, Phillips MC, Swaney JB, Tall AR, and Rothblat GH. Scavenger receptor class B type I as a mediator of cellular cholesterol efflux to lipoproteins and phospholipid acceptors. *J Biol Chem* 273: 5599–5606, 1998.
28. Kamohara M, Takasaki J, Matsumoto M, Matsumoto S, Saito T, Soga T, Matsushime H, and Furuichi K. Functional characterization of cysteinyl leukotriene CysLT(2) receptor on human coronary artery smooth muscle cells. *Biochem Biophys Res Commun* 287: 1088–1092, 2001.
29. Kita T, Kume N, Minami M, Hayashida K, Murayama T, Sano H, Moriwaki H, Kataoka H, Nishi E, Horiuchi H, Arai H, and Yokode M. Role of oxidized LDL in atherosclerosis. *Ann N Y Acad Sci* 947: 199–205, 2001.
30. Kondo K, Umemura K, Ohmura T, Hashimoto H, and Nakashima M. Suppression of intimal hyperplasia by a 5-lipoxygenase inhibitor, MK-886: studies with a photochemical model of endothelial injury. *Thromb Haemost* 79: 635–639, 1998.
31. Kuhn H and Thiele BJ. The diversity of the lipoxygenase family. Many sequence data but little information on biological significance. *FEBS Lett* 449: 7–11, 1999.
32. Kuhn H, Belkner J, Suzuki H, and Yamamoto S. Oxidative modification of human lipoproteins by lipoxygenases of different positional specificities. *J Lipid Res* 35: 1749–1759, 1994.
33. Kuhn H, Heydeck D, Hugou I, and Gniwotta C. In vivo action of 15-lipoxygenase in early stages of human atherogenesis. *J Clin Invest* 99: 888–893, 1997.
34. Kuhn H, Anton M, Gerth C, and Habenicht A. Amino acid differences in the deduced 5-lipoxygenase sequence of CAST atherosclerosis-resistance mice confer impaired activity when introduced into the human ortholog. *Arterioscler Thromb Vasc Biol* 23: 1072–1076, 2003.
35. Lusis AJ, Ji Y, Jian B, Wang N, Sun Y, Moya ML, Phillips MC, Rothblat GH, Swaney JB, and Tall AR. Atherosclerosis scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. *Nature* 407: 233–241, 2000.
36. McNally AK, Chisolm GM 3rd, Morel DW, and Cathcart MK. Activated human monocytes oxidize low-density lipoprotein by a lipoxygenase-dependent pathway. *J Immunol* 145: 254–259, 1990.
37. Mehrabian M, Allayee H, Wong J, Shi W, Wang XP, Shaposhnik Z, Funk CD, Lusis AJ, and Shih W. Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res* 91: 120–126, 2002.
38. Mellor EA, Austen KF, and Boyce JA. Cysteinyl leukotrienes and uridine diphosphate induce cytokine generation by human mast cells through an interleukin 4-regulated pathway that is inhibited by leukotriene receptor antagonists. *J Exp Med* 195: 583–592, 2002.
39. Miller YI, Chang MK, Funk CD, Feramisco JR, and Witztum JL. 12/15-Lipoxygenase translocation enhances site-specific actin polymerization in macrophages phagocytosing apoptotic cells. *J Biol Chem* 276: 19431–19439, 2001.
40. Moestrup SK, Gliemann J, Pallesen G, Tangirala RK, Mol MJ, and Steinberg D. Distribution of the alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res* 269: 375–382, 1992.
41. Natarajan R, Reddy MA, Malik KU, Fatima S, and Khan BV. Signaling mechanisms of nuclear factor-kappaB-mediated activation of inflammatory genes by 13-hydroperoxyoctadecadienoic acid in cultured vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 21: 1408–1413, 2001.
42. Niki E and Noguchi N. Effects of antioxidants against atherosclerosis. *Mol Cell Biochem* 234–235: 19–25, 2002.
43. Pech-Amsellem MA, Myara I, Pico I, Maziere C, Maziere JC, and Moatti N. Oxidative modifications of low-density lipoproteins (LDL) by the human endothelial cell line EA.hy 926. *Experientia* 52: 234–238, 1996.
