Free Rad. Res., Vol. 30, pp. 479–487
Reprints available directly from the publisher
Photocopying permitted by license only

Linoleic Acid Hydroperoxide Favours Hypochlorite- and Myeloperoxidase-Induced Lipid Peroxidation

O.M. PANASENKO[a] and J. ARNHOLD[b,*]

[a]*Research Institute of Physico-Chemical Medicine, M. Pirogovskaja 1a, Moscow, RUS-119828, Russia;*
[b]*Institute of Medical Physics and Biophysics, School of Medicine, University of Leipzig, Liebigstr. 27, D-04103 Leipzig, Germany*

Accepted by Prof. H. Sies

(Received 7 September 1998; In revised form 17 November 1998)

Liposomes composed of soybean phosphatidylcholine were peroxidized using the reagent sodium hypochlorite or the myeloperoxidase–hydrogen peroxide–Cl^- system. Linoleic acid hydroperoxide previously prepared from linoleic acid by means of lipoxidase was incorporated into liposomes. The yield of thiobarbituric acid reactive substances (TBARS) continuously increased with higher amounts of hydroperoxide groups after the initiation of lipid peroxidation by hypochlorous acid producing systems. The accumulation of TBARS was inhibited by scavengers of free radicals such as butylated hydroxytoluene and by the scavengers of hypochlorous acid, taurine and methionine. Lipid peroxidation was also prevented by sodium azide or chloride free medium in the myeloperoxidase–hydrogen peroxide–Cl^- system. Here we show for the first time that the reaction of hypochlorous acid with a biologically relevant hydroperoxide yields free radicals able to cause further oxidation of lipid molecules.

Keywords: Hypochlorous acid, myeloperoxidase, lipid peroxidation, hydroperoxides, linoleic acid, lipoxidase

INTRODUCTION

The heme enzyme myeloperoxidase (MPO) is released from stimulated polymorphonuclear leukocytes. Compound I of MPO formed after the addition of hydrogen peroxide to the native enzyme reacts easily with chloride to yield the powerful oxidizing and chlorinating species hypochlorous acid.[1] Together with other reactive oxygen species generated, enzymes and bacteriocidal proteins released by neutrophils, the myeloperoxidase–hydrogen peroxide–Cl^- system is an important part of the defence mechanisms of higher organisms against microbial invasion.

Hypochlorous acid exhibits many effects on biologically important molecules, including oxidation of sulfhydryl and thioether groups[2–4] and formation of chloramines from amino groups of

*Corresponding author. Tel.: ++49-341-9715705. Fax: ++49-341-9715709. E-mail: arnj@server3.medizin.uni-leipzig.de.

proteins,[5,6] fragmentation of polysaccharides and chlorination of their N-acetyl groups[7,8] as well as changes in nucleotides.[9] We have focused our attention on reactions of $HOCl/OCl^-$ with phospholipids during the last few years. Hypochlorous acid initiates a lipid peroxidation in model phospholipid systems[10–12] and in lipoproteins, especially low density lipoproteins.[13–15] Several lipid peroxidation products were detected in these reactions, including primary products (diene conjugates and oxygen containing species), aldehydes[11–15] and Schiff bases.[14] Hypochlorous acid reacts also with olefinic double bonds of lipids to yield chlorohydrins.[16–18]

Myeloperoxidase and hypochlorous acid have been implicated in the mechanisms of pathogenesis of arteriosclerosis. There are several aspects to their involvement, including histological findings of MPO in arteriosclerotic lesions,[19] the formation of tyrosyl radicals,[20] the formation of nitryl chloride[21] and transformation of low density lipoproteins into arteriosclerogenic particles.[22,23] $HOCl/OCl^-$ as well as other oxidants (tyrosyl radical, $^{\bullet}OH$, $^1O_2$) formed by the myeloperoxidase system are also able to initiate a lipid peroxidation.[24,25]

In order to understand the mechanisms of initiation of lipid peroxidation by $HOCl/OCl^-$ we investigated different possible pathways for the formation of reactive intermediates that are able to yield free lipid radicals and to initiate the peroxidation reaction. Neither traces of metal ions nor other reactive oxygen species ($O_2^{\bullet-}$, $H_2{}^1O_2$, $^1O_2$ $^{\bullet}OH$), were found to be responsible for this initiation reaction in our system.[11,26] On the other hand, a higher accumulation of thiobarbituric acid reactive substances (TBARS) has been observed after the addition of the organic hydroperoxides, tert-butyl or cumene hydroperoxides to phospholipids.[27] Both of these hydroperoxides react with $HOCl/OCl^-$ to give products that can only be formed assuming a homolytic cleavage of the hydroperoxide group with the formation of the alkoxy radical.[26,27]

In this investigation we used linoleic acid hydroperoxide as an example of a hydroperoxide that can be formed in biological systems. Evidence is given that liposomes containing linoleic acid hydroperoxide accumulate more TBARS after the addition of hypochlorous acid or using the MPO–hydrogen peroxide–Cl^- system.

