Participation of Cholesterol in Hypochlorite-Induced Oxidation of Cholesterol-Phosphatidylcholine Liposomes

K. T. Momynaliev, V. M. Govorun, O. M. Panasenko, and V. I. Sergienko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 516-519, May, 1996
Original article submitted March 6, 1995

The effect of cholesterol on hypochlorite-induced lipid peroxidation is studied in cholesterol-phosphatidylcholine liposomes. Cholesterol is shown to promote the accumulation of lipid peroxidation products in the presence of 0.1-3 mM hypochlorite anion in the incubation medium. The content of 2-thiobarbituric acid-reactive products is maximal at a cholesterol:phosphatidylcholine molar ratio of 1:1. On the other hand, in the presence of low hypochlorite concentrations (10-100 μM) cholesterol is found to inhibit lipid peroxidation.

Key Words: *hypochlorite; lipid peroxidation; cholesterol; phosphatidylcholine; liposomes*

Cholesterol (CH) is known to have a marked effect on the processes of lipid peroxidation (LPO) in the cell membrane. For instance, an increased content of CH in the bilayer leads to inhibition of LPO induced by UV radiation or ions of alternating valency [1] due to restriction of the molecular mobility of fatty acid residues of phospholipids (PL).

In various membrane systems LPO may also be induced by reactive oxygen species [4]. Hypochloric acid (HOCl) and its ionized form hypochlorite anion (OCl^-) have been recently shown to act as LPO inductors [2,10,11]. $HOCl/OCl^-$ is a potent oxidant produced by activated phagocytes in the reaction between Cl^- and H_2O_2 catalyzed by myeloperoxidase, and plays an important role in their antibacterial action [8]. $HOCl/OCl^-$ is known to react with CH, yielding its chlorine and hydroxy derivatives [9,13]. However, whether CH participates in $HOCl/OCl^-$-induced LPO remains unclear.

Our objective was to study the effect of CH on hypochlorite-induced LPO in liposomes with various CH/PL molar ratios.

MATERIALS AND METHODS

Liposomes were prepared by injecting an alcohol solution of yolk phosphatidylcholine (PC) and CH in a K^+-phosphate buffer, pH 7.5 [7]. The liposomes were dialyzed against 0.15 M NaCl and 0.1 M K^+-phosphate buffer (pH 7.5) at 4°C for 1 hour. The content of PL [14] and CH [6] was determined and the CH/PL molar ratio was calculated. The size of liposomes, determined by photon-correlation spectroscopy on an Autosizer IIc laser nephelometer (Malvern), ranged from 120 to 150 nm. Sodium hypochlorite (NaOCl) was obtained electrochemically using an EDO-3 apparatus [5]. For lipid oxidation NaOCl was added to a certain concentration (or an equivalent volume of 0.15 M NaCl in the control) to the liposome suspension and incubated at 37°C with access to air. The amount of secondary lipoperoxides measured in the reaction with 2-thiobarbituric acid (TBA-reactive products, TBARP) served as the measure of LPO [12]. The data were processed statistically using the Student *t* test.

RESULTS

The NaOCl-induced oxidation of PC-liposomes with various contents of CH was studied in two experimental series. In series I the total content of yolk PC and CH remained constant but the molar ratio between them was varied in such a way that the proportion of PC decreased and that of CH rose. In series II the content of PC was the same in all liposomes but the molar ratio of CH was higher. The data of these experimental series are pre-

Research Institute of Physicochemical Medicine, Russian Ministry of Health and the Medical Industry, Moscow

