Modification of Platelet Shape Change Parameter by Modified Phospholipids in Oxidized LDL

Werasak Sutipornpalangkul PhD, MD*, Supeenun Unchen PhD**,
Yupin Sanvarinda PhD**, Udom Chantharaksri PhD**

** Department of Orthopaedic Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
*** Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand*

Background: *It is believed that the oxidatively modified lipoproteins play a critical role in activating platelets and is a contributing factor in the etiology of a number of cardiovascular-related diseases.*
Objective: *Identify the active component(s) of oxidized LDL that initiated shape change in plasma-free human platelets prepared by a gel filtration method.*
Material and Method: *Shape change parameter of platelets was monitored following exposure platelets to LDL, copper sulfate-oxidized LDL, and different types of lipids extracted of the corresponding LDL.*
Results: *Oxidized LDL, but not native LDL, increased the shape-change parameter in a concentration-dependent manner. Specifically, phosphatidyl serine from oxidized LDL was responsible for this effect.*
Conclusion: *Oxidized phospholipids generated during the oxidative modification of LDL are likely to be the active components responsible for changes in platelet function.*

Keywords: *Oxidized LDL, Platelet shape change, Phospholipids, Atherosclerosis*

J Med Assoc Thai 2009; 92 (2): 229-35
Full text. e-Journal: *http://www.mat.or.th/journal*

An elevated plasma level of low-density lipoprotein (LDL) is clearly a major risk factor in the pathogenesis of atherosclerosis. It has been proposed that native LDL must undergo oxidative modification before it can give rise to foam cells, the key component of the fatty-streak lesion of atherosclerosis[1-3]. Recently, oxidized LDL (ox-LDL) has been detected *in vivo*[4-6] and considered an important substance promoting atherogenesis by damaging vascular endothelium, being deposited in atherosclerotic plaques, promoting foam cell formation and stimulating platelets[7-10].

Platelets are known to be involved in the formation of atherosclerosis lesion and its complications, and plasma lipoproteins have been shown to affect platelet activity, both *in vitro* and *in vivo*[11,12]. LDL has been reported to pose platelet-activating activities such as the enhancement of platelets sensitivity to stimuli[9,10,13-17] and the induction of platelet aggregation[18-20]. In recent years, however, platelet-activating activities were found to reside in oxidized LDL rather than in native LDL (n-LDL)[21-24]. Based on light-scattering experiments[25, 26], Zhao et al have demonstrated stimulation of disc-sphere formation of platelets by ox-LDL *in vitro*[8], but its chemical nature has yet to be characterized. Therefore, the aim of the present study was to identify the responsible biologically molecules residing in ox-LDL. Focus was placed on the early phase of platelet activation, which dictates the fate and the role of platelets, contributing to pathologic sequence of events.

Correspondence to: Sutipornpalangkul W, Department of Orthopaedic, Faculty of Medicine, Siriraj Hospital, Bangkok 10700, Thailand. Phone: 0-2419-7965, Mobile: 081-751-2428. E-mail: tewsv@mahidol.ac.th

Material and Method

Materials

Phospholipids, 2-thiobarbituric acid, butylated hydroxytoluene, bovine serum albumin (BSA), Sepharose 2B-CL, bovine serum albumin solution, HEPES, potassium bromide, apyrase, and thrombin were obtained from Sigma Chemical Co. (St. Louis, USA). Acetonitrile, chloroform, hexane, methanol, 2-propanol

(all HPLC grade) were obtained from JT Baker Inc. (Phillipsburg, NJ). Aminoprophyl solid phase extraction columns were obtained from Millipore Corp (Billerica, MA).