44. Radmark O. 5-Lipoxygenase-derived leukotrienes: mediators also of atherosclerotic inflammation. *Arterioscler Thromb Vasc Biol* 23: 1140–1142, 2003.
45. Ralph P and Nakoinz I. Phagocytosis and cytolysis by a macrophage tumour and its cloned cell line. *Nature* 257: 393–394, 1975.
46. Rankin SM, Parthasarathy S, and Steinberg D. Evidence for a dominant role of lipoxygenase(s) in the oxidation of LDL by mouse peritoneal macrophages. *J Lipid Res* 32: 449–456, 1991.
47. Reddy MA, Thimmalapura PR, Lanting L, Nadler JL, Fatima S, and Natarajan R. The oxidized lipid and lipoxygenase product 12(*S*)-hydroxyeicosatetraenoic acid induces hypertrophy and fibronectin transcription in vascular smooth muscle cells via p38 MAPK and cAMP response element-binding protein activation. Mediation of angiotensin II effects. *J Biol Chem* 277: 9920–9928, 2002.
48. Rohlmann A, Gotthardt M, Hammer RE, and Herz J. Inducible inactivation of hepatic LRP gene by Cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J Clin Invest* 101: 689–695, 1998.
49. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 340: 115–126, 1999.
50. Sakashita T, Takahashi Y, Kinoshita T, and Yoshimoto T. Essential involvement of 12-lipoxygenase in regiospecific and stereospecific oxidation of low density lipoprotein by macrophages. *Eur J Biochem* 265: 825–831, 1999.
51. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, and Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 237: 1171–1176, 1987.
52. Shen J, Kuhn H, Petho-Schramm A, and Chan L. Transgenic rabbits with the integrated human 15-lipoxygenase gene driven by a lysozyme promoter: macrophage-specific expression and variable positional specificity of the transgenic enzyme. *FASEB J* 9: 1623–1631, 1995.

53. Spanbroek R, Grabner R, Lotzer K, Hildner M, Urbach A, Ruhling K, Moos MP, Kaiser B, Cohnert TU, Wahlers T, Zieske A, Plenz G, Robenek H, Salbach P, Kuhn H, Radmark O, Samuelsson B, and Habenicht AJ. Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis. *Proc Natl Acad Sci U S A* 100: 1238–1243, 2003.
54. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, and Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 320: 915–924, 1989.
55. Steinbrecher UP. Role of superoxide in endothelial-cell modification of low density lipoproteins. *Biochim Biophys Acta* 959: 20–30, 1988.
56. Steinbrecher UP, Parthasarathy S, Leake DS, Witzum JL, and Steinberg D. Modification of low density lipoprotein by endthelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci U S A* 81: 3883–3887, 1984.
57. Sun DX and Funk CD. Disruption of 12/15-lipoxygenase expression in peritoneal macrophages—enhanced utilization of the 5-lipoxygenase pathway and diminished oxidation of low density lipoprotein. *J Biol Chem* 271: 24055–24062, 1996.
58. Swarnakar S, Beers J, Strickland DK, Azhar S, and Williams DL. The apolipoprotein E-dependent low density lipoprotein cholesteryl ester selective uptake pathway in murine adrenocortical cells involves chondroitin sulfate proteoglycans and an alpha 2-macroglobulin receptor. *J Biol Chem* 276: 21121–21128, 2001.
59. Takahashi Y and Yoshimoto T. What are the functions of mammalian 8-, 12-, and 15-lipoxygenases? *Res Adv Cancer* 2: 221–229, 2002.
60. Tangirala RK, Mol MJ, and Steinberg D. Macrophage oxidative modification of low density lipoprotein occurs independently of its binding to the low density lipoprotein receptor. *J Lipid Res* 37: 835–843, 1996.
61. Walther M, Anton M, Wiedmann M, Fletterick R, and Kuhn H. The N-terminal domain of the reticulocyte-type 15-lipoxygenase is not essential for enzymatic activity but contains determinants for membrane binding. *J Biol Chem* 277: 27360–27366, 2002.