MATERIALS AND METHODS

Chemicals

Myeloperoxidase (EC 1.11.1.7) from human polymorphonuclear leukocytes was purchased from Calbiochem (La Jolla, CA, USA). Lipoxidase (EC 1.13.11.12) Type IV from soybean and sodium hypochlorite were obtained from Sigma (Deisenhofen, Germany). Butylated hydroxytoluene (BHT), mannitol, and a colour reagent for the determination of hydroperoxides (Cat. Nr. 14350) were from Merck (Darmstadt, Germany). Linoleic acid (cis,cis-9,12-octadecadienoic acid), taurine, methionine, 5,5′-dithiobis(2-nitrobenzoic acid), sodium azide, and hydrogen peroxide were used from Fluka (Neu-Ulm, Germany). L-α-Phosphatidylcholine from soybean (SPC) was also a product from Fluka. Its fatty acid composition given by the supplier was 16 : 0 (14%), 18 : 0 (4%), 18 : 1 (12%), 18 : 2 (65%), 18 : 3 (15%). 2-Thiobarbituric acid was purchased from Serva (Heidelberg, Germany). All organic solvents (UV spectroscopy grade) and all other chemicals were from Fluka.

Preparation of Linoleic Acid Hydroperoxide

Linoleic acid was incubated with lipoxidase to yield linoleic acid hydroperoxide.[28] Briefly, linoleic acid (2.14 mmol/l, final concentration) was dissolved in 10 mmol/l phosphate buffer containing 0.14 mol/l NaCl at pH 12.0. The pH-value was lowered to 9.0 and lipoxidase (3.7 μg/ml that is 1,300 U/ml) was added. This mixture was incubated for 30 min at room

temperature. The pH was lowered to 2.0 after which linoleic acid hydroperoxide and unreacted linoleic acid were extracted with chloroform. In a similar way a chloroform extract of linoleic acid was obtained without the use of lipoxidase.

Liposomes

Multilamellar liposomes were prepared from soybean phosphatidylcholine and a mixture of linoleic acid or linoleic acid/linoleic acid hydroperoxide. Aliquots of chloroform extracts of SPC, linoleic acid and linoleic acid hydroperoxide preparation were mixed together such that the combined amount of linoleic acid and linoleic acid hydroperoxide was always 10 mol% of the total lipid content. Chloroform extracts were evaporated to dryness. Liposomes were prepared by dissolving the lipid film in 0.14 mol/l NaCl, 10 mmol/l phosphate (pH 7.4) and vortexing vigorously for 30 s.

To obtain small unilamellar vesicles multilamellar liposomes were sonicated using a Branson Sonifier 250 at 40 mW for 2 min at room temperature. In some experiments liposomes were prepared at pH 6.0.

In order to determine lipid hydroperoxides in liposome samples, 50 µl liposome suspension were mixed with 950 µl commercially available reagent for hydroperoxide determination. The concentration of hydroperoxide was determined as free iodine liberated.[29]

Incubation of Liposomes with Hypochlorous Acid

A stock solution of NaOCl was kept in the dark at 4°C. The hypochlorite concentration was determined spectrophotometrically at pH 12 using $\varepsilon_{290} = 350\,M^{-1}\,cm^{-1}$.[30] It was diluted with 0.14 mol/l NaCl, 10 mmol/l phosphate immediately prior to use and adjusted to pH 7.4. In a typical experiment liposomes (final concentration 2 mg/ml) were incubated with NaOCl at 37°C for 40 min. After incubation BHT (2 mmol/l, final concentration) was added to prevent further accumulation of peroxidation products. Control measurements showed that the pH of 7.4 did not change after the addition of NaOCl.

Incubation of Liposomes with the MPO–Hydrogen Peroxide–Cl^- System

Aliquots of myeloperoxidase dissolved in 0.14 mol/l NaCl, 10 mmol/l phosphate (pH 6.0) were stored at −80°C. The chlorinating activity of MPO was measured using taurine and 5-thio-2-nitrobenzoic acid.[31]

Liposomes (1 or 2 mg/ml) were incubated with myeloperoxidase (3.5 or 10 µg/ml, that is 0.07 or 0.2 U/ml, respectively). The reaction was started by addition of H_2O_2. The incubation was made at room temperature under constant mixing at pH 6.0. After incubation BHT (2 mmol/l, final concentration) was added to prevent further accumulation of peroxidation products. In some experiments taurine, methionine, butylated hydroxytoluene, sodium azide or mannitol were added to the incubation mixture. Control samples did not contain myeloperoxidase, hydrogen peroxide or chloride. In order to obtain chloride free solutions, Cl^- was replaced by SO_4^{2-} in all solutions used for liposome and MPO preparations.