0007-4888/96/0005-0468$15.00

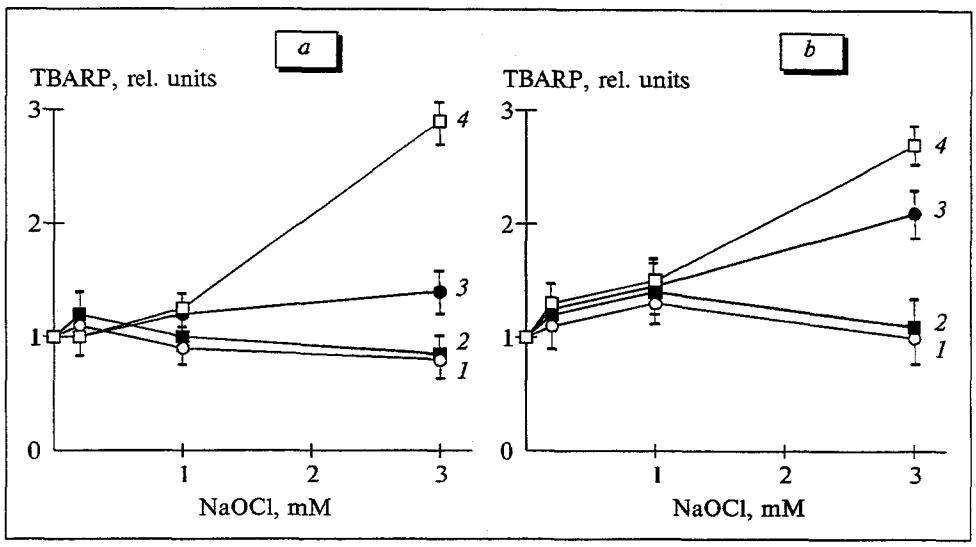

Fig. 1. Content of TBARP in CH/PL liposomes incubated with NaOCl. *a*) total concentration of PC and CH 1.2 mg/ml; *b*) concentration of PC 0.6 mg/ml. CH/PL molar ratio: *1*) 0; *2*) 0.2; *3*) 0.5; *4*) 1.0. TBARP content is expressed as the ratio of TBARP in the experimental sample to that in the control. In the control samples NaOCl was replaced with NaCl.

sented in Fig. 1, *a* and *b*, respectively. Increasing the ratio of CH in liposomes is seen to result in a more pronounced accumulation of TBARP even when the total lipid content in the sample remains constant (i.e., the absolute amount of PC decreases as CH increases, Fig. 1, *a*). At the molar ratio CH/PL=1 the content of TBARP surpassed that in the control 2.9-fold. A sharp rise of TBARP occurs in liposomes containing 50% CH or more (Fig. 1, *3*, *4*). This may be due to the well-known ability of CH to form clusters as its concentration in liposomes increases, and to segregation of PL domains [3].

Figure 2 shows kinetic curves of TBARP accumulation in liposomes prepared from yolk PC and in liposomes with a molar content of CH/PL=1 at a NaOCl concentration of 3 mM. In the CH-containing liposomes, oxidation of PL is more pronounced (a 3.5-fold rise of TBARP, Fig. 2, *3*). TBARP did not accumulate in a CH suspension incubated with NaOCl under the same conditions, i.e., the secondary LPO products did not arise from CH.

It should be noted that the accumulation of TBARP in the CH-containing liposomes was noticeable only at a relatively high concentration of NaOCl (3 mM). In order to evaluate the effect of CH on $HOCl/OCl^-$-induced LPO of PL in the presence of low concentrations of NaOCl (10-100 μM) the incubation was prolonged to 24 h, since a 2-hour incubation was not enough to detect TBARP accumulation spectrophotometrically. As follows from Fig. 3, the increase of the CH content in the liposomes inhibited TBARP accumulation in comparison with PC liposomes. No accumulation of LPO products was observed in liposomes with the CH/PL=1 molar ratio (Fig. 3, *1*).

Thus, CH inhibits LPO in the presence of low concentrations of $HOCl/OCl^-$ and, conversely, activates it at high concentrations. There are two possible explanations for these phenomena. First, the relationship between the accumulation of TBARP in PL liposomes and the NaOCl concentration usually yields a curve with an extremum [2,10,11] (Fig. 1, *b*, *1*, *2*), where the ascending portion is due to TBARP accumulation and the descending part (at relatively high concentrations of NaOCl) is due to the reaction between the excess NaOCl with TBARP [15], converting aldehydes into TBA-inactive acids. NaOCl is known to react with CH, yielding various hydroxy and chlorine derivatives [9,13], which do not form a stained complex with TBARP (Figs. 2, *1* and 3, *1*). Taking the above into consideration, we may assume that CH incorporated into PL liposomes will react with NaOCl, thereby reducing its concentration

