REVIEWS

Hypochlorite, Oxidative Modification of Plasma Lipoproteins, and Atherosclerosis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 5, pp. 484-494, May, 2001
Original article submitted May 29, 2000

Hypochlorite produced *in vivo* by activated neutrophils, monocytes, and macrophages modifies blood lipoproteins and intensifies free radical lipid peroxidation, which probably plays a key role in the early stages of atherosclerotic vascular damages.

Key Words: *hypochlorite; oxidative modification; lipid peroxidation; free radicals; atherosclerosis*

Studies performed in the late 1980s [37,48,55,90] indicated that oxidatively modified blood lipoproteins (LP) are involved in the early stages of atherosclerotic vascular damages [22,30,54,59,76]. However, the cause of formation of oxidized blood LP was unclear. It was hypothesized that native LP interact with reactive oxygen species (ROS) $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and hypochlorite ion HOCL/OCl^- produced by vascular wall and blood cells [55,89,90]. $O_2^{\bullet-}$ was suggested to play a key role in LP modification [55,90].

At the same time, $O_2^{\bullet-}$ and H_2O_2 formed after $O_2^{\bullet-}$ dismutation are weak oxidizers not initiating lipid peroxidation (LPO) [5,7]. Therefore, $OH^{\bullet}$ and HOCL/OCl^- can act as oxidizers. $OH^{\bullet}$ is produced in the reaction of $O_2^{\bullet-}$ or H_2O_2 with metals of alternating valence (Fig. 1). HOCL/OCl^- is synthesized in the reaction catalyzed by myeloperoxidase (MPO, donor H_2O_2 oxidoreductase, EC 1.11.1.7):

$$Cl^- + H^+ + H_2O_2 = HOCl + H_2O \qquad \textbf{(1)}.$$

Active $OH^{\bullet}$ initiates free radical reactions. However, its involvement in LPO activation by phagocytes was in doubt due to the use of nonspecific traps [42].

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Strong oxidizer hypochlorite reacts with various biologically active compounds, including proteins, lipids, nucleic acids, and polysaccharides [1,20,36,72, 86-88,92-94,97], with the formation of free radical intermediates [33,39,56,60].

Here we focused our attention on monocytes and neutrophils containing and secreting MPO, which catalyzes hypochlorite formation in reaction **(1)**.

Possible Mechanisms Underlying Formation of Oxidized Blood LP

In vitro experiments showed that incubation of low-density lipoproteins (LDL) with monocytes or neutrophils even in the absence of activating agents leads to accumulation of LPO products in these particles. Monocytes were incubated with LDL oxidized to a different extent. The higher was the degree of LDL oxidation, the higher was the yield of LPO products. We suggested that oxidized LDL stimulate cells and promote oxidative modification of LDL [76,81].

Special experiments demonstrated that hypochlorite-modified or autoxidized LP activate monocytes and neutrophils, which stimulates $O_2^{\bullet-}$ production and increases chemiluminescence of cells [76,81].

On the one hand, MPO-containing monocytes and macrophages cause oxidative modification of LDL; on the other, oxidized LDL activate phagocytes and con-

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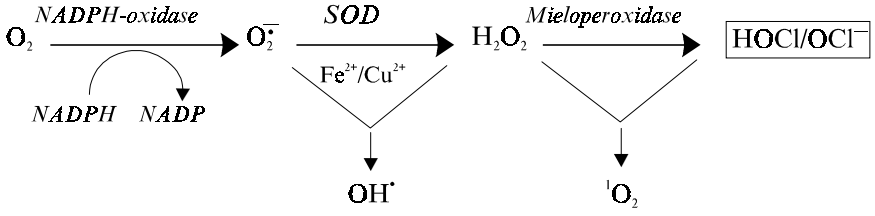

Fig. 1. Transformation of reactive oxygen species after activation of neutrophils or monocytes.

tribute to secretion of ROS, MPO, and hypochlorite in the extracellular medium. These processes aggravate oxidative modification of LDL.

Our experiments on the model of perfused rabbit liver demonstrated that oxidized blood LP can be produced by hepatocytes under conditions of LP hypercholesterolemia and increased content of LPO products [29,31]. The content of LPO products in liver perfusate from rabbits with alimentary atherosclerosis 3-fold surpassed that in intact animals. Moreover, the content of LPO products in LP isolated from the liver perfusate of rabbits with experimental atherosclerosis (density >1.065 g/cm^3) was 2 times higher than in intact animals [29,31]. Therefore, during atherosclerosis LPO modifies physicochemical properties of LP at the stage of their synthesis and secretion by hepatocytes.

Figure 2 illustrates the mechanisms underlying the formation of oxidized LP. LPO activation in the liver during hypercholesterolemia leads to the synthesis and secretion of very low-density lipoproteins (VLDL) with increased content of lipid peroxides (pathway *1*). Oxidized VLDL and LDL enter the circulation (pathway *2*). Oxidized LDL stimulate monocytes or neutrophils, which is accompanied by ROS generation and MPO secretion into the extracellular medium (pathway *3*). Native LP contacting with monocytes or neutrophils undergo oxidative modification (pathway *4*).

The inhibitory assay with mannite (OH• trap), desferrioxamine and ethylenediaminetetraacetic acid (iron ion chelators), superoxide dismutase (SOD) and catalase eliminating $O_2^{\bullet-}$ and H_2O_2 (respectively), and butylated hydroxytoluene (ionol, radical trap) showed that LPO initiation in LP stimulated by neutrophils and monocytes is mediated by a free radical mechanism not involving OH• [30,76,81]. $O_2^{\bullet-}$ and H_2O_2 are not involved in LPO activation by phagocytes [7,49]. Taking into account the scheme of ROS transformations (Fig. 1), it can be suggested that hypochlorite produced by activated phagocytes plays an important role in oxidative modification of LP.