Lipid Peroxidation Products

Thiobarbituric acid reactive substances (TBARS) were determined using a colorimetric assay.[32] TBARS were calculated from the increase in extinction at 532 nm ($\varepsilon_{532} = 1.56 \times 10^5\,M^{-1}\,cm^{-1}$) over the baseline in the absorption spectra.

RESULTS

Effects of Linoleic Acid Hydroperoxide on $HOCl/OCl^-$-induced Lipid Peroxidation

The incubation of liposomes composed of phosphatidylcholines from soybean and linoleic acid (10 mol%) with hypochlorous acid leads to an

increase of thiobarbituric acid reactive substances (TBARS). The production of lipid peroxidation products increased with the concentration of $HOCl/OCl^-$ (Figure 1). A similar increase of TBARS has been also reported in egg yolk phosphatidylcholine and low density lipoproteins after treatment with the reagent hypochlorous acid.[11–15]

The addition of hydroperoxides gives rise to a much higher accumulation of lipid peroxidation products induced by $HOCl/OCl^-$. A mixture of linoleic acid and linoleic acid hydroperoxide was incorporated into the lipid matrix such that their total amount was 10 mol%. A mixture of SPC with linoleic acid (without any linoleic acid hydroperoxide) contained 5.7 nmol hydroperoxides per mg lipid. These hydroperoxides resulted from pre-existing oxidation products in SPC. Figure 1 compares the production of TBARS induced by $HOCl/OCl^-$ in liposomes containing respectively 5.7 and 30.1 nmol hydroperoxides per mg lipid. Over three times as much TBARS has accumulated in some of the hydroperoxide treated samples.

The dependence of TBARS accumulation on the concentration of hydroperoxides is shown in Figure 2. Although there was an increase in TBARS with increase in hydroperoxide concentration in control samples (no addition of $HOCl/OCl^-$), a much greater accumulation of these lipid peroxidation products was observed at all data points after addition of hypochlorous acid.

Tert-butyl and cumene hydroperoxides have been reported to increase the production of TBARS under hypochlorous acid.[27] Our data show that linoleic acid hydroperoxide, a more common biological hydroperoxide, also favours

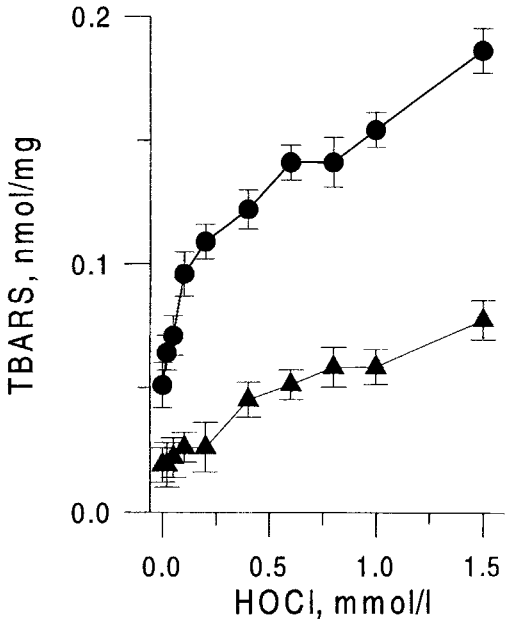

FIGURE 1 Accumulation of TBARS in liposomes (total lipid concentration 2 mg/ml) composed of SPC and either 10 mol% linoleic acid (▲) or 10 mol% of a mixture of linoleic acid and linoleic acid hydroperoxide (●) as a function of the concentration of hypochlorous acid. The initial concentrations of hydroperoxides were 5.7 (▲) or 30.1 (●) nmol/mg lipid. Liposomes were prepared in 0.14 mol/l NaCl, 10 mmol/l phosphate buffer, pH 7.4. Liposomes were incubated with NaOCl at 37°C during 40 min. All data are given as means and standard deviation ($n = 4$).