Methods

Isolation of LDL

Blood was drawn from fasting volunteers into 0.1% EDTA. Plasma was separated by centrifugation at 2,000 g for 15 min at 4°C. Lipoproteins were separated by sequential ultracentrifugation in a Beckman TL-100 Beckman tabletop ultracentrifuge using a fixed angle TLA-100.2 rotor[27]. LDL was dialyzed overnight against PBS (Phosphate Buffer Saline) pH 7.4 with a least two changes of dialysate. Protein concentration was determined by a modified Lowry method[28], using BSA as a standard. LDL was stored at -70°C and used within 2 weeks.

Oxidation of LDL and phospholipids

Oxidation of LDL and phospholipids were conducted in a shaking water bath at 37°C. One hundred μg/ml of LDLs and phospholipids were incubated with $CuSO_4$ (10-100 μM) in 10 mM PBS (pH 7.4). Oxidation was terminated by the addition of 0.1 mM EDTA and the solution was immediately frozen. The extent of lipoprotein and phospholipids oxidation was measured by determining ratios of thiobarbituric acid reactive substances (TBARs) before and after incubation with $CuSO_4$[29].

Lipid extraction

Lipids were extracted using a modified method of Bligh and Dyer[30]. To 1 ml aliquot of the aqueous lipid-containing suspension, 7 volumes of chloroform/methanol (2:1) were added. The mixture was vortexed 1-2 min and then centrifuged at 1,200 g for 15 min at 4°C. The chloroform phase was carefully removed and 5 volumes of chloroform were added to the residual mixture for another round of extraction and combined with the previous extract.

Solid phase extraction chromatography

Phospholipids, free fatty acids and neutral lipids (cholesterol, cholesteryl esters, mono-, di-, triacylglycerides) were separated according to the method of Kaluzny et al[31] utilizing aminopropyl solid phase extraction (SPE) chromatography. Total lipid extracts were dried under nitrogen at 37°C and resuspended in 500 μl of chloroform. The extracts were then added to aminopropyl SPE column that had previously been washed with 3 ml of methanol and preconditioned with 6 ml of hexane. Neutral lipids were eluted with 3 ml of chloroform/2-propanol (2:1), free fatty acids with 3 ml of 3% acetate in ethyl ether, and phospholipids with 3 ml of methanol.

Preparation of platelets

Blood was obtained by venipuncture from healthy human donors who had not been on medication for 2 weeks before donation. Blood was anticoagulanted with ACD (Anticoagulant Citrate Dextrose) (6:1 v/v) and centrifuged at 250 x g for 12 min to obtain platelet-rich plasma. Platelets were then separated from plasma by the albumin-cushion method[32]. Platelet were purified further by gel filtration, using a Sepharose 2B-CL column and resuspended in platelet buffer (3.5 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 5.5 mM glucose, 1 mM $MgCl_2$, 0.35% BSA, pH 7.4). The platelet suspension was incubated for 30 min at 37°C to restore platelets to resting state.

Shape-change analysis

The shape change of platelets in suspension was determined by recording light transmission using a Chrono-Log Corp Whole Blood Aggregometer Model 550 (Chrono-Log Corp, Japan). Light transmission of a suspension of asymmetric particles (discoid platelets) increases with stirring, whereas that of spherical, symmetric particles with or without uniformly distributed pseudopods, is insensitive to stirring, assuming that the total volume does not change upon stimulation[25,26,33,34].

An aliquot of 500 μl of 10^5 cells/μl was used as test sample and 600 μl of 5-7x10^4 cells/μl as control sample. Before and after incubation of platelets with oxidized LDL and phospholipids at 37°C for 5 minutes, light transmission was recorded. The electronic stirrer was alternatively turned on and off every 30 seconds for 5 minutes. The shape-change parameter was calculated as (1 - (test defection / control defection) x 100%) as described by Beaumont et al[25].

Results

Dose-and time-effect of copper for generating platelet shape changes in LDL

The dose of copper sulfate that is required for LDL oxidation and induction of LDL for platelet shape change was investigated by monitoring the formation of oxidized lipids, TBARs, and shape-change parameter. These parameters were measured following incubation of copper sulfate and LDL (100 μg/ml) for 6

hours. $CuSO_4$ up to 100 μM showed an all or none effect, e.g., the oxidation of LDL and platelet shape change could be initiated at 30 μM $CuSO_4$ (Fig. 1).