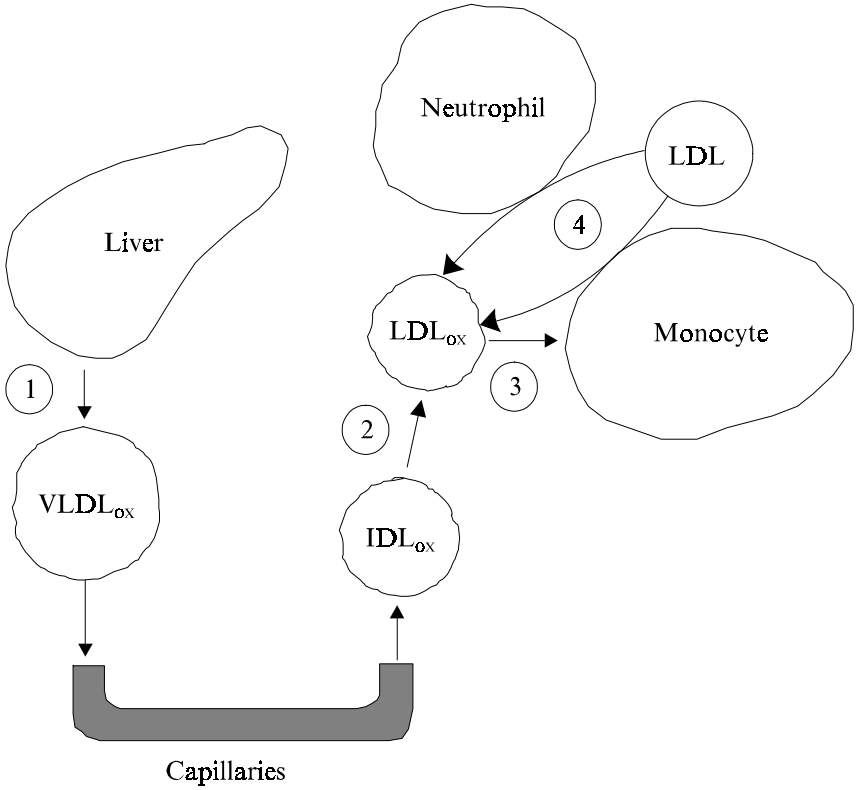

Fig. 2. Formation of oxidized blood lipoproteins. Here and in Figs. 5 and 6: LDL_{ox}: oxidized LDL; IDL_{ox}: oxidized intermediate-density lipoproteins; and $VLDL_{ox}$: oxidized VLDL.

Mechanism of Hypochlorite-Induced LPO

We demonstrated that hypochlorite added to the incubation medium containing human blood LP potentiates accumulation of thiobarbituric acid-reactive substances (TBARS) and fluorescent LPO products [25]. T. Stelmaszynska *et al*. [91] revealed that hypochlorite stimulates accumulation of TBARS and conjugated dienes in LDL. A comparative assay showed that atherogenic LP (LDL and VLDL) are most sensitive to hypochlorite-induced LPO, while high-density lipoprotein (HDL) are least oxidized [4, 78]. Similar results were obtained in measurements of TBARS content in LP incubated in the MPO+H_2O_2+ Cl^- system [78]. LP oxidation in this system was induced by MPO, since the effect was not found in the absence of any enzyme substrate. Similarly to exogenous hypochlorite, the reaction capacity of LP decreased in the order: VLDL—LDL—HDL [78].

To study the mechanism underlying hypochlorite-induced LPO of fatty acids, we used phosphatidylcholine liposomes. Incubation of hypochlorite with phosphatidylcholine liposomes led to accumulation of LPO products (hydroperoxides, conjugated dienes, and TBARS), which was accompanied by a decrease in the number of phospholipid double bonds [10,13-16,18, 34,72,74,75].

Since hypochlorite-induced peroxidation of LP and phosphatidylcholine liposomes is inhibited by radical trap α-tocopherol and butylated hydroxytoluene, this process involves free radicals [13,74].

All free radical reactions have the stage of initiation, which can occur in the aqueous or lipid phase of the liposomal suspension (Fig. 3). Initiation in the aqueous phase is associated with the reaction between hypochlorite and H_2O_2, $O_2^{\bullet-}$, or metal ions of alternating valency formed or present as admixtures in the incubation medium [33,72]. Hypochlorite reacts with H_2O_2 with the formation of singlet oxygen ($^1O_2$); the reaction of hypochlorite with $O_2^{\bullet-}$ or metal ions yields $OH^{\bullet}$ (Fig. 3). Initiation in the lipid phase is related to the interaction between hypochlorite and functional groups of phosphatidylcholine, including polar phosphocholine group, saturated (-CH_2-CH_2-) or unsaturated (-HC=CH-) bonds in acyl chains, or minor components of unsaturated lipids (primarily LPO products, Fig. 3).

Previous studies showed that initiation of hypochlorite-induced LPO is not determined by its interaction with water-soluble and low-molecular-weight compounds $O_2^{\bullet-}$, H_2O_2, $^1O_2$, and oxochlorates (OCl_2^-, OCl_3^-, and OCl_4^-), which are formed or present as admixtures in the reaction medium [13,19,74]. Figure 3 shows that hypochlorite reacts with Fe^{2+} (bimolecular rate constant 114±7/mol/sec, pH 7.2, Fig. 3). However, Fe^{2+} and Fe^{3+} are not involved in the initiation of hypochlorite-induced LPO [13,23,74]. At the same time, chemiluminescence data suggest that Fe^{2+} play a role in hypochlorite-induced LPO [23,79].

Hypochlorite attacks -HC=CH- bonds, but not phosphocholine or carboxylic group and saturated bonds in acyl chains. ^{1}H-Nuclear magnetic resonance showed that this reaction yields chlorohydrin [34]:

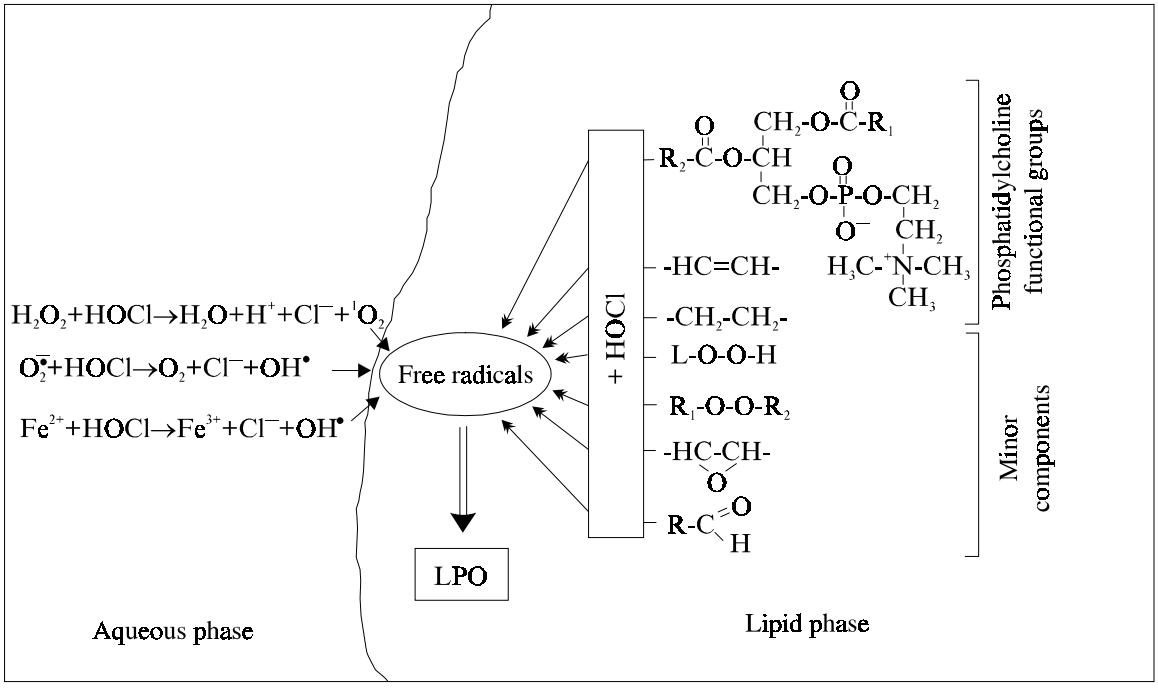

Fig. 3. Initiation of hypochlorite-induced LPO in aqueous and lipid phases of phosphatidylcholine liposome suspension.

$$-HC{=}CH-+HOCl \rightarrow HC(Cl)-CH(OH)-.$$

According to current concepts, this reaction is mediated by the mechanism of electrophilic binding by the double bond, involves no free radicals, and does not yield activators of free radical LPO [20].

Studies of the kinetics of interaction between hypochlorite and preoxidized (to a different degree) liposomes showed that hypochlorite reacts with LPO products, including malonic dialdehyde [4,96]. However, it remains unclear whether hypochlorite can react with aldehydes with the formation of free radical intermediates. This reaction is probably mediated by a molecular mechanism including oxidation of aldehyde to the corresponding acid.

Unsaturated lipids also contain other LPO products, *e.g.*, hydroperoxides, dialkyl peroxides, diacyl peroxides, alkylacyl peroxides, and epoxides (Fig. 3). The hydroperoxide group is the only oxygen-containing functional group reacting with hypochlorite [17, 21]. Incubation of liposomes containing unsaturated -HC=CH- bonds and tert-butyl, cumene, or linoleic acid hydroperoxide with hypochlorite or in the MPO+H_2O_2+Cl^- system was accompanied by accumulation of LPO products in liposomes [21,72,73,75]. The increase in TBARS content was completely inhibited by butylated hydroxytoluene, hypochlorite traps methionine and taurine, and MPO inhibitor azide, but not by $OH^\bullet$ trap mannitol [73].

Thus, the hydroperoxide group was the only oxygen-containing organic compound reacting with hypochlorite. This reaction resulted in accumulation of LPO products in unsaturated lipids. Hydroperoxide in a concentration of 2.5-30 nmol/mg lipid produced significant effects [73,75]. It should be emphasized that the content of hydroperoxides in native and freshly isolated LDL estimated by various methods varies from 10 to 20 nmol/mg LDL [45,46,57,71,85].

The mechanisms underlying the reaction between hypochlorite and hydroperoxide group remain unclear. Two possible mechanisms were hypothesized. The process probably leads to the formation of $^1O_2$ (similarly to H_2O_2, Fig. 3) [21] or synthesis of free radical intermediates:

$$HOCl+LOOH- \begin{cases} \rightarrow {}^1O_2+LOH+H^++Cl^- \\ \rightarrow \text{Free radicals } (LO^\bullet \text{ and } LOO^\bullet). \end{cases}$$

These reactions can activate LPO.

Special experiments showed that no $^1O_2$ is generated in the reaction of hypochlorite with hydroperoxide [21]. Di-tert-butyl peroxide is the main product of the reaction between hypochlorite and tert-butyl hydroperoxide [21,34]. Its synthesis is probably related to the formation of alkoxyl ($LO^\bullet$) and peroxyl ($LOO^\bullet$) radicals. This assumption was confirmed by experiments with spin traps demonstrating the formation of alkyl, peroxyl, and alkoxyl radicals after incubation of tert-butyl hydroperoxide with human polymorphonuclear leukocytes or in the MPO+H_2O_2+Cl^- system [14]. The formation of radical adducts was inhibited by azide. Polymorphonuclear leukocytes with low MPO activity produced only 20-30% radical intermediates compared to normal cells [41].

Thus, initiation of hypochlorite-induced LPO is realized through the reaction of hypochlorite produced by activated neutrophils or monocytes with organic hydroperoxides (LOOH). This reaction results in the formation of free radical intermediates (but not $^1O_2$), including strong LPO activators alkoxyl and peroxyl radicals (Fig. 4).

Hypochlorite-Induced Modification of Blood LP

In vivo hydroperoxide groups are localized in the hydrophobic region of the lipid phase adjacent to unsaturated bonds in acyl chains. The question arises: whether water-soluble hypochlorite can enter the lipid phase of LP and biological membranes? Experiments with spin-labeled stearic acid analogues used as paramagnetic probes showed that hypochlorite penetrates at least the surface phospholipid layer of LDL and oxidizes radical fragments of probes localized at various distances from the surface of these particles [26]. This property probably contributes to high potency of hypochlorite in initiating LPO in LP [4,11,25,28,32, 77-79].