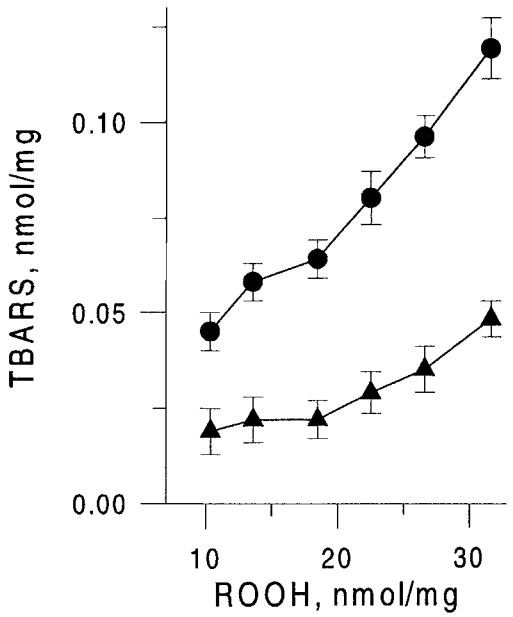

FIGURE 2 Accumulation of TBARS in SPC liposomes in relation to the concentration of hydroperoxides in the presence (●) or absence (▲) of NaOCl (200 μmol/l, final concentration). The concentration of hydroperoxide groups was modified by the incorporation of linoleic acid hydroperoxide into liposomes. All other conditions were as in Figure 1. All data are given as means and standard deviation ($n = 4$).

the initiation of lipid peroxidation. The phenolic antioxidant butylated hydroxytoluene (BHT) inhibited completely at a concentration of 20 μmol/l the accumulation of TBARS induced by $HOCl/OCl^-$ (200 μmol/l) in soybean phosphatidylcholine liposomes containing linoleic acid hydroperoxide (18 nmol/mg lipid) while 50% inhibition occurred at 2 μmol/l BHT (data not shown). A similar inhibition profile by BHT has been found in $HOCl/OCl^-$-induced peroxidation of egg yolk phosphatidylcholine.[11,12]

Effects of Linoleic Acid Hydroperoxide on Lipid Peroxidation Induced by the MPO–Hydrogen Peroxide–Cl^- System

In place of sodium hypochlorite the MPO–hydrogen peroxide–Cl^- system was used in all further experiments. Small unilamellar liposomes composed of soybean phosphatidylcholine and linoleic acid (10 mol%) were incubated with myeloperoxidase (MPO) and H_2O_2 in the presence of chloride anions. Our liposomes contained 5.7 nmol hydroperoxides per mg lipid. In this sample, the accumulation of TBARS depended on the concentration of MPO and increased with time up to 10 min (3.5 μg/ml MPO) and 30 min (10 μg/ml MPO) (Figure 3). No accumulation of TBARS was found neither in a chloride-free medium nor in the absence of hydrogen peroxide (data not shown).

The effect of MPO concentration on TBARS accumulation can be explained assuming a preferable binding of hydroperoxides by the enzyme. This hypothesis is also supported by the fact that

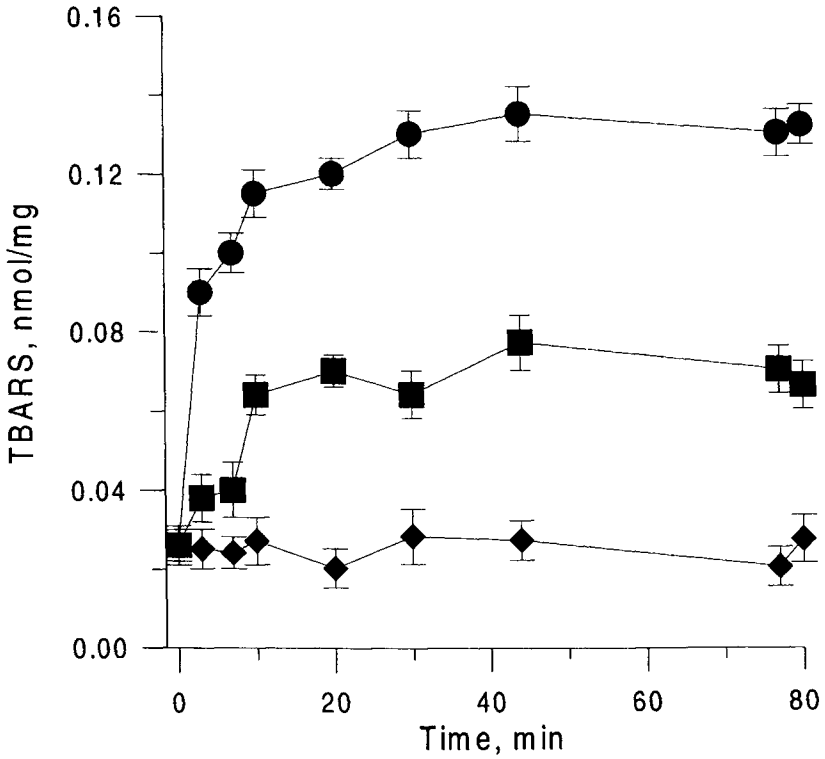

FIGURE 3 Kinetics of accumulation of TBARS in liposomes (total lipid concentration 1 mg/ml) composed of SPC and 10 mol% linoleic acid using the MPO–hydrogen peroxide–Cl^- system to produce HOCl. Small unilamellar liposomes and MPO were prepared in 0.14 mol/l NaCl, 10 mmol/l phosphate buffer, pH 6.0. The concentration of MPO was 3.5 μg/ml (■) or 10 μg/ml (●, ◆). The production of HOCl was started by addition of 250 μmol/l H_2O_2. Some experiments (◆) were performed in the absence of chloride. Incubation was made at 25°C. All data are given as means and standard deviation ($n = 4$).

the reagent NaOCl causes smaller effects on TBARS accumulation at 250 μmol/l in comparison to the MPO–hydrogen peroxide–Cl^- system using 250 μmol/l H_2O_2.

