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Copper-induced LDL peroxidation: interrelated dependencies of the kinetics on the concentrations of copper, hydroperoxides and tocopherol

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Abstract Excessive uptake of oxidized low density lipoprotein plays a role in the onset of atherosclerosis. Lipid-associated antioxidants, the most abundant of which is tocopherol (vitamin E), are therefore believed to have anti-atherogenic properties. By contrast, hydroperoxides enhance the peroxidation of low density lipoprotein. We demonstrate that none of these compounds markedly affect the maximal rate of oxidation of low density lipoprotein, whereas the lag preceding rapid oxidation is prolonged by tocopherol but shortened by hydroperoxides. The corresponding 'prolongation' and 'shortening' can be compensated by each other in low density lipoprotein preparations enriched with both these compounds. The dependence of the balance between the effects of tocopherol and hydroperoxides on the copper concentration indicates that the antioxidative effect of vitamin E increases with the oxidative stress.

Key words: Low density lipoprotein; Peroxidation; Tocopherol; Hydroperoxide; Atherosclerosis

1. Introduction

The peroxidation of low density lipoprotein (LDL) and the subsequent excessive uptake of the oxidatively-modified LDL via the scavenger receptor are believed to play an important role in atherogenesis [1]. The sensitivity of LDL to oxidation is commonly assayed in vitro by monitoring the kinetics of formation of oxidation products upon exposure of the fractionated LDL to an initiator of the chain reaction such as copper ions or organic generators of free radicals [2]. The kinetics of peroxidation are usually described in terms of the time interval preceding 'bulk oxidation' of the LDL lipids, the maximal rate of oxidation and the maximal accumulation of oxidation products [3]. Although the relevance of any of the in vitro models to in vivo oxidation is questionable, several lines of evidence indicate that the kinetic parameters used to describe the in vitro oxidation correlate with different pathologies [1].

The susceptibility of LDL to oxidation depends, among other factors, on the presence and concentrations of two major groups of compounds, namely lipid-associated pro-oxidants and antioxidants [4]. The most important pro-oxidants are hydroperoxides (LOOH) and related products of oxidation pre-formed in circulating LDL [2]. Thus, enrichment of LDL by LOOH or its pre-treatment by lipoxygenase reduce the lag preceding LDL oxidation [5]. Furthermore, several pathological states associated with a high susceptibility of LDL to peroxidation are characterized by an increased concentration of 'oxidized LDL', namely by elevated concentrations of primary oxidation products of LDL [6–8].

The most abundant radical-scavenging antioxidant present in LDL is α-tocopherol (vitamin E). Although detailed examinations revealed that under certain conditions, this potent antioxidant exhibits pronounced pro-oxidative effects [9], it can still be regarded as the most important lipidic 'line of defence' against lipid oxidation [10].

For a given LDL preparation, the kinetics of oxidation of course depends on the 'oxidative stress', which for in vitro assays can be varied by varying the concentration of the initiator of chain peroxidation [11].

The aim of the current investigation was to characterize systematically the interrelated effects of the concentrations of Cu(II), vitamin E and LOOH on the kinetics of LDL peroxidation. By varying the copper concentration and enrichment of LDL by hydroperoxylinoleic acid and/or vitamin E, we simulated the peroxidation of LDL under different oxidative stress in the presence of different quantities of LOOH and tocopherol. The results shed light on the influence of the LDL composition on its oxidative status.

2. Materials and methods

13-hydroperoxyoctadeca-9,11-dienoic acid was purchased from ICN pharmaceuticals (USA). All the other reagents were obtained from Sigma Chemical.

Human plasma was recovered following blood drawing from individuals fasting for 12 h. The blood was centrifuged in chilled tubes containing Na_2EDTA (0.1% w/v final concentration, pH 7.4) and LDL was then isolated as previously described [12]. Briefly, the plasma underwent sequential flotation at 4°C in KBr density solutions ($d = 1.019$–1.050 g/ml), containing 1 mM Na_2EDTA, pH 7.4, in a LB-70 Beckman ultracentrifuge (Beckman Instruments, Mountain View, CA, USA) using a 70.1 rotor at 40 000 rpm for 20 h. LDL was recovered by tube slicing and re-isolated at the limiting density under the same conditions. Subsequently, LDL was dialyzed at 4°C in the dark for 24 h against five changes of 200 volumes of phosphate-buffered solution (PBS) containing 143 mM NaCl, 3.3 mM Na_2HPO_4 and 3.3 mM NaH_2PO_4 (pH adjusted up to 7.4). The Na_2EDTA was added into the dialysis medium so that after dilution of the LDL to 0.1 μM, the medium contained 0.5 μM EDTA. The 'total copper concentration' (Cu_T) as given in this study for the various oxidation experiments is in fact equal to the total copper concentration minus 0.5 μM. The bound copper (Cu_B) was computed from the previously-determined binding parameters [13].