Hypochlorite entering the surface layer of LP interacts with antioxidants α-tocopherol, carotenoids,

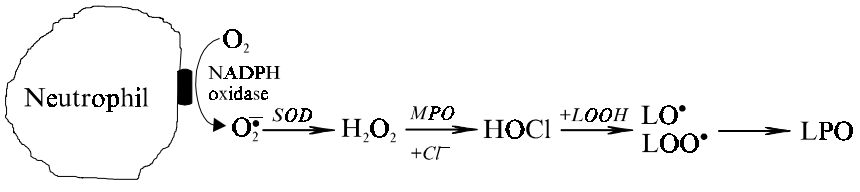

Fig. 4. Initiation of hypochlorite-induced LPO by neutrophils. LOOH: lipid hydroperoxide; $LO^\bullet$ and $LOO^\bullet$: alkoxyl and peroxyl radicals, respectively. Here and in Figs. 5 and 6: MPO, myeloperoxidase.

and xanthophylls [28]. Degradation of antioxidants impairs LP resistance to free radical LPO [28]. On the one hand, it leads to accumulation of primary (conjugated dienes), secondary (TBARS), and end fluorescent products of LPO [4,25,78,79]. On the other hand, this process is accompanied by impairment of physicochemical properties of the lipid phase manifested in decreased mobility and increased polarity up to C_{12} of the acyl chain [27], increased content of mobile protein SH groups, and increased negative surface charge of LP [24]. These changes are similar to those revealed in autoxidized LP after incubation at 37°C in air [30, 76,80].

Modification of physicochemical properties and structural reorganization of LP impair their interaction with the cells and lipid transport. Experiments with cultured J774.2 macrophages showed that hypochlorite-modified LDL cause more pronounced accumulation of cholesterol than native LDL. This effect increased with increasing the concentration of LDL [77].

Incubation of human blood monocytes with native and oxidized LDL increases the contents of phospholipids (by 18.3±8.1 and 16.0±6.1%, respectively) and cholesterol (by 99.6±12.4 and 166.2±23.5%, respectively) compared to incubation without LDL. During incubation of monocytes with oxidized LDL, at least 50% cholesterol were directly transferred into cell membrane without endocytosis [30,76].

Erythrocytes, whose plasma membranes do not contain LP receptors, are a convenient model for studying the nonspecific transfer of cholesterol from LP. We revealed that native LDL act as cholesterol donors in relation to erythrocytes. Autoxidized or hypochlorite-preoxidized LDL more intensively transported cholesterol into various cells. This effect increased with increasing in the degree of LDL oxidation. Incubation of erythrocytes with HDL decreased cholesterol content in cells. Preoxidation of HDL inhibited their cholesterol-acceptor properties [20,30,76].

Thus, peroxidative modification of LP intensifies cholesterol transfer from LDL to cells and completely blocks cholesterol-acception by HDL. These changes promote cholesterol accumulation in cells.

It should be emphasized that blood LP from patients with coronary heart disease are similar to oxidized LP: cholesterol easily migrates from LDL, but is poorly accepted by HDL [22,30,80]. Probably, peroxidative modification of LDL and HDL during atherosclerosis is accompanied by a decrease in the effective volume of hydrophobic regions in the lipid phase (similarly to the lipid phase of liposomes) [3].

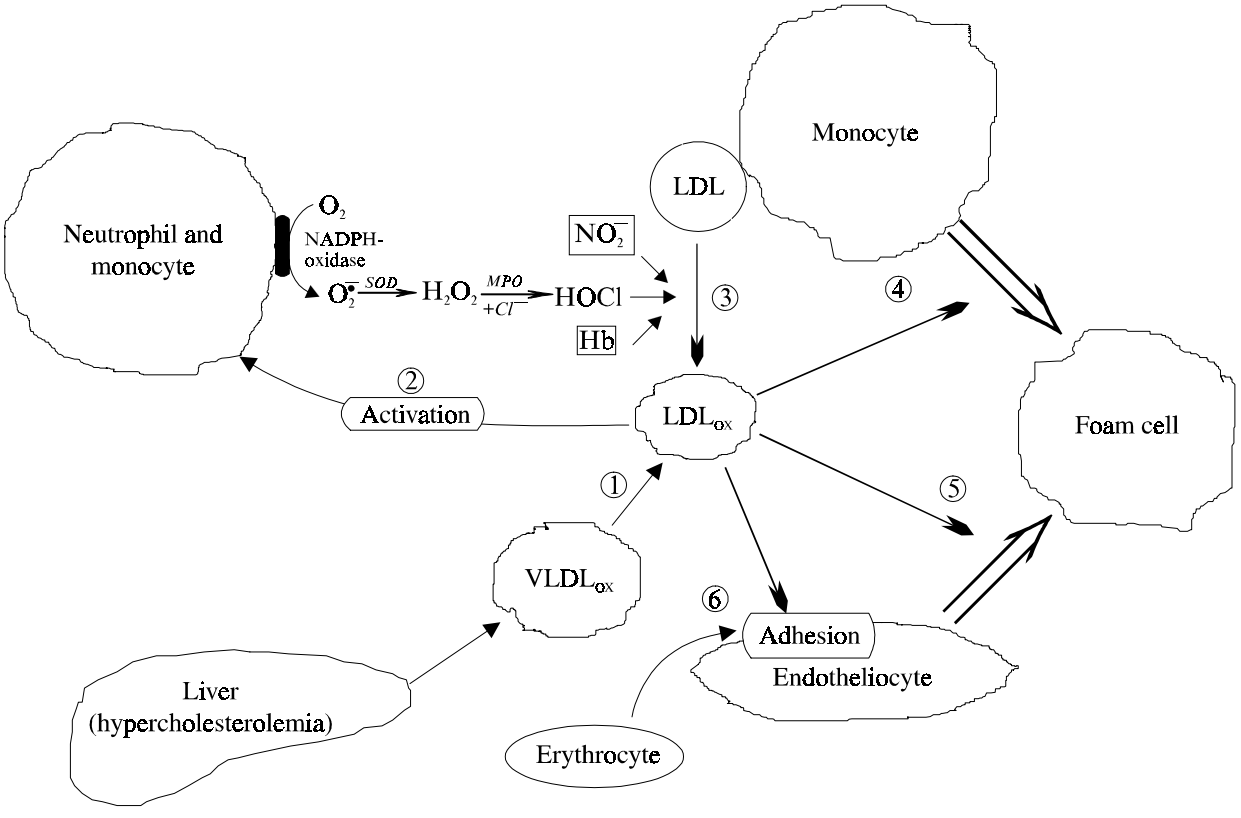

Fig. 5. Formation of oxidized blood LP and their involvement in activation of monocytes and neutrophils and development of early stages of atherosclerosis.