A much greater production of TBARS occurred in liposomes containing additionally linoleic acid hydroperoxides (Figure 4). Control experiments revealed that there was no increase in TBARS during incubation of liposomes without any additives, or after addition of MPO in the absence of either H_2O_2 or Cl^-. In Figure 4 only data for the Cl^--free medium are shown. Only when using the complete MPO–hydrogen peroxide–Cl^- system, there was an additional accumulation of TBARS dependent on the concentration of hydroperoxides in the liposome samples. These facts indicate that hypochlorous acid produced by the MPO–hydrogen peroxide–Cl^- system is responsible for the initiation of lipid peroxidation.

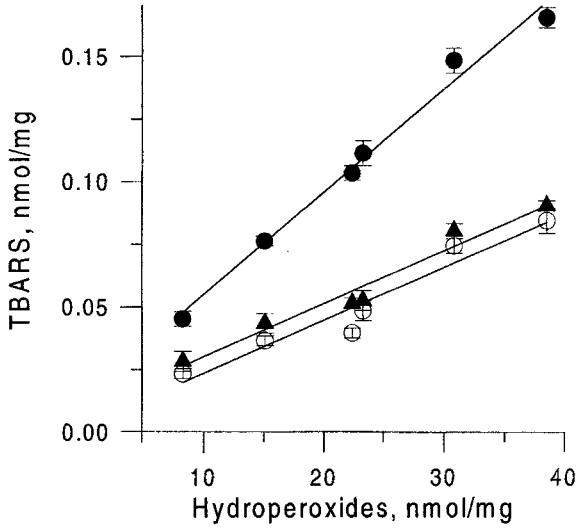

FIGURE 4 Accumulation of TBARS in SPC liposomes (total lipid concentration 2 mg/ml) as a function of the concentration of hydroperoxides using the MPO–hydrogen peroxide–Cl^- system to produce HOCl. The concentration of hydroperoxide groups was adjusted by incorporation of linoleic acid hydroperoxide into liposomes. Liposomes (●,▲) were incubated with MPO (3.5 μg/ml) and H_2O_2 (50 μmol/l, a second addition of H_2O_2 was made after 15 min) at 25°C for 35 min. Some experiments (▲) were performed in the absence of chloride. Initial values (○) of TBARS are also given. All other conditions were as in Figure 3. All data are given as means and standard deviation ($n = 4$).

Further evidence for the role of hypochlorous acid produced by MPO was obtained using different inhibitors (Table I). Taurine and methionine inhibited the accumulation of TBARS in our liposomes. Both compounds are known as scavengers of hypochlorous acid.[33] The MPO inhibitor sodium azide[5] also inhibited the accumulation of TBARS. On the other hand, mannitol, a known scavenger for hydroxyl radicals,[34] was ineffective at all concentrations used. Butylated hydroxytoluene effectively inhibited the accumulation of lipid peroxidation products.

DISCUSSION

It is known that lipid peroxidation products accumulate in vesicles composed of egg yolk phosphatidylcholine[10–13] and human blood lipoproteins[13–15] upon the action of exogenous hypochlorous acid or myeloperoxidase and H_2O_2 in Cl^--containing solutions. Free radicals appear to be involved in hypochlorite-induced lipid peroxidation because two well-known scavengers of free radicals, α-tocopherol and butylated hydroxytoluene, completely inhibit the accumulation of TBARS.[11,12]

Our data show that soybean phosphatidylcholine is also peroxidized by sodium hypochlorite or the MPO–hydrogen peroxide–Cl^- system. Moreover, the yield of TBARS is continuously increased with increasing concentrations of hydroperoxide groups in the prepared liposomes. The content of hydroperoxide in liposomes was changed by adding linoleic acid hydroperoxide previously prepared from linoleic acid by means of lipoxidase. The inhibition of additional TBARS formation by taurine and methionine, both effective scavengers of hypochlorous acid,[33] and by the use of Cl^--free medium indicate, that HOCl produced by the MPO–hydrogen peroxide–Cl^- system is the reactive species in initiation of lipid peroxidation in our experiments.

