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Vitamin E Slows the Rate of Free Radical-Mediated Lipid Peroxidation in Cells[1]

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Much of what is known about the antioxidant mechanism of vitamin E has been learned from studies of lipid dispersions, solutions, or subcellular organelles. We have investigated the effect of vitamin E supplementation on intact live eucaryotic cells. L1210 murine leukemia cells were exposed to an oxidative stress induced by 20 μM Fe^{2+} and 100 μM ascorbic acid introduced immediately before oxidative measurements were begun, and the kinetics of the generation of lipid-derived free radicals, as measured by EPR spin trapping (a product) and O_2 consumption (a reactant) were measured. Cells grown for 24 h with supplemental (5–100 μM) vitamin E in their media had a slower rate of lipid radical generation compared to cells grown without vitamin E supplementation; this inhibition in the rate of oxidation was generally dependent upon the amount of vitamin E supplementation. In complementary studies measuring O_2 consumption, 5–100 μM vitamin E slowed the rate of oxidation (10-fold with 100 μM supplemental vitamin E) consistent with the EPR studies. The membrane active drug edelfosine accentuated the vitamin E effects; vitamin E introduced a discernible lag phase (time delay) in both lipid radical generation and O_2 consumption that was not seen in the absence of edelfosine. Vitamin E supplementation of cells also altered the kinetics of ascorbate free radical formation. We conclude that vitamin E inhibits lipid peroxidation in cells by slowing the rate of lipid peroxidation; but with iron/ascorbate as the initiating system, vitamin E does not delay the onset of peroxidation. Of special interest is that these free radical peroxidation events parallel cell membrane damage as detected using trypan blue exclusion. These observations are consistent with the free radical events preceding and causing the observed membrane damage.

Key Words: vitamin E; lipid peroxidation; free radicals; oxygen consumption; ascorbate radical; electron paramagnetic resonance; iron; spin trapping; edelfosine.

Vitamin E is thought to be the major nonenzymatic antioxidant present in the lipid structures of cells (1). It is a donor antioxidant (reductant), which appears to react with peroxyl radicals to inhibit the propagation cycle of lipid peroxidation. Its characteristics as an antioxidant in organic solutions of fatty acids are well studied. In lipid solutions and dispersions, it inhibits radical formation linearly with time until it is depleted, and then oxidation accelerates, taking place at the same rate as if vitamin E had not been present (2, 3). Its inhibition of oxidation of liposomal membranes (4) and lipoproteins (5–7) has the same general features.

Much less is known about the action of vitamin E on intact cells and tissues since most previous studies of oxidation related to free radicals have been performed on isolated cell components or liposomes (8). To study the action of vitamin E in cells, we enriched L1210 murine leukemia cells with polyunsaturated fatty acids and varying amounts of vitamin E and then subjected them to an oxidative stress. Using these intact cells, measurements of lipid peroxidation were made in real time. We followed the formation of lipid-derived free radical ($L^{\bullet}$)[3] by EPR spin trapping and compared these

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[3] Abbreviations used: $Asc^{\bullet -}$, ascorbate radical; $[Asc^{\bullet -}]_{ss}$, ascorbate radical at steady state; EPR, electron paramagnetic resonance; $L^{\bullet}$, lipid-derived radical; MBI, methylene bridge index, the mean number of bis-allylic methylene positions/fatty acid; POBN, α-(4-pyridyl-1-oxide)-*N*-tert-butylnitrone.

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kinetics to that of oxygen consumption and ascorbate radical generation. Our results indicate that the mechanism of action of vitamin E in intact cells has major differences from those of vitamin E in lipid solution or liposomes.

MATERIALS AND METHODS

Cell culture and lipid modification. L1210 murine leukemia cells were fatty acid-modified as previously described (9, 10). Briefly, cells were grown for 48 h in RPMI 1640 media and 5% heat-inactivated fetal bovine serum (Sigma Chemical Co., St. Louis, MO) supplemented with 32 μM docosahexaenoic acid (22:6ω3) in the *cis* form (Nu Prep Chek, Inc., Elysian, MN) . The effects of fatty acid modification in this cell line have been thoroughly characterized as to fatty acid profile changes, methylene bridge index (MBI, the mean number of *bis*-allylic methylene positions/fatty acid) and effects on cellular lipid peroxidation (9–12). Twenty-four hours after fatty acid modification was started, the culture media was supplemented with various concentrations of vitamin E acetate. This fortification was accomplished by adding vitamin E acetate at various concentrations to the culture media using ethanol as a vehicle at 1 μl/ml of culture media (0.1 % v/v final ethanol concentration) (10, 13). Cells were incubated an additional 24 h at 37°C after the addition of vitamin E. Analysis of cells after supplementing the growth media with vitamin E acetate is known to increase cellular α-tocopherol concentration (13). Immediately before the lipid peroxidation experiments, the cells were washed, suspended in 0.9% NaCl, and counted using a Coulter Model Zf cell counter (Coulter, Inc., Hialeah, FL). Cell densities were adjusted to 5×10^6 cells/ml. Cell membrane integrity was measured using trypan blue dye exclusion.