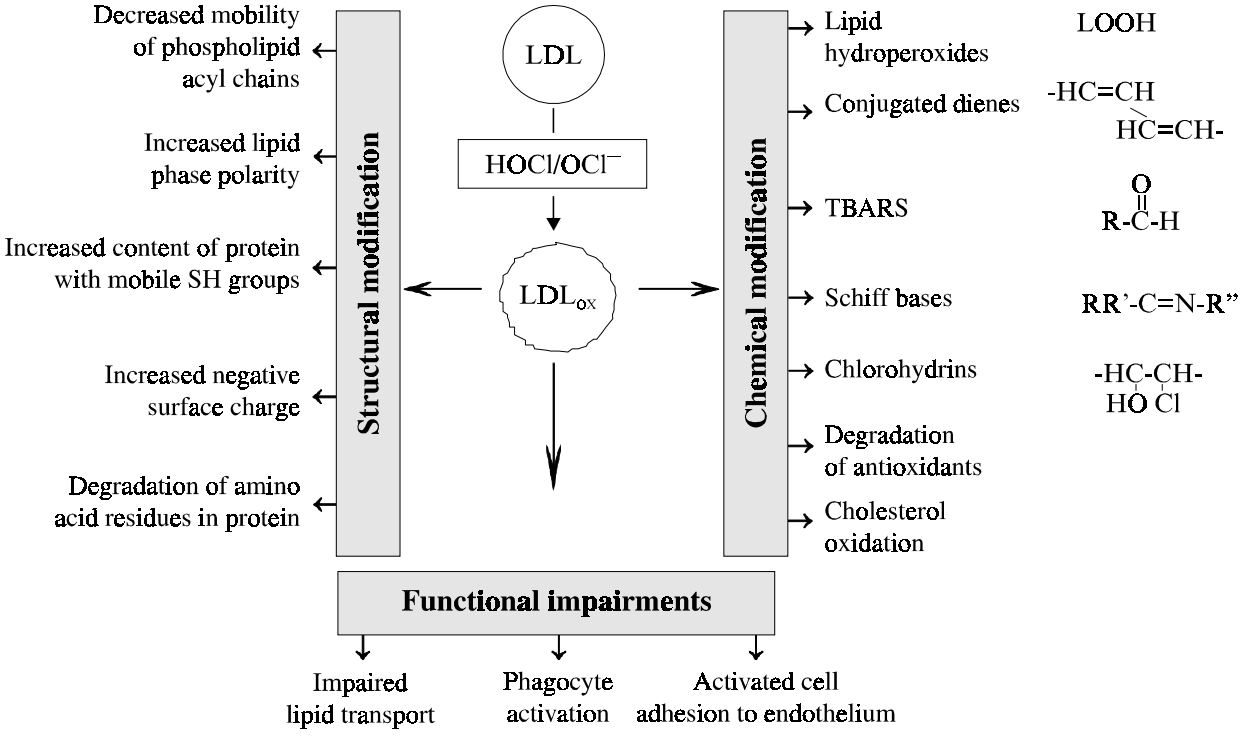

Fig. 6. Hypochlorite-induced changes in chemical composition, structure, and functions of LDL.

The distribution of spin label 3-doxyl-17β-hydroxy-5α-androstane between the aqueous and lipid phases in the suspension of native and oxidized LDL confirms our assumption. It was found that oxidation decreases the number of binding sites in LDL, where hydrophobic molecules of the probe and, probably, stearin can be localized [30,80]. Therefore, cholesterol easily migrates from LDL, but is poorly accepted by HDL. Increased polarity of the lipid phase and high surface charge in autoxidized and hypochlorite-oxidized LP also promote these changes [24,27,30,80].

Thus, free radical modification of LP intensifies cholesterol accumulation in cells containing (monocytes and macrophages) and not containing (erythrocytes) LP receptors. The direct transfer of cholesterol from LDL to plasma membranes not mediated by endocytosis plays an important role in these changes. Formation of foam cells and pathogenesis of atherosclerotic vascular damages are probably associated with nonspecific cholesterol migration to monocytes/macrophages.

Hypochlorite-Induced Adhesion of Blood Cells to the Endothelium

Oxidized LDL act as chemoattractants for blood cells, cause expression of adhesion molecules [40,43] and, therefore, stimulate adhesion of leukocytes [62-64], neutrophils [63], and other cells to the endothelium. Cell adhesion to the vascular surface reflects the early stage of cardiovascular diseases, including atherosclerosis [9,68,99]. We studied the effects of hypochlorite and LDL modified by $HOCl/OCl^-$ in various concentrations on adhesion of blood cells to the endothelium. Experiments were performed on the simplest cell model of human erythrocytes, whose adhesion to the vascular surface plays an important role in the formation of atheromatous plaques [8].

Preincubation of endothelial cell (EC) monolayer with $HOCl/OCl^-$ intensified erythrocyte adhesion of the endothelial surface. This effect increased with increasing $HOCl/OCl^-$ concentration to 50 μM [2]. Preincubation of EC with LDL modified by $HOCl/OCl^-$ (up to 250 μM) increased the cholesterol/phospholipid molar ratio in EC and stimulated erythrocyte adhesion to the endothelium. $HOCl/OCl^-$ in a concentration above 50 μM or LDL modified by 500 μM $HOCl/OCl^-$ produced a cytotoxic effect on EC, decreased the cholesterol/phospholipid molar ratio, and inhibited erythrocyte adhesion to the endothelium. Therefore, $HOCl/OCl^-$ in physiological concentrations stimulates adhesion of blood cells to the endothelium and promotes cholesterol accumulation in vascular wall EC by affecting the endothelium or modifying LDL [2]. These changes can play an important role in the pathogenesis of cardiovascular diseases.

Since free radical traps prevent LDL oxidation [40,67] (including that induced by hypochlorite [73,

79]), expression of adhesion molecules [43], and cell adhesion to the endothelium [47], it can be suggested that the effects of hypochlorite and hypochlorite-modified LDL on the endothelium are mediated by free radical LPO. This is consistent with previous data that LDL preoxidized with hypochlorite stimulate leukocyte adhesion to the endothelium, which depends on the content of LPO products in LDL, but not on the degree of protein oxidation [63].