Enrichment of LDL with either LOOH and/or vitamin E was achieved by adding the calculated volumes of ethanolic solutions of 13-hydroperoxyoctadeca-9,11-dienoic acid and/or tocopherol to LDL dispersions. The mixtures were kept at room temperature for 30 min before the addition of $CuCl_2$. The quantities of the additives were chosen to obtain a 2–4-fold enrichment over their average concentrations in native LDL. Specifically, each LDL particle was enriched on

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Abbreviations: LDL, low density lipoprotein; LOOH, conjugated hydroperoxides

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average by 10 or 20 LOOH molecules and/or by 12 or 24 tocopherol molecules.

Oxidation was monitored using a Kontron double beam spectrophotometer (Uvikon 933) equipped with a 12 cell automatic cell changer. Measurements were carried out at 37°C in 1 cm quartz cuvettes, following the addition of freshly-prepared $CuCl_2$ solutions to LDL solutions (0.1 μM) in PBS (final volume 1.5 ml, pH 7.4). A PBS solution was used as a reference. The studied range of $CuCl_2$ concentrations was 0.5–5 μM. The apparent reproducibility of *OD* measurements and subsequent calculations was usually better than 2%.

The concentrations of reaction products in the time course of LDL peroxidation were determined from the UV absorption at four wavelengths (234, 245, 250 and 268 nm), as previously described [13,14]. The subsequent analysis revealed that the characteristic time points (see below) were quite independent of whether they were evaluated from the absorption at 234 nm or at 245 nm or from the calculated profiles of the accumulation of LOOH. The data presented in this paper were therefore derived from the generally accepted measurements of UV absorption at 234 nm.

Numerical calculations, regression analysis and simulations were performed using Microsoft Excell 97, Mathcad Pro 8 and Microcal Origin 5 software.

2.1. Definition of kinetic parameters

The upper panel of Fig. 1 depicts a typical time course of peroxidation. Characteristic time points were defined from this curve and from the time-dependence of the rate (lower panel) as follows:

1. lag, the intercept of the derivative in the point of the maximal propagation rate on the time axis [15].
2. t_{max}, the time at which the rate of propagation of bulk lipid peroxidation was maximal $(d(OD)/dt)_{max}$ [16].
3. t_{ODmax}, the time at which the accumulation of oxidation products was maximal (OD_{max}), i.e. when $d(OD)/dt = 0$.

The consecutive phases of the copper-induced peroxidation of LDL lipids can be defined by the time intervals between the characteristic time points (lag, t_{max}–lag and t_{ODmax}–t_{max}). These phases differ in their sensitivity towards the inhibitory or pro-oxidative effect of enrichment by tocopherol and LOOH, respectively (see below).

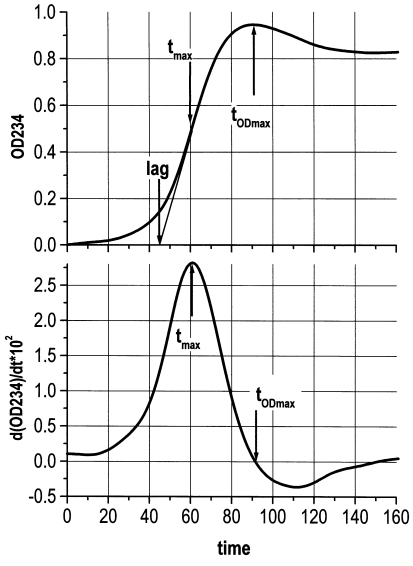

Fig. 1. Definition of the 'characteristic time points' of the kinetics of copper-induced oxidation of LDL, as monitored by the measurement of UV absorption of LDL oxidation products at 234 nm. The upper panel is a typical time course of absorbance (i.e. of product accumulation). The lower panel is the first derivative of this time course and is therefore a time-dependency of the rate of accumulation of absorbing products.