The shape change activity formed in LDL by oxidation was elevated at 3 hours and increased with further oxidation in a time-dependent manner (data not shown). This effect of oxidized LDL was dose dependent (Fig. 2). After 5 minutes of incubation with oxidized LDL up to 20 μg/ml, the shape change parameter of gel-filtered platelets clearly increased. However, native LDL (20 μg/ml) had only a minimal effect on platelet shape change. Thrombin (0.1 U/ml) was used as positive control.

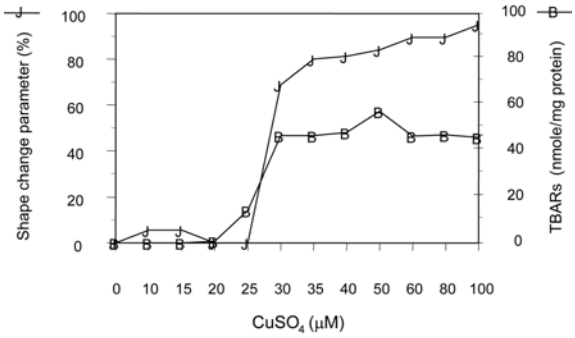

Fig. 1 Dose-effect of copper sulfate on oxidation of LDL and shape-change parameter. One hundred μg/ml of LDL were incubated with 0-100 mM $CuSO_4$ in PBS pH 7.4 for 6 hr. Twenty μg/ml of modified LDL were used in stimulating platelets. This is a single experiment that is representative of three replicate experiments (TBARs = thiobarbituric acid reactive substances)

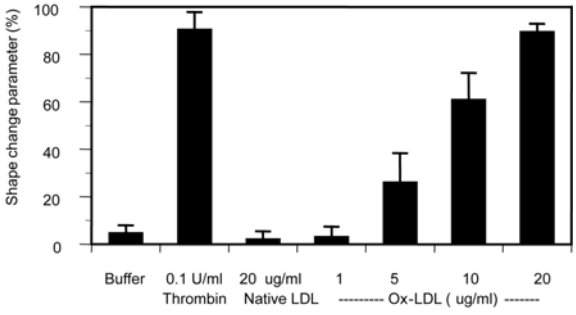

Fig. 2 Effect of native LDL and ox-LDL on shape change parameter. Ox-LDL was prepared by incubation 100 μg/ml of LDL with 50 μM $CuSO_4$ for 24 hr in PBS pH 7.4. Values represent means ± SD (n = 5)

Determination of components in oxidized LDL causing platelet shape change

Lipids were extracted from oxidized LDL and neutral lipids, fatty acids, and phospholipids were separated using SPE chromatography. Only lipids extracted from oxidized and not native LDL had activity causing shape change of platelets (Fig. 3). This ability to induce shape change of platelets was present entirely in the phospholipids fraction (Fig. 4).

Effect of oxidized phospholipids on platelet shape change

To determine which type of oxidized phospholipids in ox-LDL induced platelet shape change, the effects of oxidation by $CuSO_4$ of individual species of phospholipids were examined. To evaluate the extent of oxidation, TBARs values were monitored. The TBARs content of phosphatidyl serine, phosphatidyl ethanolamine, phosphotidyl choline, and sphingomyelin after oxidation with 100 μM $CuSO_4$ for 24 hours was 2.36 ± 0.27, 1.51 ± 0.16, 0.18 ± 0.09 and 0.13 ± 0.09 nmol/ml, respectively (Table 1). However, only oxidized phosphatidyl serine caused shape change of platelets. This effect of oxidized phosphotidyl serine on platelet shape change was dose-dependent (data not shown). Unmodified phospholipids did not show any TBARs production or platelet shape change activity.