62. Walther M, Wiesner R, and Kuhn H. Investigations into calcium-dependent membrane association of 15-lipoxygenase-1. Mechanistic roles of surface-exposed hydrophobic amino acids and calcium. *J Biol Chem* 279: 3717–3725, 2004.
63. Wickelgren I. Heart disease. Gene suggests asthma drugs may ease cardiovascular inflammation. *Science* 303: 941, 2004.
64. Willnow TE, Tangirala RK, Mol MJ, and Steinberg D. The low-density lipoprotein receptor gene family: multiple roles in lipid metabolism. *J Mol Med* 77: 306–315, 1999.
65. Witztum JL and Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* 88: 1785–1792, 1991.
66. Woods JW, Evans JF, Ethier D, Scott S, Vickers PJ, Hearn L, Heibein JA, Charleson S, and Singer I. 5-Lipoxygenase and 5-lipoxygenase-activating protein are localized in the nuclear envelope of activated human leukocytes. *J Exp Med* 178: 1935–1946, 1993.
67. Xu W, Takahashi Y, Sakashita T, Iwasaki T, Hattori H, and Yoshimoto T. Low density lipoprotein receptor-related protein is required for macrophage-mediated oxidation of low density lipoprotein by 12/15-lipoxygenase. *J Biol Chem* 276: 36454–36459, 2001.
68. Yamamoto S. Mammalian lipoxygenases: molecular structures and functions. *Biochim Biophys Acta* 1128: 117–131, 1992.
69. Yla-Herttuala S, Rosenfeld ME, Parthasarathy S, Glass CK, Sigal E, Witztum JL, and Steinberg D. Colocalization of 15-lipoxygenase mRNA and protein with epitopes of oxidized low density lipoprotein in macrophage-rich areas of atherosclerotic lesions. *Proc Natl Acad Sci U S A* 87: 6959–6963,1990.
70. Yla-Herttuala S, Rosenfeld ME, Parthasarathy S, Sigal E, Sarkioja T, Witztum JL, and Steinberg D. Gene expression in macrophage-rich human atherosclerotic lesions. *J Clin Invest* 87: 1146–1152, 1991.
71. Yoshimoto T and Takahashi Y. Arachidonate 12-lipoxygenases. *Prostaglandins Other Lipid Mediat* 68–69: 245–262, 2002.
72. Zhao L, Cuff CA, Moss E, Wille U, Cyrus T, Klein EA, Pratico D, Rader DJ, Hunter CA, Pure E, and Funk CD. Selective interleukin-12 synthesis defect in 12/15-lipoxygenase-deficient macrophages associated with reduced atherosclerosis in a mouse model of familial hypercholesterolemia. *J Biol Chem* 277: 35350–35356, 2002.
73. Zhu H, Takahashi Y, Xu W, Kawajiri H, Murakami T, Yamamoto M, Iseki S, Iwasaki T, Hattori H, and Yoshimoto T. Low density lipoprotein receptor-related protein-mediated membrane translocation of 12/15-lipoxygenase is required for oxidation of low density lipoprotein by macrophages. *J Biol Chem* 278: 13350–13355, 2003.

Address reprint requests to:
Yoshitaka Takahashi, Ph.D.
Department of Nutritional Science
Faculty of Health and Welfare Science
Okayama Prefectural University
Kuboki 111, Soja
Okayama 719-1197, Japan

E-mail: ytaka@fhw.oka-pu.ac.jp

Received for publication September 25, 2004; accepted October 9, 2004.