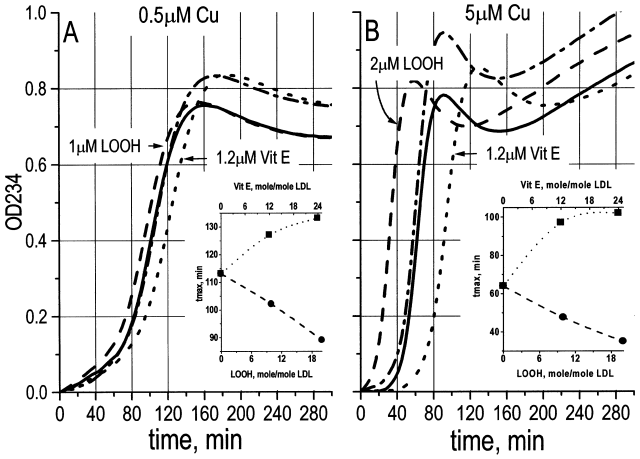

Fig. 2. Kinetic profiles of oxidation of 0.1 μM LDL, native (solid lines) or enriched by different concentrations of vitamin E (dotted lines) or hydroperoxylinoleic acid (dashed lines), as indicated on the figure. Dashed and dotted lines relate to the oxidation kinetics of the preparations, enriched by both vitamin E and LOOH. Insets show the dose-dependent changes of t_{max} upon enrichment of LDL by vitamin E (upper curves) or LOOH (lower curves). A and B represent data for 0.5 μM and 5 μM total copper, respectively.

3. Results

Addition of LOOH to native LDL resulted in a dose-dependent reduction of t_{max}. For each copper concentration, this reduction depended apparently linearly on the concentration of the added LOOH throughout the studied range of LOOH. The decrease of t_{max} was only slightly dependent on the concentration of copper (Fig. 2, insets). By contrast, addition of vitamin E prolonged t_{max} (Fig. 2) and the dose-dependence of t_{max} on vitamin E was more pronounced at high copper concentrations (insets).

Notably, in spite of these marked effects of both LOOH and tocopherol on t_{max} (as well as on the lag and t_{ODmax}), neither of these additives altered significantly either the maximal rates ($(d(OD)/dt)_{max}$) or the maximal accumulation of oxidation products (OD_{max}) (Fig. 2, Table 1. Hence, increasing the initial concentration of either LOOH or vitamin E results in a shift of the whole kinetic curve without affecting the kinetic profile of peroxidation. It thus appears that both these compounds affect only the initial stages of peroxidation. In other words, after a certain stage, the kinetics of peroxidation become independent of either tocopherol or LOOH added to the LDL before its exposure to copper.

In all our experiments, the maximal accumulation of peroxidation products did not depend substantially on the concentrations of either LOOH or vitamin E. Similarly, the maximal accumulation did not depend on the concentration of copper (Fig. 2), although increasing the copper concentration resulted in shortening of the lag, enhanced maximal rate of peroxidation and reduced accumulation of peroxidation products during the initial phases of peroxidation (prior to the lag) in agreement with several previous systematic studies, including ours [13,15,16].

In an attempt to evaluate whether the effects of tocopherol enrichment relate only to the tocopherol/LDL ratio and to the bound copper/LDL ratio or to the absolute concentrations as well, we have studied the effects of tocopherol on the kinetics of oxidation of a 3-fold lower LDL concentration. Similar to

Table 1
Dependence of 'characteristic time points' as defined in Fig. 1 on enrichment of 0.1 μM LDL by vitamin E and LOOH for different copper concentrations