Discussion

Previous studies of the effects of LDL on platelet function have reported various results, although most workers have found stimulatory effect[8,21,35]. The presented experiments confirmed that the oxidatively modified LDL but not native LDL could induce platelet

Table 1. Effect of oxidized phospholipids on platelet shape change parameter

Lipid	TBARs (nmole/ml)	Shape change parameter (%)
Phosphatidyl serine	2.36 ± 0.27	45.58 ± 4.55
Phosphatidyl ethanolamine	1.51 ± 0.16	1.45 ± 2.51
Phosphatidyl choline	0.18 ± 0.09	4.15 ± 3.88
Sphingomyelin	0.13 ± 0.09	1.52 ± 2.63

One hundred μg/ml of phospholipids were incubated with 100 μM $CuSO_4$ in PBS (pH 7.4) at 37°C for 24 hrs. Gel-filtered platelets were treated with 20 μg/ml of oxidized phospholipids. Values represent means ± SD from three independent experiments

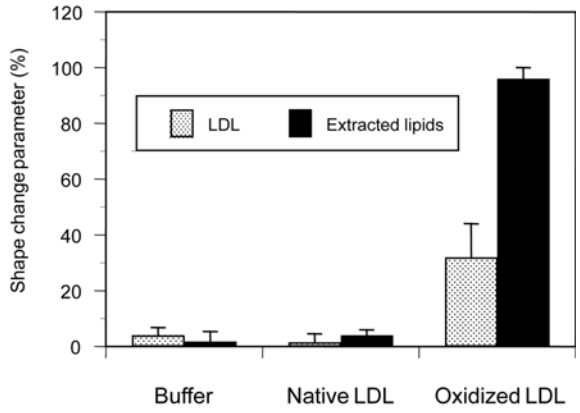

Fig. 3 Effects of LDL and lipid extracts on platelet shape-change parameter. 100 μg/ml LDL was oxidized with 50 μM $CuSO_4$ for 6 hr. All samples then underwent a Bligh-Dyer extraction procedure. Lipids were suspended in PBS (pH 7.4) for analysis of shape-change parameter of platelets. Values are means ± SD (n = 4)

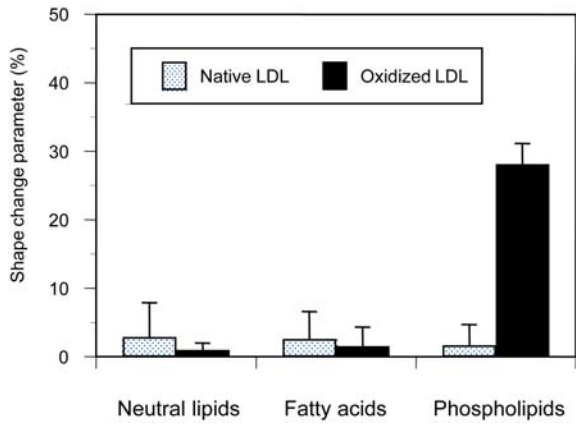

Fig. 4 Effects of oxidized lipids on platelet shape change parameter. Oxidized LDL was prepared by incubation 100 μg/ml LDL with 50 μM $CuSO_4$ for 6 hr. Total lipids in oxidized LDL were extracted and separated by SPE chromatography as described in Material and Method. Each lipid fraction was suspended in PBS pH 7.4 for analysis of shape-change parameter of platelets. Values are means ± SD (n = 3)

shape change in a dose-dependent manner. The authors demonstrated that generation of platelet shape change activity in oxidized LDL induced by copper sulfate was a specific process, as it required a critical amount of copper to initiate the production of bioactive compounds in LDL. From a biological perspective, it indicates that the oxidation of LDL *in vitro* may generate compounds that cause platelet shape change. This could have important consequences in the pathogenesis of atherosclerosis as activation of platelet is a feature of the disease, and because LDL is thought to be oxidatively modified at the site of atherosclerosis[1,2,4]. The authors directly demonstrated that phospholipids with platelet shape change activity are generated by the oxidation of phospholipids in LDL. Moreover, the authors showed that the generation of oxidized phosphatidyl serine in LDL could be responsible for the LDL-induced platelet shape change.