Conclusion

Figure 5 illustrates the interrelation between the formation of oxidized blood LP and activation of monocytes and neutrophils. The involvement of hypochlorite-modified LP in the early stages of atherosclerosis is hypothesized.

VLDL with increased content of lipid peroxides are secreted by liver cells and transformed into oxidized LDL (Fig. 5, pathway *1*). Oxidized LDL activate monocytes, neutrophils and, probably, other cells (pathway *2*). Activated neutrophils and monocytes secrete MPO and $O_2^{\bar{\bullet}}$ that undergoes dismutation into H_2O_2. Since physiological concentration of Cl^- is high, these changes provide optimal conditions for reaction **(1)**. Hypochlorite-induced modification of native LP (pathway *3*) is promoted by physiologically active substances, including oxidized NO metabolite (nitrite, NO_2^-) [77] and hemoglobin (Hb) [12]. Marked changes in physicochemical properties of LP are manifested in degradation of antioxidants, increase in LP sensitivity to oxidative modification, accumulation of lipid and protein oxidation products, structural reorganization of apolipoproteins and lipid phase, and rise in the negative surface charge of LP. Figure 6 summarizes our data on changes in the chemical composition, structural reorganization of the lipid and protein phases, and hypochlorite-induced impairment of LP functions.

Modified LP interact with monocytes and macrophages by the receptor-independent or scavenger receptor-mediated mechanism, potentiate cholesterol accumulation, and transform them into foam cells, which reflects the early stage of atherosclerosis (Fig. 5, pathway *4*). Modified LP activate monocytes and neutrophils, intensify free radical LPO, and close a vicious cycle of the formation of oxidized blood LP (pathway *2*). Modified LP increase cholesterol content in EC (pathway *5*), stimulate adhesion of erythrocytes and, probably, other blood cells to the endothelium (pathway *6*), and promote atherosclerotic vascular damages.

Recent studies confirm out hypothesis that hypochlorite-modified LP are involved in atherogenesis. Experimental and clinical observations indicate that atherosclerosis is accompanied by hyperactivity of neutrophils [6,61] and enhanced production and secretion of MPO [70,95]. At the same time, *in vivo* stimulation of phagocytes activates LPO in the plasma, intensifies proliferation of aortic intima cells, and promotes the development of atherosclerosis [50]. Moreover, active MPO was found in atherosclerotically modified human vessels and tissues [44,66].

Experiments showed that neutrophil-derived hypochlorite causes dysfunction of isolated guinea pig heart. Hypochlorite traps taurine [83,84] and methionine [38, 82] prevented the neutrophil-dependent effects of hypochlorite.

Experiments with monoclonal antibodies to hypochlorite-treated LDL demonstrated accumulation of HOCl-modified protein in the arteries of patients with atherosclerosis. Epitopes were mainly associated with monocytes, macrophages, smooth muscle cells, and EC. Their molecular weight was similar to that of apo-B *in vitro* modified with hypochlorite [51,65].

The content of 3-chloro-L-tyrosine (specific marker of hypochlorite-modified protein) in atherosclerotically damaged tissues 6-fold surpasses that in intact aortic intima. Moreover, its concentration in LDL isolated from the aortic intima of patients with atherosclerosis was 30 times higher than in circulating LDL from healthy donors [52,53].

Hypochlorite-modified LDL induce and enhance reactivity of phagocytes, stimulate synthesis of chemokines and chemotaxis of neutrophils [98] and, therefore, promote leukocyte infiltration playing an important role in the early stages of atherosclerosis.

This work was partially supported by the Russian Foundation for Basic Research (grant No. 99-04-48239) and Alexander von Humboldt Foundation (Bonn, Germany).

REFERENCES

1. N. Yu. Govorova, B. P. Sharonov, and S. N. Lyzlova, *Biokhimiya*, **53**, 2025-2032 (1998).
2. E. A. Gorbatenkova, G. M. Artman, and O. M. Panasenko, *Biol. Membrany*, **16**, 437-444 (1999).
3. A. I. Deev, G. E. Dobretsov, J. Arnhold, and Yu. A. Vladimirov, *Ibid*., **6**, 1227-1231 (1989).
4. S. A. Evgina, O. M. Panasenko, V. I. Sergienko, and Y. A. Vladimirov, *Ibid*., **9**, 946-953 (1992).
5. V. E. Kagan, O. N. Orlov, and L. L. Prilipko, *Itogi Nauki i Tekhniki VINITI. Biofizika*, **18**, 5-135 (1986).
6. G. I. Klebanov, *Effects of Lipid and Cholesterol Peroxidation on the Structure and Functions of Membranes and Lipoproteins*, Abstract of Doct. Biol. Sci. Dissertation, Moscow (1991).
7. G. I. Klebanov, Yu. A. Vladimirov, L. Ts. Benov, and S. R. Ribarov, *Byull. Eksp. Biol. Med.*, **105**, No. 6, 674-677 (1988).
8. A. N. Klimov, *Preventive Cardiology* [in Russian], Moscow (1987), pp. 239-316.