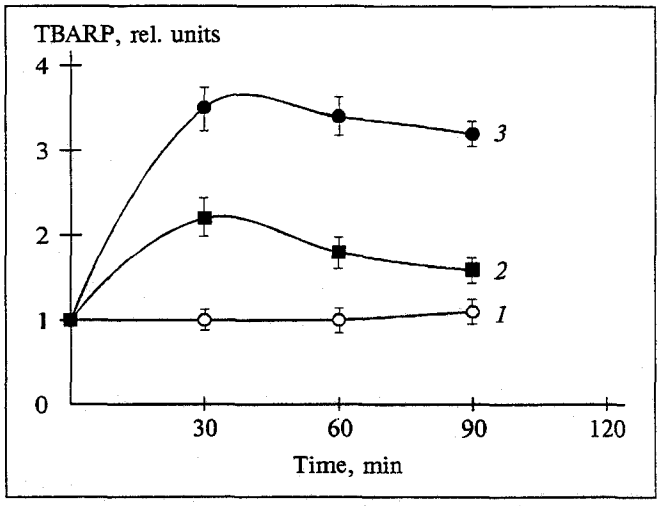

Fig. 2. Accumulation of TBARP in PC (*2*) and CH/PC (*3*) liposomes (CH/PL= 1) incubated in the presence of 3 mM NaOCl. The content of TBARP is expressed as the ratio of TBARP at any time point to the initial content of TBARP. *1*) accumulation of TBARP in CH suspension incubated with 3 mM NaOCl.

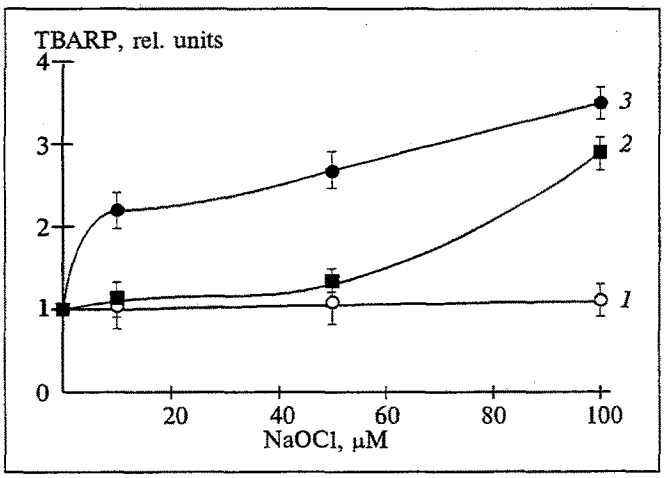

Fig. 3. Content of TBARP in CH/PC liposomes as a function of concentration of NaOCl. Concentration of PC=0.6 mg/ml. CH/PL molar ratio: *1*) 1; *2*) 0.5; *3*) 0. Incubation time 24 h.

in the reaction mixture. At low NaOCl concentrations this results in a lowered level of TBARP (Fig. 1). At higher concentrations of HOCl/OCl^-, when the decrease of TBARP due to direct oxidation by NaOCl becomes noticeable, the excess HOCl/OCl^- participating in the oxidation of TBARP will be utilized in the reaction with CH. This results in a higher level of TBARP in CH-containing liposomes in comparison with liposomes consisting solely of PL.

The second explanation can be summarized as follows: CH or products of its reaction with NaOCl, upon being incorporated into the PL bilayer, somehow affect the HOCl/OCl^--induced LPO, for instance, by changing the physicochemical properties of the bilayer.

In order to verify the above assumption, we prepared liposomes from PC or dimyristoyl-PC (DMPC) alone or in combination with CH (PC/CH and DMPC/CH liposomes, respectively) and incubated them in dialysis tubes that were placed in flasks with HOCl/OCl^- solution, after which the level of TBARP was measured in each tube. The oxidation system consisted of two dialysis tubes filled with PC and DMPC/CH liposomes (experiment 1), or PC/CH and DMPC liposomes (experiment 2), or only PC liposomes (experiment 3). The data are presented in Table 1.