Various possible explanations for the platelet-activating action of ox-LDL have been described[36,37]. These include lipoperoxide formation, activation of phospholipase A_2 present in LDL, fragmentation of fatty acids, formation of aldehydes, and conjugation of aldehydes to apolipoprotein B and phospholipids. In the authors' experiments with extensively oxidized preparation of ox-LDL, platelet-activating activity was found to reside with phospholipids, indicating that biologically active fragments of fatty acids or other substances are unlikely to be contributing factors. Modified apoliprotein B may also not be contributing factor as platelet shape change could be induced by ox-HDL (data not shown), which does not contain apolipoprotein B but contains apolipoprotein A-I. Several studies have suggested that one of the active principles of ox-LDL seems to reside in a class of oxidized phospholipids that are PAF-like substances[38-40]. It is, therefore, likely that the active components of ox-LDL in the present studies may belong to this class of PAF-like substances.

The authors' experiments showed that bioactive phospholipids were not formed using low concentrations of copper sulfate for inducing oxidative modification of LDL at any time of oxidation up to 6 hours. However, when LDL was incubated with high concentrations of copper sulfate, these bioactive phospholipids were detected during the first 3 hours of oxidation. A possible explanation is that PAF-like oxidized phospholipids may be generated in minimally modified LDL, but has been hydrolyzed by the action of PAF acetlyhydrolase[39,41]. However, this protective mechanism is lost in strongly oxidizing environment as PAF acetylhydrolase is completely inactivated by strong oxidants[42-44].

The present studies indicated that only oxidized phosphatidyl serine could induce disc-sphere

transformation of platelets. This is in contrast to previous results showing that platelet activation is generated by oxidation of phosphatidyl choline containing a *sn*-2 polyunsaturated fatty acyl residue[45-47]. The lack of platelet-activation activity of oxidized phosphatidyl choline in the present study may have resulted from an inability of copper sulfate to induced oxidation of the phosphatidyl choline used. This was confirmed by lack of TBARs production when compared between unmodified and oxidized phosphatidyl choline. Thus, the authors suggest the oxidized phospholipids that can induce disc-sphere transformation of platelets could be generated by oxidation of both phosphatidyl serine and phosphatidyl choline in LDL, although, the mechanism by which oxidized phosphatidyl serine causes platelet shape change is unknown. Oxidation of phosphatidyl serine, causes marked increase in phospholipid-dependent thrombin generation[48]. These results suggest that phosphalidyl serine is a potentially important phospholipid in LDL because it may give insight into a mechanism by which oxidized LDL, platelet function and coagulation factors interact to cause atherosclerosis and thrombosis[49].

Acknowledgements

This study was supported by the Medical Scholars Program, Mahidol University, Thailand. The technical supports of Robert I Grossman, Jeerawan Dangdoungjai and staff at the Blood Bank, Ramathibodi Hospital, Bangkok, Thailand are gratefully acknowledged.