TBARS arise from the breakdown of polyunsaturated fatty acid residues with three and more

TABLE I Effects of inhibitors on the oxidation of liposomes containing linoleic acid hydroperoxide using the system $MPO + H_2O_2 + Cl^-$

Incubation system[a]	Inhibitors, mmol/l	TBARS, nmol/mg lipid[b]	Inhibition of TBARS accumulation, %
Only liposomes	—	0.059 ± 0.003	—
+ MPO + Cl^-	—	0.061 ± 0.004	—
+ MPO + H_2O_2	—	0.060 ± 0.003	—
+ MPO + Cl^- + H_2O_2	—	0.107 ± 0.005	—
+ MPO + Cl^- + H_2O_2 + BHT	0.002	0.060 ± 0.003	100
	0.01	0.059 ± 0.004	100
	0.1	0.058 ± 0.002	100
+ MPO + Cl^- + H_2O_2 + mannitol	0.1	0.108 ± 0.003	0
	1	0.106 ± 0.004	2.1
	10	0.103 ± 0.004	8.5
+ MPO + Cl^- + H_2O_2 + taurine	0.1	0.093 ± 0.005	29.8
	1	0.064 ± 0.003	91.5
	10	0.060 ± 0.002	100
+ MPO + Cl^- + H_2O_2 + methionine	0.1	0.067 ± 0.005	85.1
	1	0.059 ± 0.002	100
	10	0.058 ± 0.003	100
+ MPO + Cl^- + H_2O_2 + azide	0.1	0.080 ± 0.004	57.4
	1	0.063 ± 0.006	100
	10	0.059 ± 0.007	100

[a]Liposomes containing 25.1 nmol hydroperoxide per mg lipid were used. All other conditions were as in Figure 4.
[b]All data are given as means and standard deviation ($n = 4$).

double bonds.[34] Therefore, polyunsaturated phosphatidylcholine, and not linoleic acid, is the source for TBARS additionally formed in our study.

Experiments with linoleic acid hydroperoxide confirm earlier data showing enhanced increase of TBARS in egg yolk phosphatidylcholine liposomes in the presence of tert-butyl or cumene hydroperoxides.[27] Linoleic acid hydroperoxide is a more common biological species than tert-butyl or cumene hydroperoxides. We used this compound as a model for the many possible hydroperoxides formed from phospholipids and fatty acids in biological membranes under conditions of oxidative stress. Only the reaction of $HOCl/OCl^-$ with organic hydroperoxides yields alkoxyl radicals that are able to induce further free radical reactions, including the addition of oxygen, in the lipid matrix.[26,27] $HOCl/OCl^-$ did not react with dialkyl-, diacyl- or alkylacylperoxides nor with epoxides.[27] Other reactive oxygen species such as $O_2^{\bullet -}$, H_2O_2, $^1O_2$, $^\bullet OH$ as well as traces of metal ions could also be excluded from playing any role in $HOCl/OCl^-$-induced lipid peroxidation of phospholipids.[11,26] However, detail mechanisms of the reaction between organic hydroperoxides and hypochlorous acid are unknown to us. The hydroperoxide group is apparently split homolytically with the formation of an alkoxyl radical. A reaction mechanism known for hypochlorous acid and hydrogen peroxide to yield singlet oxygen is not applicable to our case. There was no production of singlet oxygen during the reaction of hypochlorous acid with tert-butyl or cumene hydroperoxide.[26,35]

There is an apparent discrepancy between the concentration of NaOCl (200 μmol/l) used or the amount of HOCl (50–250 μmol/l) produced by the myeloperoxidase–hydrogen peroxide–Cl^- system and the amount of additional TBARS (0.2–0.3 μmol/l) formed in the presence of linoleic acid hydroperoxide. This may be explained by the fact that most of hypochlorous acid reacts with double bonds to yield chlorohydrins.[16,18]

Furthermore, although TBARS can be easily detected, they are only a minor product in lipid peroxidation.

Regarding biological significance, it is of interest that the effect of linoleic acid hydroperoxide on the initiation of lipid peroxidation by $HOCl/OCl^-$ appears at concentrations comparable with values found *in vivo*. Various tissues of animals or human beings contain from 0.4 to 19.6 nmol hydroperoxides per mg lipids.[36] Hydroperoxides are regarded as primary products of autoxidation, metal-catalysed oxidation and other types of lipid peroxidation.[37] They are also a product of the action of lipoxidases.[38] An increased level of hydroperoxides in biological membranes and lipoproteins would make these lipids more susceptible to new peroxidation reactions induced by the MPO–hydrogen peroxide–Cl^- system of neutrophils under conditions of oxidative stress.

Acknowledgements

This work was supported by the German Research Council (Grants 436 RUS 113/222/0 and INK 23 A1-1).