This article has been cited by:

1. Yuki Kawakami, Tomoko Hosokawa, Tomoko Morinaka, Shioka Irino, Shiori Hirano, Hirokazu Kobayashi, Akiko Yoshioka, Toshiko Suzuki-Yamamoto, Miyuki Yokoro, Masumi Kimoto, Hideaki Tsuji, Hiromi Yamashita, Shinobu Doi, Chikao Yutani, Rina Kato, Hiroyuki Itabe, Teruyuki Kanada, Takahiko Hada, Yoshitaka Takahashi. 2011. Antiatherogenic effect of guava leaf extracts inhibiting leucocyte-type 12-lipoxygenase activity. *Food Chemistry* . [CrossRef]
2. Magomed Khaidakov, Jawahar L. Mehta. 2011. Do Atherosclerosis and Obesity-Associated Susceptibility to Cancer Share Causative Link to oxLDL and LOX-1?. *Cardiovascular Drugs and Therapy* . [CrossRef]
3. Lili Yin, Xingwei Wu, Yuanyuan Gong, Yuhua Shi, Yating Qiu, Hongmei Zhang, Xiaojuan Liu, Qing Gu. 2011. OX-LDL Up-Regulates the Vascular Endothelial Growth Factor-to-Pigment Epithelium-Derived Factor Ratio in Human Retinal Pigment Epithelial Cells. *Current Eye Research* **36**:4, 379-385. [CrossRef]
4. Manar A. Nader, Dina S. El-Agamy, Ghada M. Suddek. 2010. Protective effects of propolis and thymoquinone on development of atherosclerosis in cholesterol-fed rabbits. *Archives of Pharmacal Research* **33**:4, 637-643. [CrossRef]
5. Alice L. Yu, Reinhard L. Lorenz, Christos Haritoglou, Anselm Kampik, Ulrich Welge-Lussen. 2009. Biological effects of native and oxidized low-density lipoproteins in cultured human retinal pigment epithelial cells. *Experimental Eye Research* **88**:3, 495-503. [CrossRef]
6. 2008. Leukotrienes. *New England Journal of Medicine* **358**:7, 746-746. [CrossRef]
7. E.O. Farombi, O.O. Ige. 2007. Hypolipidemic and antioxidant effects of ethanolic extract from dried calyx of Hibiscus sabdariffa in alloxan-induced diabetic rats. *Fundamental & Clinical Pharmacology* **21**:6, 601-609. [CrossRef]
8. Fung Ping Leung, Suk Ying Tsang, Chi Ming Wong, Lai Ming Yung, Yau Chi Chan, Hok Sum Leung, Xiaoqiang Yao, Yu Huang. 2007. RALOXIFENE, TAMOXIFEN AND VASCULAR TONE. *Clinical and Experimental Pharmacology and Physiology* **34**:8, 809-813. [CrossRef]
9. Konstantin G. Birukov. 2006. Oxidized lipids: The two faces of vascular inflammation. *Current Atherosclerosis Reports* **8**:3, 223-231. [CrossRef]
10. S SAMPAIO, T ALBALOUREIRO, P BRIGATTE, R LANDGRAF, E SANTOS, R CURI, Y CURY. 2006. Lipoxygenase-derived eicosanoids are involved in the inhibitory effect of Crotalus durissus terrificus venom or crotoxin on rat macrophage phagocytosis. *Toxicon* **47**:3, 313-321. [CrossRef]
11. Elisabeth Koller, Ivo Volf, Aner Gurvitz, Franz Koller. 2006. Modified Low-Density Lipoproteins and High-Density Lipoproteins. *Pathophysiology of Haemostasis and Thrombosis* **35**:3-4, 322-345. [CrossRef]
12. Vilasinee Hirunpanich, Anocha Utaipat, Noppawan Phumala Morales, Nuntavan Bunyapraphatsara, Hitoshi Sato, Angkana Herunsale, Chuthamanee Suthisisang. 2006. Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of Hibiscus sabdariffa L. in hypercholesterolemic rats. *Journal of Ethnopharmacology* **103**:2, 252-260. [CrossRef]
13. Yoshitaka Takahashi, Hong Zhu, Wanpeng Xu, Takashi Murakami, Tadao Iwasaki, Hiroaki Hattori, Tanihiro Yoshimoto. 2005. Selective uptake and efflux of cholesteryl linoleate in LDL by macrophages expressing 12/15-lipoxygenase. *Biochemical and Biophysical Research Communications* **338**:1, 128-135. [CrossRef]
14. Kiyoshi Nose . 2005. Redox Control of Protein Trafficking. *Antioxidants & Redox Signaling* **7**:3-4, 303-307. [Citation] [Full Text PDF] [Full Text PDF with Links]