Cu_T ($Cu_{Ba)}$, μM	Additives, (mole/mole LDL)		lag, min	t_{max}, min	t_{ODmax}, min	$(d(OD)/dt)_{max}$, OD234/min	$OD234_{max}$
	LOOH	Vitamin E					
0.5 (0.28)	0	0	71	113	172	0.011	0.76
		12	82	127	185	0.012	0.84
		24	84	133	193	0.011	0.80
	10	0	61	102	158	0.012	0.77
		12	70	111	171	0.012	0.83
		24	72	112	175	0.012	0.79
	20	0	51	89	143	0.012	0.76
		12	57	98	159	0.013	0.87
		24	59	98	154	0.012	0.83
1 (0.51)	0	0	58	92	137	0.015	0.80
		12	67	99	147	0.016	0.85
		24	85	121	168	0.015	0.86
	10	0	44	74	114	0.015	0.76
		24	63	96	140	0.016	0.85
	20	0	41	71	118	0.015	0.80
		12	42	76	112	0.018	0.91
		24	48	84	136	0.014	0.84
5 (1.22)	0	0	45	64	91	0.024	0.77
		12	73	97	130	0.022	0.85
		24	79	102	130	0.024	0.83
	10	0	27	48	71	0.027	0.80
		12	61	83	110	0.027	0.90
		24	79	101	126	0.026	0.88
	20	0	18	35	58	0.028	0.79
		12	44	64	91	0.029	0.95
		24	72	92	119	0.026	0.88

[a]The bound copper concentration (Cu_B) was calculated according to our previously published data on copper binding parameters [13].

our previous results [13], the kinetics of oxidation, as observed in the absence of added tocopherol, depended on the bound copper/LDL ratio. More importantly, at bound copper/LDL ratios of 2.8 and 12.2, the dependence of the kinetics of oxidation on the tocopherol/LDL ratio was similar to that observed at 0.1 μM LDL (not shown). We therefore conclude that at any LDL concentration below 0.1 μM, the effects of tocopherol are dependent only on the ratios of concentrations and independent of the absolute concentrations of copper, LDL and tocopherol. This conclusion is not likely to be valid at LDL concentrations above 0.14 μM because at higher LDL, the oxidation kinetics are complicated by additional unknown factors, as previously reported [13].

Since both LOOH and vitamin E cause a dose-dependent offset of the whole kinetic curve, without affecting substantially the shape of the kinetic curve at time points exceeding the lag time, the pro-oxidative effect of added LOOH could be neutralized by adding an appropriate concentration of vitamin E (Fig. 2). For any given concentration of copper, the LOOH/vitamin E molar ratio at which these two additives compensated the effect of each other was about constant. Nonetheless, these 'compensating' concentrations depended markedly on the concentration of copper. As an example, upon exposure of 0.1 μM LDL to 5 μM Cu(II), 1.2 μM tocopherol was sufficient to revert the shortening of the lag by the addition of 2 μM LOOH (Fig. 2B), whereas upon exposure of the same LDL to 0.5 μM Cu(II), the same concentration of tocopherol was merely sufficient to revert the shortening of the lag by 1 μM LOOH (Fig. 2A).

4. Discussion

Interpretation of the dependence of t_{max} on the concentration of LOOH can be based on our previously proposed model of auto-accelerated peroxidation [13]. This model assumes that after complete consumption of the antioxidant, copper-catalyzed decomposition of pre-formed LOOH induces propagation of LDL peroxidation. Therefore, propagation of the reaction is determined primarily by the growth of the concentration of LOOH. Increasing the LOOH content of the LDL prior to its exposure to copper is equivalent to inducing earlier auto-accelerated oxidation without changing the relevant rate constants. Therefore, enrichment of LDL by LOOH could have been expected to result in an offset of the whole kinetic curve to earlier times without affecting the shape of the kinetic curve and particularly the maximal rate and maximal accumulation of LOOH. The observed experimental results (Table 1) are indeed consistent with these considerations.

The observed influence of both added LOOH and vitamin E on the characteristic time points of LDL peroxidation can be empirically described by multiple linear regression analysis. Specifically, for each copper concentration, each of the characteristic times (t) can be presented as a function of the concentrations of LOOH and vitamin E

$$t(LOOH, E, Cu) = t_0(cu) - A(cu) \times LOOH + B(Cu) \times E \qquad (1)$$

where the coefficients A and B characterize the dependence of the given characteristic time (t) on the concentrations of LOOH ($LOOH$) and vitamin E (E), respectively. The copper-dependent value of t_0 represents the extrapolated value for a zero concentration of added LOOH and/or vitamin E and cannot be explicitly interpreted because it accumulates all the inaccuracies of the linear approximation. The copper-dependent coefficients A and B may be considered as being the products of decomposition of the complex functional depend-