References

1. Kita T, Kume N, Minami M, Hayashida K, Murayama T, Sano H, et al. Role of oxidized LDL in atherosclerosis. Ann N Y Acad Sci 2001; 947: 199-206.
2. Galle J, Hansen-Hagge T, Wanner C, Seibold S. Impact of oxidized low density lipoprotein on vascular cells. Atherosclerosis 2006; 185: 219-26.
3. Itabe H. Oxidized low-density lipoproteins: what is understood and what remains to be clarified. Biol Pharm Bull 2003; 26: 1-9.
4. Palinski W, Rosenfeld ME, Yla-Herttuala S, Gurtner GC, Socher SS, Butler SW, et al. Low density lipoprotein undergoes oxidative modification *in vivo*. Proc Natl Acad Sci U S A 1989; 86: 1372-6.
5. Ueba T, Nomura S, Nishikawa T, Kajiwara M, Yamashita K. Circulating oxidized LDL, measured with FOH1a/DLH3 antibody, is associated with metabolic syndrome and the coronary heart disease risk score in healthy Japanese. Atherosclerosis 2008 Jun 3 [Epub ahead of print].
6. Andican G, Seven A, Uncu M, Cantasdemir M, Numan F, Burcak G. Oxidized LDL and anti-oxLDL antibody levels in peripheral atherosclerotic disease. Scand J Clin Lab Invest 2008; 68: 473-8.
7. Thomas JP, Geiger PG, Girotti AW. Lethal damage to endothelial cells by oxidized low density lipoprotein: role of selenoperoxidases in cytoprotection against lipid hydroperoxide- and iron-mediated reactions. J Lipid Res 1993; 34: 479-90.
8. Zhao B, Dierichs R, Liu B, Holling-Rauss M. Functional morphological alterations of human blood platelets induced by oxidized low density lipoprotein. Thromb Res 1994; 74: 293-301.
9. Aviram M. Modified forms of low density lipoprotein affect platelet aggregation *in vitro*. Thromb Res 1989; 53: 561-7.
10. Ardlie NG, Selley ML, Simons LA. Platelet activation by oxidatively modified low density lipoproteins. Atherosclerosis 1989; 76: 117-24.
11. Relou IA, Hackeng CM, Akkerman JW, Malle E. Low-density lipoprotein and its effect on human blood platelets. Cell Mol Life Sci 2003; 60: 961-71.
12. Akkerman JW. From low-density lipoprotein to platelet activation. Int J Biochem Cell Biol 2008; 40: 2374-8.
13. Zhao B, Dierichs R, Liu JF, Zhu ZM, Berkes P, Frye S. Influence of low density lipoproteins on cytosolic free Ca2+ concentration and dense tubular system in human platelets. Thromb Res 1993; 72: 33-7.
14. Bochkov VN, Voino-Yasenetskaya TA, Tkachuk VA. Epinephrine potentiates activation of human platelets by low density lipoproteins. Biochim Biophys Acta 1991; 1097: 123-7.
15. Surya II, Gorter G, Mommersteeg M, Akkerman JW. Enhancement of platelet functions by low density lipoproteins. Biochim Biophys Acta 1992; 1165: 19-26.
16. van Willigen G, Gorter G, Akkerman JW. LDLs increase the exposure of fibrinogen binding sites on platelets and secretion of dense granules. Arterioscler Thromb 1994; 14: 41-6.
17. Vlasova II. The effect of oxidatively modified low-density lipoproteins on platelet aggregability and membrane fluidity. Platelets 2000; 11: 406-14.
18. Andrews HE, Aitken JW, Hassall DG, Skinner VO, Bruckdorfer KR. Intracellular mechanisms in the activation of human platelets by low-density lipoproteins. Biochem J 1987; 242: 559-64.