9. A. N. Klimov and N. G. Nikul'cheva, *Lipids, Lipoproteins, and Atherosclerosis* [in Russian], St. Petersburg (1995).
10. K. T. Momynaliev, V. M. Govorun, O. M. Panasenko, and V. I. Sergienko, *Byull. Eksp. Biol. Med*., **121**, No. 5, 516-519 (1996).
11. K. T. Momynaliev, O. M. Panasenko, V. M. Govorun, and V. I. Sergienko, *Biol. Membrany*, **12**, 385-390 (1995).
12. A. N. Osipov, E. V. Bryukhanova, T. V. Vakhrusheva, *et al*., *Biofizika*, **42**, 400-407 (1997).
13. O. M. Panasenko and J. Arnhold, *Biol. Membrany*, **13**, 89-99 (1996).
14. O. M. Panasenko, J. Arnhold, K. Arnol'd, *et al*., *Ibid*., **12**, 294-302 (1995).
15. O. M. Panasenko, J. Arnhold, K. Arnol'd, *et al*., *Biofizika*, **41**, 334-341 (1996).
16. O. M. Panasenko, J. Arnhold, Yu. A. Vladimirov, *et al*., *Ibid*., **40**, 1234-1242 (1995).
17. O. M. Panasenko, J. Arnhold, Yu. A. Vladimirov, and V. I. Sergienko, *Biokhimiya*, **60**, 1419-1428 (1995).
18. O. M. Panasenko, J. Arnhold, and V. I. Sergienko, *Biofizika*, **43**, 463-469 (1998).
19. O. M. Panasenko, J. Arnhold, and V. I. Sergienko, *Biol. Membrany*, **14**, 207-211 (1997).
20. O. M. Panasenko, J. Arnhold, V. I. Sergienko, *et al*., *Ibid*., **13**, 271-281 (1996).
21. O. M. Panasenko, J. Arnhold, and Yu. Shiller, *Biokhimiya*, **62**, 1111-1121 (1997).
22. O. M. Panasenko, T. V. Vol'nova, O. A. Azizova, and Yu. A. Vladimirov, *Byull. Eksp. Biol. Med*., **106**, No. 9, 277-280 (1988).
23. O. M. Panasenko, S. A. Evgina, E. S. Dremina, *et al*., *Biol. Membrany*, **12**, 191-199 (1995).
24. O. M. Panasenko, S. A. Evgina, S. V. Nikitin, and V. I. Sergienko, *Biofizika*, **40**, 545-550 (1995).
25. O. M. Panasenko, S. A. Evgina, and V. I. Sergienko, *Electrochemical Methods in Medical Practice* [in Russian], Moscow (1991), pp. 7-8.
26. O. M. Panasenko, S. A. Evgina, and V. I. Sergienko, *Byull. Eksp. Biol. Med.*, **115**, No. 4, 358-360 (1993).
27. O. M. Panasenko, S. A. Evgina, and V. I. Sergienko, *Ibid*., **116**, No. 8, 153-155 (1993).
28. O. M. Panasenko, O. O. Panasenko, K. Briviba, and G. Sis, *Biokhimiya*, **62**, 1332-1338 (1997).
29. O. M. Panasenko, A. G. Sdvigova, V. I. Sergienko, and Yu. M. Lopukhin, *Byull. Eksp. Biol. Med*., **113**, No. 2, 143-144 (1992).
30. O. M. Panasenko and V. I. Sergienko, *Biol. Membrany*, **10**, 341-382 (1993).
31. A. G. Sdvigova, O. M. Panasenko, I. V. Luk'yashchenko, *et al*., *Vopr. Med. Khimii*, **39**, No. 2, 30-33 (1993).
32. V. I. Sergienko, M. A. Murina, O. M. Panasenko, *et al*., *Vestn. Ros. Akad. Med. Nauk*, No. 3, 48-53 (1995).
33. E. Sh. Yakutova, E. S. Dremina, S. A. Evgina, *et al*., *Biofizika*, **39**, 275-279 (1994).
34. J. Arnhold, O. M. Panasenko, J. Schiller, *et al*., *Chem. Phys. Lipids*., **78**, 55-64 (1995).
35. J. Arnhold, O. M. Panasenko, J. Schiller, *et al*., *Z. Naturforsch*., **51c**, 386-394 (1996).
36. O. I. Aruoma and B. Halliwell, *Biochem. J.*, **248**, 973-976 (1987).
37. P. Avogaro, G. Bon Bittolo, and G. Cazzolato, *Arteriosclerosis*, **8**, 79-87 (1988).
38. D. Bagchi, D. K. Das, R. M. Engelman, *et al*., *Eur. Heart J*., **11**, 800-813 (1990).
39. C. Bernofsky and S. W. O'Dea, *Free Radic. Res. Commun*., **2**, 129-136 (1986).
40. A. L. Catapano, *Angiology*, **48**, 39-44 (1997).
41. W. Chamulitrat, M. S. Cohen, and R. P. Mason, *Free Radic. Biol. Med*., **11**, 439-445 (1991).
42. M. S. Cohen, B. E. Britigan, D. J. Hassett, and G. M. Rosen, *Ibid*., **5**, 81-88 (1988).
43. L. Cominacini, U. Garbin, A. F. Pasini, *et al*., *Ibid*., **22**, 117-127 (1997).
44. A. Daugherty, J. L. Dunn, D. L. Rateri, J. W. Heinecke, *J. Clin. Invest*., **94**, 437-444 (1994).
45. M. El-Saadani, H. Esterbauer, M. El-Sayed, *et al*., *J. Lipid Res*., **30**, 627-630 (1989).
46. H. Esterbauer, J. Gebicki, H. Puhl, and G. Jurgens, *Free Radic. Biol. Med*., **13**, 341-390 (1992).
47. G. A. Ferns, L. Forster, A. Stewart Lee, *et al*., *Atherosclerosis*, **100**, 171-181 (1993).
48. A. M. Fogelman, I. Shechter, J. Scager, *et al*., *Proc. Natl. Acad. Sci. USA*, **77**, 2214-2218 (1980).
49. B. Garner, R. T. Dean, and W. Jessup, *Biochem. J*., **301**, 421-428 (1994).
50. P. Gorog, F. J. Semeria, and D. A. Gorog, *Atherosclerosis*, **111**, 47-53 (1994).
51. L. J. Hazell, L. Arnold, D. Flowers, *et al*., *J. Clin. Invest*., **97**, 1535-1544 (1996).
52. S. L. Hazen, J. R. Crowley, D. M. Mueller, and J. W. Heinecke, *Free Radic. Biol. Med*., **23**, 909-916 (1997).
53. S. L. Hazen and J. W. Heinecke, *J. Clin. Invest*., **99**, 2075-2081 (1997).
54. J. W. Heinecke, *Free Radic. Biol. Med*., **3**, 65-73 (1987).
55. J. W. Heinecke, L. Baker, H. Rosen, and A. Chait, *J. Clin. Invest*., **77**, 757-761 (1986).
56. J. W. Heinecke, W. Li, H. L. Daehnke, and J. A. Goldstein, *J. Biol. Chem*., **268**, 4069-4077 (1993).
57. N. Hogg, C. Rice Evans, V. Darley Usmar, *et al*., *Arch. Biochem. Biophys*., **314**, 39-44 (1994).
58. E. G. Janzen, L. T. Jandrisits, and D. L. Barber, *Free Radic. Res. Commun*., **4**, 115-123 (1987).
59. G. Jurgens, H. F. Hoff, G. M. Chisolm, and H. Esterbauer, *Chem. Phys. Lipids*., **45**, 315-336 (1987).
60. B. Kalyanaraman and P. G. Sohnle, *J. Clin. Invest*., **75**, 1618-1622 (1985).
61. P. Kienbaum, M. Braun, T. Hohlfeld, *et al*., *J. Cardiovasc. Pharmacol*., **25**, 774-781 (1995).
62. H. A. Lehr, C. Hubner, D. Nolte, *et al*., *Res. Exp. Med. (Berl.).*, **191**, 85-90 (1991).
63. L. Liao, T. Y. Aw, P. R. Kvietys, and D. N. Granger, *Arterioscler. Thromb. Vasc. Biol*., **15**, 2305-2311 (1995).
64. L. Liao and D. N. Granger, *Am. J. Physiol*., **268**, H1643-H1650 (1995).
65. E. Malle, L. Hazell, R. Stocker, *et al*., *Arterioscler. Thromb. Vasc. Biol*., **15**, 982-989 (1995).
66. E. Malle, C. Woenckhaus, G. Waeg, *et al*., *Am. J. Pathol*., **150**, 603-615 (1997).
67. J. Matz, T. L. Andersson, G. A. Ferns, and E. E. Anggard, *Atherosclerosis*, **110**, 241-249 (1994).
68. G. M. McGorisk and C. B. Treasure, *Curr. Opin. Cardiol*., **11**, 341-350 (1996).