TABLE 1. Content of TBARP in Different Types of Liposomes after Incubation with NaOCl (*M±m*, *n*=6)

No. of experiment	Composition of liposomes in one oxid system	TBARP, μmol/g PL
1	PC	88.1±5.6
	DMPC/CH	20.3±2.4
2	PC/CH	179.2±12.8
	DMPC	22.5±2.9
3	PC	36.4±4.0
4	Control	20.7±2.5

Note. Concentration of PC, DMPC, and CH in liposomes=1.04 mg/ml. PC/CH and DMPC/CH=1. Liposomes were incubated for 2 h at 37°C in the presence of 3 mM NaOCl. The control is the sample without liposomes.

It should be noted that no accumulation of TBARP was observed in DMPC and DMPC/CH liposomes. A comparison of the data of experiments 1 and 2 shows that CH incorporated in PC/CH liposomes promotes the TBARP accumulation much more in comparison with the situation where CH is present in the same oxidation system but is spatially separated from the PC liposomes. This seems to confirm the assumption that physicochemical changes in the bilayer induced by CH (or products of its reaction with NaOCl) may affect the HOCl/OCl^--induced LPO in liposomes composed of unsaturated PC, thereby promoting the accumulation of secondary LPO products.

Our findings suggest that the effect of CH on the HOCl/OCl^--induced accumulation of TBARP in liposomes composed of unsaturated PL may be mediated, first, through direct interaction between CH and NaOCl, and second, through interaction between CH or its chlorine and hydroxy derivatives and the PL bilayer, which possibly modulates its physicochemical properties and may affect the LPO process. However, the present study does not provide complete insight into the possible mechanisms of the LPO-stimulating effect of CH in the bilayer at relatively high concentrations of HOCl/OCl^- (3 mM) and calls for further investigation of this phenomenon.

REFERENCES

1. Yu. A. Vladimirov and A. I. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972), pp. 1-252.
2. S. A. Evgina, O. M. Panasenko, V. I. Sergienko, and Yu. A. Vladimirov, *Biol. Membr.*, **9**, 946-953 (1992).
3. V. G. Ivkov and G. N. Berestovskii, in: *Dynamic Structure of the Lipid Bilayer* [in Russian], Moscow (1981), pp. 40-244.
4. O. M. Panasenko and V. I. Sergienko, *Biol. Membr.*, **10**, 341-382 (1993).
5. V. I. Sergienko, A. K. Martynov, Yu. B. Vasil'ev, *et al.*, *Vopr. Med. Khim.*, **36**, 28-32 (1990).
6. *Biological Membranes. A Practical Approach*, Eds. J. B. C. Findlay and W. H. Evans, IRL Press, Ltd. Oxford - Washington, DC (1987).
7. S. Batary and E. D. Korn, *Biochim. Biophys. Acta*, **298**, 1015-1019 (1973).
8. S. W. Edwards, *Biochemistry and Physiology of Neutrophils*, Cambridge (1994).
9. J. W. Heinecke, D. M. Mueller, A. Bohrer, and J. Turk, *Biochemistry*, **33**, 10127-10136 (1994).
10. O. M. Panasenko, J. Arnhold, J. Schiller, *et al.*, *Biochim. Biophys. Acta*, **1215**, 259-266 (1994).
11. O. M. Panasenko, S. A. Evgina, R. K. Aidyraliev, *et al.*, *Free Radic. Biol. Med.*, **16**, 143-148 (1994).
12. M. Uchiyama and M. Michara, *Anal. Biochem.*, **86**, 271-278 (1978).
13. J. J. Van der Berg, C. C. Winterbourn, and F. A. Kuypers, *J. Lipid Res.*, **34**, 2005-2012 (1993).
14. V. E. Vaskovsky, E. J. Kostetsky, and J. M. Vasendin, *J. Chromatogr.*, **114**, 129-141 (1975).
15. C. C. Winterbourn and A. C. Carr, *Arch. Biochem. Biophys.*, **302**, 461-467 (1993).