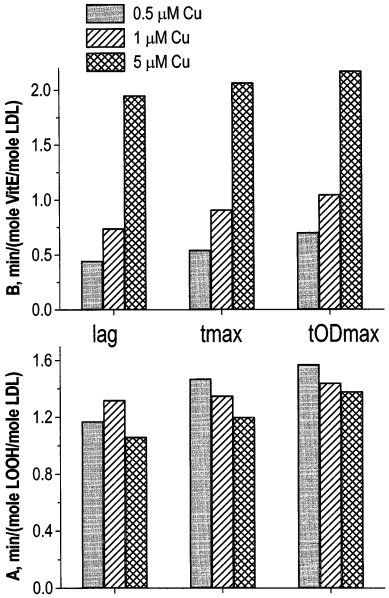

Fig. 3. The efficiencies of acceleration of peroxidation of LDL by excessive LOOH (lower panel) and of inhibition of peroxidation by added vitamin E (upper panel), as characterized by regression coefficients A and B. The data relate to three different total concentrations of copper at consecutive characteristic time points as indicated on the figure.

ency of time on the concentrations of LOOH and vitamin E into power series. Although the linear dependence given by Eq. 1 is not based on theoretically derived equations, we believe that the obtained linear approximations for the studied range of concentrations ($R^2 > 0.92$) reflect semi-quantitatively the dependence of the characteristic time points on the LDL content of vitamin E and LOOH.

To elucidate the influence of LOOH and vitamin E on the time coordinate of peroxidation, we have analyzed the results obtained for each copper concentration in terms of the coefficients A and B. Fig. 3 depicts the values of these coefficients, as observed for the three characteristic time points (lag, t_{max}, t_{ODmax}) at three copper concentrations. Two obvious conclusions can be drawn from this figure. First, for any given copper concentration, the value of both regression coefficients A and B are almost independent of the characteristic time. Thus, analyses of the kinetics of oxidation in terms of three periods, namely the lag and the following two periods (t_{max}–lag and t_{ODmax}–t_{max}), reveal that the latter two periods are only slightly dependent, if at all, on the LDL content of either LOOH or vitamin E (Fig. 4). These results must mean that at time periods after the lag, the continuation of the peroxidation process does not depend on the concentration of either LOOH or vitamin E in the LDL prior to its exposure to copper. In other words, the 'memory of the LDL' with respect to the initial concentrations of LOOH and vitamin E is restricted to the lag period.

This observation is not unexpected because(i) vitamin E is known to be consumed at 20–40% of the lag time [11] and (ii) the formation of LOOH during the propagation phase of peroxidation is much larger than their initial quantities.

The second conclusion from Fig. 3 is that the regression coefficient A, which characterizes the dose-dependence of the pro-oxidative effect of LOOH, is only slightly dependent on the concentration of copper, whereas the coefficient B, which characterizes the dose-dependence of the antioxidative effect of vitamin E, increases with the copper concentration and is very low under conditions of low oxidative stress (e.g. at 0.5 μM Cu(II)). These results can be expressed in terms of the 'relative efficiency' of inhibition of peroxidation by vitamin E, as given by the ratio of coefficients B/A (mol LOOH/mol vitamin E). This ratio expresses the number of LOOH molecules whose pro-oxidative effect can be neutrolized by one molecule of vitamin E. Fig. 5 depicts the dependence of the ratio B/A on Cu_B. It reveals that at $[Cu_B] = 0.28$ μM, one molecule of vitamin E is only capable of neutralizing the effect of ~0.4 LOOH molecules whereas at $[Cu_B] = 1.22$ μM, one molecule of vitamin E is sufficient to revert the shortening of the lag by two molecules of LOOH (Fig. 5).

The effect of the copper concentration on the 'relative efficiency' of vitamin E can be explained on the basis of the known 'dual activity' of chain-breaking antioxidants, such as tocopherol [9]. Briefly, these antioxidants protect oxidizable lipids from chain peroxidation by quenching the peroxy radicals involved in the propagation of oxidation [3]:

$$\mathrm{TocH} + \mathrm{LOO}\bullet \rightarrow \mathrm{Toc}\bullet + \mathrm{LOOH} \quad (\mathrm{R1})$$

$$\mathrm{Toc}\bullet + \mathrm{LOO}\bullet \rightarrow \mathrm{Non-reactive\ products} \quad (\mathrm{R2})$$