References

[1] S.J. Klebanoff (1988). Phagocytic cells: products of oxygen metabolism. In *Inflammation. Basic Principles and Clinical Correlates* (Eds. J.I. Gallin, I.M. Goldstein and R. Snyderman), Raven Press, New York, pp. 391–443.

[2] M.F. Tsan (1982). Myeloperoxidase-mediated oxidation of methionine. *Journal of Cell Physiology*, **111**, 49–54.

[3] C.C. Winterbourn (1985). Comparative reactivities of various biological compounds with myeloperoxidase–hydrogen peroxide–chloride, and similarity of the oxidant to hypochlorite. *Biochimica et Biophysica Acta*, **840**, 204–210.

[4] J. Arnhold, S. Hammerschmidt and K. Arnold (1991). Role of functional groups of human plasma and luminol in scavenging of NaOCl and neutrophil-derived hypochlorous acid. *Biochimica et Biophysica Acta*, **1097**, 145–151.

[5] S.J. Weiss, M.B. Lampert and S.T. Test (1982). Long-lived oxidants generated by human neutrophils: characterization and bioactivity. *Science*, **222**, 625–628.

[6] M.B. Grisham, M.M. Jefferson, D.J. Melton and E.L. Thomas (1984). Chlorination of endogenous amines by isolated neutrophils. *The Journal of Biological Chemistry*, **259**, 10404–10412.

[7] J. Schiller, J. Arnhold, W. Gründer and K. Arnold (1994). The action of hypochlorous acid on polymeric components of cartilage. *Biological Chemistry Hoppe-Seyler*, **375**, 167–172.

[8] J. Schiller, J. Arnhold and K. Arnold (1995). NMR studies of the action of hypochlorous acid on native pig articular cartilage. *European Journal of Biochemistry*, **233**, 672–676.

[9] W.A. Prütz (1998). Interactions of hypochlorous acid with pyrimidine nucleotides, and secondary reactions of chlorinated pyrimidines with GSH, NADH, and other substrates. *Archives of Biochemistry and Biophysics*, **349**, 183–191.

[10] S.M. Sepe and R.A. Clark (1985). Oxidant membrane injury by the neutrophil myeloperoxidase system. I. Characterization of a liposome model and injury by myeloperoxidase, hydrogen peroxide, and halides. *Journal of Immunology*, **134**, 1888–1895.

[11] O.M. Panasenko, J. Arnhold, J. Schiller, K. Arnold and V.I. Sergienko (1994). Peroxidation of egg yolk phosphatidylcholine liposomes by hypochlorous acid. *Biochimica et Biophysica Acta*, **1215**, 259–266.

[12] O.M. Panasenko and J. Arnhold (1996). Mechanisms of hypochlorite-induced peroxidation in phospholipid liposomes. *Membrane and Cell Biology*, **10**, 93–104.

[13] T. Stelmaszynska, E. Kukovetz, G. Egger and R.J. Schaur (1992). Possible involvement of myeloperoxidase in lipid peroxidation. *International Journal of Biochemistry*, **24**, 121–128.

[14] O.M. Panasenko, S.A. Evgina, R.K. Aidyraliev, V.I. Sergienko and Yu.A. Vladimirov (1994). Peroxidation of human blood lipoproteins induced by exogenous hypochlorite or hypochlorite generated in the system of "myeloperoxidase + H_2O_2 + Cl^-". *Free Radical Biology and Medicine*, **16**, 143–148.

[15] O.M. Panasenko, S.A. Evgina, E.S. Driomina, V.S. Sharov, V.I. Sergienko and Yu.A. Vladimirov (1995). Hypochlorite induces lipid peroxidation in blood lipoproteins and phospholipid liposomes. *Free Radical Biology and Medicine*, **19**, 133–140.

[16] C.C. Winterbourn, J.J.M. Van den Berg, E. Roitman and F.A. Kuypers (1992). Chlorohydrin formation from unsaturated fatty acids reacted with hypochlorous acid. *Archives of Biochemistry and Biophysics*, **296**, 547–554.

[17] J.W. Heinecke, W. Li, D.M. Mueller, A. Bohrer and J. Turk (1994). Cholesterol chlorohydrin synthesis by the myeloperoxidase–hydrogen peroxide–chloride system: potential markers for lipoproteins oxidatively damaged by phagocytes. *Biochemistry*, **33**, 10127–10136.

[18] J. Arnhold, O.M. Panasenko, J. Schiller, Yu.A. Vladimirov and K. Arnold (1995). The action of hypochlorous acid on phosphatidylcholine liposomes in dependence on the content of double bonds. Stoichiometry and NMR analysis. *Chemistry and Physics of Lipids*, **78**, 55–64.