19. Hassall DG, Owen JS, Bruckdorfer KR. The aggregation of isolated human platelets in the presence of lipoproteins and prostacyclin. Biochem J 1983; 216: 43-9.
20. Dunn RC, Schachter M, Miles CM, Feher MD, Tranter PR, Bruckdorfer KR, et al. Low-density lipoproteins increase intracellular calcium in aequorin-loaded platelets. FEBS Lett 1988; 238: 357-60.
21. Weidtmann A, Scheithe R, Hrboticky N, Pietsch A, Lorenz R, Siess W. Mildly oxidized LDL induces platelet aggregation through activation of phospholipase A_2. Arterioscler Thromb Vasc Biol 1995; 15: 1131-8.
22. Takahashi Y, Chiba H, Matsuno K, Akita H, Hui SP, Nagasaka H, et al. Native lipoproteins inhibit platelet activation induced by oxidized lipoproteins. Biochem Biophys Res Commun 1996; 222: 453-9.
23. Chen LY, Mehta P, Mehta JL. Oxidized LDL decreases L-arginine uptake and nitric oxide synthase protein expression in human platelets: relevance of the effect of oxidized LDL on platelet function. Circulation 1996; 93: 1740-6.
24. Liapikos TA, Antonopoulou S, Karabina SP, Tsoukatos DC, Demopoulos CA, Tselepis AD. Platelet-activating factor formation during oxidative modification of low-density lipoprotein when PAF-acetylhydrolase has been inactivated. Biochim Biophys Acta 1994; 1212: 353-60.
25. Beaumont DO, Whitten KW, Mock RG, Slattery CW. Measuring platelet function with platelet shape change, an early event in aggregation. Thromb Res 1989; 53: 109-27.
26. Latimer P, Born GV, Michal F. Application of light-scattering theory to the optical effects associated with the morphology of blood platelets. Arch Biochem Biophys 1977; 180: 151-9.
27. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955; 34: 1345-53.
28. Markwell MA, Haas SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 1978; 87: 206-10.
29. Smith JB, Ingerman CM, Silver MJ. Malondialdehyde formation as an indicator of prostaglandin production by human platelets. J Lab Clin Med 1976; 88: 167-72.
30. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37: 911-7.
31. Kaluzny MA, Duncan LA, Merritt MV, Epps DE. Rapid separation of lipid classes in high yield and purity using bonded phase columns. J Lipid Res 1985; 26: 135-40.
32. Grossman I. The effect of dengue-2 virus on the function of normal human platelets separated from plasma [Thesis]. Bangkok: Mahidol University; 1993.
33. Ozaki Y, Jinnai Y, Yatomi Y, Kume S. Protein kinase C inhibitors suppress disc-sphere changes of human platelets, as assessed with the shape-change parameter. Eur J Pharmacol 1993; 235: 255-65.
34. Patscheke H, Dubler D, Deranleau D, Luscher EF. Optical shape change analysis in stirred and unstirred human platelet suspensions. A comparison of aggregometric and stopped-flow turbidimetric measurements. Thromb Res 1984; 33: 341-53.
35. Zhao B, Dierichs R, Harrach-Ruprecht B, Winterhorff H. Oxidized LDL induces serotonin release from blood platelets. Am J Hematol 1995; 48: 285-7.
36. Witztum JL. The role of oxidized LDL in atherosclerosis. Adv Exp Med Biol 1991; 285: 353-65.
37. Korporaal SJ, Van Eck M, Adelmeijer J, Ijsseldijk M, Out R, Lisman T, et al. Platelet activation by oxidized low density lipoprotein is mediated by CD36 and scavenger receptor-A. Arterioscler Thromb Vasc Biol 2007; 27: 2476-83.
38. Heery JM, Kozak M, Stafforini DM, Jones DA, Zimmerman GA, McIntyre TM, et al. Oxidatively modified LDL contains phospholipids with platelet-activating factor-like activity and stimulates the growth of smooth muscle cells. J Clin Invest 1995; 96: 2322-30.
39. Watson AD, Navab M, Hama SY, Sevanian A, Prescott SM, Stafforini DM, et al. Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein. J Clin Invest 1995; 95: 774-82.
40. Marathe GK, Prescott SM, Zimmerman GA, McIntyre TM. Oxidized LDL contains inflammatory PAF-like phospholipids. Trends Cardiovasc Med 2001; 11: 139-42.
41. Stremler KE, Stafforini DM, Prescott SM, McIntyre TM. Human plasma platelet-activating factor acetylhydrolase. Oxidatively fragmented phospholipids as substrates. J Biol Chem 1991; 266: 11095-103.
42. Stafforini DM, Zimmerman GA, McIntyre TM,