Nonetheless, the tocopheryl radical may enhance peroxidation of LDL lipids via the TMP mechanism [9]:

$$\mathrm{Toc}\bullet\mathrm{LH} \xrightarrow{\mathrm{O_2}} \mathrm{TocH} + \mathrm{LOO}\bullet \quad (\mathrm{R3})$$

The balance between these two pathways is primarily determined by the intensity of the oxidative stress. Specifically, at high oxidative stress (high copper) the TMP is negligible [9] and each tocopherol molecule can scavenge up to two peroxyl radicals, as expected from Eq. R1 and R2. Notably, the max-

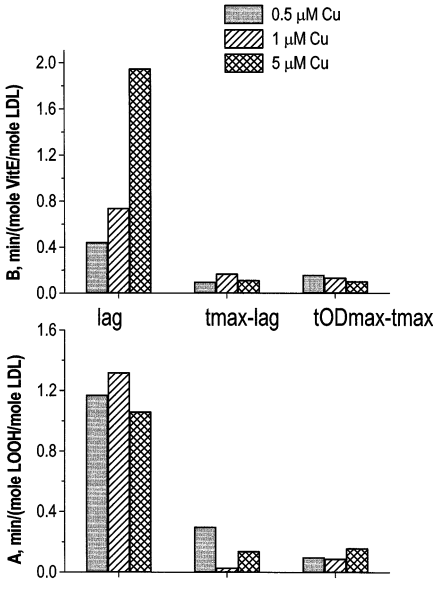

Fig. 4. The efficiencies of acceleration of peroxidation of LDL by excessive LOOH (lower panel) and of inhibition of peroxidation by added vitamin E (upper panel), as characterized by regression coefficients A and B. The data relate to three different total concentrations of copper at consecutive characteristic time periods as indicated on the figure.

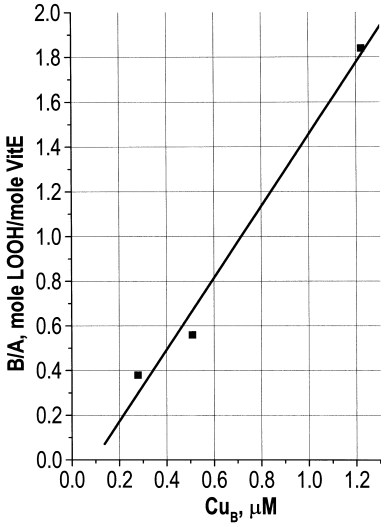

Fig. 5. Dependence of the relative efficiency of inhibition of peroxidation by vitamin E and acceleration by LOOH on the concentration of copper bound to LDL. The relative efficiency is characterized by the ratio of regression coefficients B and A. The presented points relate to the values calculated for the lag time point.

imal value of B/A at 1.25 μM bound copper (Fig. 5) is close to two (two LOOH molecules neutralized by one tocopherol), in good agreement with the expected maximum [3]. By contrast, at low oxidative stress, oxidation via a TMP mechanism makes a marked contribution [9]. This accelerates the peroxidation by enhancing accumulation of LOOH (via Eq. R3) and by that partially compensates for the inhibitory effect of tocopherol. It may explain why the antioxidative effect of tocopherol, as defined by Eq. 1, decreases upon decreasing the copper concentration.

Interestingly, the effects of both LOOH and tocopherol on the kinetics of LDL peroxidation induced by free radical generators, such as AAPH, are quite different from those described above [3,4]. Specifically, in contrast to copper-induced peroxidation, AAPH-induced peroxidation is not affected significantly by changes in the LOOH content of LDL [4] and its inhibition by tocopherol, as expressed by prolongation of the lag preceding uninhibited peroxidation, is more pronounced at low AAPH-induced oxidative stress [3]. To explain these differences, it is important to note that, unlike in the case of copper-induced peroxidation, peroxidation induced by free radical generators does not depend substantially on the production of free radical from the copper-catalyzed decomposition of LOOH. Accordingly, the lag preceding the uninhibited phase of AAPH-induced peroxidation is not shortened by LOOH whereas its prolongation by tocopherol, which reflects the time required for consumption of added tocopherol, is relatively small at high AAPH concentrations.