69. H. F. McMurray, S. Parthasarathy, and D. Steinberg, *J. Clin. Invest*., **92**, 1004-1008 (1993).
70. A. Mohacsi, B. Kozlovszky, I. Kiss, *et al*., *Biochim. Biophys. Acta*, **1316**, 210-216 (1996).
71. V. J. O'Leary, V. M. Darley Usmar, L. J. Russell, and D. Stone, *Biochem. J.*, **282**, 631-634 (1992).
72. O. M. Panasenko, *Biofactors*, **6**, 181-190 (1997).
73. O. M. Panasenko and J. Arnhold, *Free Radic. Res*., **30**, 479-487 (1999).
74. O. M. Panasenko, J. Arnhold, J. Schiller, *et al*., *Biochim. Biophys. Acta*, **1215**, 259-266 (1994).
75. O. M. Panasenko, J. Arnhold, Y. A. Vladimirov, *et al*., *Free Radic. Res*., **27**, 1-12 (1997).
76. O. M. Panasenko, O. A. Azizova, and Yu. A. Vladimirov, *Sov. Med. Rev. Sect. B*., **3**, Pt. 3, 1-74 (1992).
77. O. M. Panasenko, K. Briviba, L.-O. Klotz, and H. Sies, *Arch. Biochem. Biophys*., **343**, 254-259 (1997).
78. O. M. Panasenko, S. A. Evgina, R. K. Aidyraliev, *et al*., *Free Radic. Biol. Med*., **16**, 143-148 (1994).
79. O. M. Panasenko, S. A. Evgina, and E. S. Driomina, *Ibid*., **19,** 133-140 (1995).
80. O. M. Panasenko, T. V. Vol'nova, O. A. Azizova, and Yu. A. Vladimirov, *Ibid*., **10**, 137-148 (1991).
81. O. M. Panasenko, T. V. Vol'nova, A. N. Osipov, *et al*., *Biomed. Sci*., **2**, 581-589 (1991).
82. K. Prasad, R. Kapoor, and J. Kalra, *Circ. Shock*, **36**, 265-276 (1992).
83. P. Raschke, B. F. Brcker, B. Leipert, *et al*., *Bas. Res. Cardiol*., **88**, 321-339 (1993).
84. P. Raschke, P. Massoudy, and B. F. Brcker, *Free Radic. Biol. Med*., **19**, 461-471 (1995).
85. C. Rice-Evans, D. Leake, K. R. Bruckdorfer, and A. T. Diplock, *Free Radic. Res.*, **25**, 285-311 (1996).
86. J. Schiller, J. Arnhold, W. Grunder, and K. Arnold, *Biol. Chem. Hoppe Seyler*, **375**, 167-172 (1994).
87. I. Schraufstatter, P. A. Hyslop, J. H. Jackson, and C. G. Cochrane, *J. Clin. Invest.*, **82**, 1040-1050 (1988).
88. B. P. Sharonov, N. I. Govorova, S. N. Lyzlova, *Biochem. Int*., **19**, 27-35 (1989).
89. U. P. Steinbrecher, *Biochim. Biophys. Acta*, **959**, 20-30 (1988).
90. U. P. Steinbrecher, S. Parthasarathy, D. S. Leake, *et al*., *Proc. Natl. Acad. Sci. USA*, **81**, 3883-3887 (1984).
91. T. Stelmaszynska, E. Kukovetz, G. Egger, and R. J. Schaur, *Int. J. Biochem*., **24**, 121-128 (1992).
92. J. J. Van den Berg, C. C.Winterbourn, and F. A. Kuypers, *J. Lipid Res*., **34**, 2005-2012 (1993).
93. C. E. Van Rensburg, A. M. Van Staden, and R. Anderson, *Free Radic. Biol. Med*., **11**, 285-291 (1991).
94. C. E. Van Rensburg, A. M. Van Staden, R. Anderson, E. J. Van Rensburg, *Mutat. Res*., **265**, 255-261 (1992).
95. M. T. Ventura, I. Puzziferri, C. Tortorella, *et al.*, *Rec. Prog. Med*., **84**, 503-510 (1993).
96. C. C. Winterbourn and A. C. Carr, *Arch. Biochem. Biophys*., **302**, 461-467 (1993).
97. C. C. Winterbourn, J. J. van den Berg, E. Roitman, F. A. Kuypers, *Arch. Biochem. Biophys.*, **296**, 547-555 (1992).
98. C. Woenckhaus, A. Kaufmann, D. Bussfeld, *et al*., *Clin. Immunol. Immunopathol.*, **86**, 27-33 (1998).
99. K. K. Wu and P. Thiagarajan, *Annu. Rev. Med*., **47**, 315-331 (1996).