[19] A. Daugherty, J.L. Dunn, D.L. Rateri and J.W. Heinecke (1994). Myeloperoxidase, a catalyst for lipid peroxidation, is expressed in human atherosclerotic lesions. *Journal of Clinical Investigation*, **94**, 437–444.

[20] J.W. Heinecke, W. Li, H.L. Daehnke and J.A. Goldstein (1993). Dityrosine, a specific marker of oxidation, is synthesized by the myeloperoxidase–hydrogen peroxide system of human neutrophils and macrophages. *The Journal of Biological Chemistry*, **268**, 4069–4077.

[21] J.P. Eiserich, M. Hristova, C.E. Cross, A.D. Jones, B.A. Freeman, B. Halliwell and A. van der Vliet (1998). Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature*, **391**, 393–397.

[22] L.J. Hazell and R. Stocker (1993). Oxidation of low-density lipoprotein with hypochlorite causes transformation of

the lipoprotein into a high-uptake form for macrophages. *Biochemical Journal*, **290**, 165–172.

[23] L.J. Hazell, J.J.M. Van den Berg and R. Stocker (1994). Oxidation of low-density lipoprotein with hypochlorite causes aggregation that is mediated by modification of lysine residues rather than lipid peroxidation. *Biochemical Journal*, **302**, 297–302.

[24] M.S. Cohen, B.E. Britigan, D.J. Hassett and G.M. Rosen (1988). Phagocytes, O_2 reduction, and hydroxyl radical. *Reviews of Infectious Diseases*, **10**, 1088–1096.

[25] M.I. Savenkova, D.M. Mueller and J.W. Heinecke (1994). Tyrosyl radical generated by myeloperoxidase is a physiological catalyst for the initiation of lipid peroxidation in low density lipoprotein. *The Journal of Biological Chemistry*, **269**, 20394–20400.

[26] J. Arnhold, O.M. Panasenko, J. Schiller, K. Arnold, Yu.A. Vladimirov and V.I. Sergienko (1996). Reaction of hypochlorous acid with hydrogen peroxide and tert-butyl hydroperoxide. ^{1}H-MM spectroscopy and chemiluminescence analyses. *Zeitschrift für Naturforschung*, **51**, 386–394.

[27] O.M. Panasenko, J. Arnhold, Yu.A. Vladimirov, K. Arnold and V.I. Sergienko (1997). Hypochlorite-induced peroxidation of egg yolk phosphatidylcholine is mediated by hydroperoxides. *Free Radical Research*, **27**, 1–12.

[28] M. Maiorino, C. Gregolin and F. Ursini (1990). Phospholipid hydroperoxide glutathione peroxidase. *Methods in Enzymology*, **168**, 448–457.

[29] M. El-Saadani, H. Esterbauer, M. El-Sayed, M. Goher, A.Y. Nassar and G.A. Jürgens (1989). A spectrophotometric assay for lipid peroxides in serum lipoproteins using a commercially available reagent. *Journal of Lipid Research*, **30**, 627–630.

[30] J.C. Morris (1966). The acid ionization constant of HOCl from 5 to 35°. *Journal of Physical Chemistry*, **195**, 133–140.

[31] A.J. Kettle and C.C. Winterbourn (1994). Assays for the chlorination activity of myeloperoxidase. *Methods in Enzymology*, **233**, 502–512.

[32] M. Uchiyama and M. Michara (1978). Determination of malondialdehyde precursor in tissues by thiobarbituric test. *Analytical Biochemistry*, **86**, 271–278.

[33] E.L. Thomas (1979). Myeloperoxidase, hydrogen peroxide, chloride antimicrobial system: nitrogen-chlorine derivatives of bacterial components in their actions against *Escherichia coli*. *Infection and Immunity*, **25**, 110–116.

[34] H. Esterbauer, R.J. Schaur and H. Zollner (1991). Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radical Biology and Medicine*, **11**, 81–128.

[35] O.M. Panasenko, J. Arnhold and J. Schiller (1997). Hypochlorite reacts with an organic hydroperoxide forming free radicals, but not singlet oxygen, and thus initiates lipid peroxidation. *Biochemistry (Moscow)*, **62**, 951–957.

[36] V.E. Kagan, O.N. Orlov and L.L. Prilipko (1986). The problem of endogenous lipid peroxidation products. *Itogi Nauki i Techniki. Serija Biofizika (Russian)*, **18**, 1–36.

[37] G. Carlin (1985). Peroxidation of linoleic acid promoted by human polymorphonuclear leukocytes. *Free Radical Biology and Medicine*, **1**, 255–262.

[38] P. Walstra, J. Verhagen, M.A. Vermeer, G.A. Veldink and J.F.G. Vliegenhart (1987). Demonstration of a 12-lipoxygenase activity in bovine polymorphonuclear leukocytes. *Biochimica et Biophysica Acta*, **921**, 312–319.