In conclusion, the pro-oxidative effect of LOOH is almost independent of the oxidative stress (Fig. 3, lower panel), whereas the antioxidative effect of vitamin E and its efficiency in preventing the pro-oxidative effect of pre-formed LOOH is high only under conditions of high oxidative stress (Fig. 3, upper panel). For a slow chronic process such as the development of atherosclerotic plaques, the benefit of vitamin E supplementation may therefore be quite limited [17]. This accords with Witztum's proposal that the mixed results of the intervention trials conducted thus far can be explained by 'the inclusion of population that would not be expected to benefit from antioxidant supplementation' [18]. Further epidemiological studies will have to be conducted to define criteria by which possible beneficiaries of vitamin E supplementation can be differentiated from other individuals. Whether such criteria can be based on the oxidizability of serum lipids [19], in vitro immunological assays of minimally oxidized LDL [20] or urinary isoprostane secretion [18,21] remains to be evaluated. Based on the present study, we are tempted to speculate that those patients whose serum lipids are relatively susceptible to copper-induced peroxidation in vitro are likely to benefit from vitamin E supplementation.

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References

[1] Esterbauer, H. and Scmidt, R. (1997) Adv. Pharmacol. 38, 425–455.
[2] Rice-Evans, C., Leake, D. and Bruckdorfer, K.R. (1996) Free Radic. Res. 25, 285–311.
[3] Esterbauer, H. and Jurgens, G. (1993) Curr. Opin. Lipidol. 4, 114–124.
[4] Frei, B. and Gaziano, M. (1993) J. Lipid Res. 34, 2135–2144.
[5] O'Leary, V.J., Darley-Usmar, V.M, Russel, L.J. and Stone, D. (1992) Biochem. J. 282, 631–634.
[6] Holvoet, P., Vanhaeke, J., Janssens, S., Van de Werf, F. and Collen, D. (1998) Circulation 98, 1487–1494.
[7] Jankowski, J., Nofer, J.-R., Tepel, M., Griewel, B., Schluter, H., Assmann, G. and Zidek, W. (1998) Clin. Sci. 95, 489–495.
[8] Polidori, M.C., Frei, B., Cherubini, A., Nelles, G., Rordorf, G., Keaney, J.F., Schwamm, L., Mecocci, P., Koroshetz, W.J. and Beal, M.F. (1998) Free Radic. Biol. Med. 25, 561–567.
[9] Neuzil, J., Thomas, S.R. and Stocker, R. (1997) Free Radic. Biol. Med. 22, 57–71.
[10] Noguchi, N. and Niki, E. (1998) Free Radic. Res. 28, 561–572.
[11] Abuja, P.M. and Esterbauer, H. (1995) Chem. Res. Toxicol. 8, 753–763.
[12] Fainaru, M., Schafer, Z., Gavish, D., Harel, A. and Schwartz, M. (1988) Comp. Biochem. Physiol. 91B, 331–338.
[13] Pinchuk, I., Schnitzer, E. and Lichtenberg, D. (1998) Biochim. Biophys. Acta 1389, 155–172.
[14] Pinchuk, I. and Lichtenberg, D. (1996) Free Radic. Res. 24, 351–360.
[15] Esterbauer, H. and Ramos, P. (1995) Rev. Physiol. Biochem. Pharmacol. 127, 31–64.
[16] Ramos, P., Gieseg, S.P., Schuster, B. and Esterbauer, H. (1995) J. Lipid Res. 36, 2113–2128.
[17] Steinberg, P. and Witztum, J.L. (1998) in: Molecular Basics of Cardiovascular Disease (Chien, K.R., Ed.), pp. 458–475, W.B. Saunders, Philadelphia, MS, USA.
[18] Witztum, J.L. (1998) Circulation 98, 2785–2787.
[19] Schnitzer, E., Pinchuk, I., Bor, A., Fainaru, M., Samuni, A.M. and Lichtenberg, D. (1998) Chem. Phys. Lipids 92, 151–170.
[20] Holvoet, P., Vanhaeke, J., Janssens, S., Van de Werf, F. and Collen, D. (1998) Circulation 98, 1487–1494.
[21] Reilly, M., Pratico, D., Delanty, N., DiMinno, G., Tremoli, E., Rader, D.J., Kapoor, S., Rokach, J., Lawson, J. and FitzGerald, G.A. (1998) Circulation 98, 2822–2828.