Prescott SM. The platelet-activating factor acetylhydrolase from human plasma prevents oxidative modification of low-density lipoprotein. Trans Assoc Am Physicians 1992; 105: 44-63.
43. Dentan C, Lesnik P, Chapman MJ, Ninio E. PAF-acether-degrading acetylhydrolase in plasma LDL is inactivated by copper- and cell-mediated oxidation. Arterioscler Thromb 1994; 14: 353-60.
44. Ambrosio G, Oriente A, Napoli C, Palumbo G, Chiariello P, Marone G, et al. Oxygen radicals inhibit human plasma acetylhydrolase, the enzyme that catabolizes platelet-activating factor. J Clin Invest 1994; 93: 2408-16.
45. Tokumura A, Tanaka T, Yotsumoto T, Tsukatani H. Formation of PAF-like compounds by peroxidation of phospholipids from bovine brain. J Lipid Mediat 1992; 5: 127-30.
46. Smiley PL, Stremler KE, Prescott SM, Zimmerman GA, McIntyre TM. Oxidatively fragmented phosphatidylcholines activate human neutrophils through the receptor for platelet-activating factor. J Biol Chem 1991; 266: 11104-10.
47. Siess W. Platelet interaction with bioactive lipids formed by mild oxidation of low-density lipoprotein. Pathophysiol Haemost Thromb 2006; 35: 292-304.
48. Rota S, McWilliam NA, Baglin TP, Byrne CD. Atherogenic lipoproteins support assembly of the prothrombinase complex and thrombin generation: modulation by oxidation and vitamin E. Blood 1998; 91: 508-15.
49. Hazen SL. Oxidized phospholipids as endogenous pattern recognition ligands in innate immunity. J Biol Chem 2008; 283: 15527-31.

การเปลี่ยนรูปร่างของเกล็ดเลือดโดยฟอสโฟไลปิดในออกซิไดซ์แอลดีแอล

วีระศักดิ์ สุทธิพรพลางกูร, สุภีนันท์ อัญเชิญ, ยุพิน สังวรินทะ, อุดม จันทรารักษ์ศรี

ปัจจุบันเป็นที่ทราบกันว่าออกซิไดซ์แอลดีแอล มีบทบาทสำคัญในการทำให้เกิดโรคหลอดเลือดอุดตัน และการแข็งตัวของเกล็ดเลือด การวิจัยนี้มีจุดมุ่งหวังในการหาสารที่อยู่ในออกซิไดซ์แอลดีแอล และทำให้การเปลี่ยนรูปร่างของเกล็ดเลือด โดยวัดการเปลี่ยนรูปร่างของเกล็ดเลือดที่ถูกแยกจากน้ำเลือดแล้วหลังจากถูกกระตุ้นด้วย แอลดีแอลปกติ, ออกซิไดซ์แอลดีแอล, ไขมันชนิดต่าง ๆ ที่สกัดจากแอลดีแอล การทดลองพบว่าเฉพาะออกซิไดซ์แอลดีแอลเท่านั้นที่สามารถกระตุ้นการเปลี่ยนรูปร่างของเกล็ดเลือด นอกจากนี้เฉพาะไขมันที่สกัดจากออกซิไดซ์แอลดีแอลเท่านั้นที่มีฤทธิ์ดังกล่าว โดยฟอสโฟไลปิดชนิดซีรินเป็นสารในออกซิไดซ์แอลดีแอลที่ทำให้เกิดการกระตุ้นเกล็ดเลือด จากผลการทดลองนี้แสดงให้เห็นว่าออกซิไดซ์ฟอสโฟไลปิด ที่เกิดขึ้นในขบวนการออกซิเดชั่นของแอลดีแอล น่าจะเป็นองค์ประกอบในออกซิไดซ์แอลดีแอล ที่ทำให้เกิดการทำงานที่ผิดปกติของเกล็ดเลือด และนำไปสู่การเกิดโรคหลอดเลือดอุดตัน และการแข็งตัวของเกล็ดเลือด