EPR studies of lipid peroxidation. EPR lipid peroxidation experiments were as previously described (10–12). Briefly, to 5×10^6 cells/ml in 0.9% NaCl were added the following final concentrations of reagents in respective order: α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (50 mM) (POBN) (Sigma, Chemical Co., St. Louis, MO), ascorbic acid (100 μM) (Fisher Scientific Co., Fair Lawn, NJ), and $FeSO_4 \cdot 7H_2O$ (20 μM) (Sigma). A 4-ml aliquot of the cell sample was then aspirated into a quartz flat cell that previously had been positioned in a TM_{110} cavity of a Bruker ESP-300 EPR spectrometer. Thirty seconds after the introduction of Fe^{2+}, EPR scans were initiated and sequentially collected at 23.5- to 30-s intervals. In some experiments, 40 μM 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH_3, edelfosine, Medmark GmbH, Grünwald, Germany, kindly supplied by Dr. R. Nordström) was added to the remaining sample 218 s after initiation of peroxidation and aspirated into the EPR flat cell displacing the previous portion of the sample. In experiments without the addition of ether lipids, EPR scans were taken at 30-s intervals and the entire 8 ml was aspirated into the flat cell in the EPR cavity without further additions. EPR instrument settings were as follows: 40 mW microwave power at a frequency of 9.77 GHz; modulation frequency of 100 kHz; receiver gain 2.5×10^5; modulation amplitude 1.0 G; scanning 60 G/23.5 s with a time constant of 164 ms.

EPR lipid radical-POBN adduct (POBN/L$^\bullet$) peak areas were quantitated using the first peak in the low field doublet with 3-carboxyproxyl (3-CP) (Aldrich Chemical Co., Milwaukee, WI) as a standard (14). Rates of POBN/L$^\bullet$ formation were calculated from the linear portion of the plots of POBN adduct formation over time and are expressed as the rate of POBN/L$^\bullet$ formation per second (12).

Oxygen uptake. Oxygen uptake experiments were performed in conditions identical to the EPR experiments using the same cell densities, reactant concentrations, and times (12). Oxygen consumption experiments were done at room temperature (25°C) using air-saturated solutions and cell samples in a Model 53 biological oxygen monitor (Yellow Springs Instruments, Yellow Spring, OH). Rates of oxygen uptake were taken from the linear portions of oxygraph recordings during maximal rates of oxygen uptake.

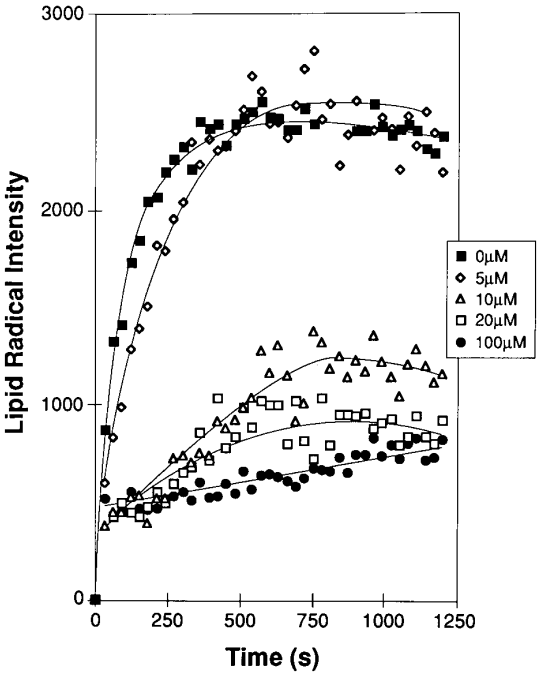

FIG. 1. Vitamin E slows the rate of cellular lipid radical formation. Cellular incorporation of α-tocopherol slows the rate, but induces no apparent lag phase in lipid radical formation during lipid peroxidation as shown by EPR-detectable POBN/L$^\bullet$ adducts. L1210 cells (5×10^6 cells/ml) enriched with 22:6ω3 and supplemented with various concentrations of α-tocopherol acetate were subjected to the oxidative stress presented by 20 μM Fe^{2+} and 100 μM ascorbate in the presence of 50 mM of the spin trap 4-POBN. Each data point is the mean of 5–7 experiments and represents the EPR peak height of the first peak of the low field doublet. The lipid radical intensity units of the ordinate are arbitrary values; 1000 corresponds to 0.24 μM POBN/L$^\bullet$.

α-Tocopherol content of cells. Cellular free tocopherol content was determined by HPLC (13). The extraction method was modified from our previous method. The cells were extracted in 8 ml 3:2 hexane:isopropanol (HPLC grade, Fisher Scientific, Fair Lawn, NJ) with a 30-s vortex. Following phase separation, the organic hexane fraction was removed and the aqueous phase containing the cells was extracted twice more using 4 ml 3:2 hexane-isopropanol. The pooled organic phase was dried, 400 μl 99:1 hexane:isopropanol added, vortexed, and 100-μl aliquots were injected into the HPLC.

RESULTS

α-Tocopherol Slows Lipid-Derived Radical (L$^\bullet$) Formation during Lipid Peroxidation of Intact Cells

We have developed an EPR spin trapping system, using POBN as the spin trap that allows the real time detection of lipid-derived free radicals from intact cells undergoing oxidative stress (12, 15). In this study, we have used this system to examine the effect of vitamin E on *in vitro* cellular lipid peroxidation. In Fig. 1 the

effects of cellular α-tocopherol enrichment on the kinetics of POBN/L$^{\bullet}$ formation can be seen. The rate and extent of L$^{\bullet}$ generation in 22:6-enriched L1210 leukemia cells during oxidative stress (20 μM Fe^{2+} and 100 μM ascorbic acid) is blunted in the presence of increasing concentrations of vitamin E. In the absence of supplemental vitamin E, [POBN/L$^{\bullet}$] rises above baseline almost immediately after the initiation of oxidative stress and continues to increase in a linear manner until about 400–500 s when it plateaus. In contrast, when 100 μM supplemental vitamin E is present, radical generation increases slowly at a linear rate and reaches a plateau after about 1800 s (not shown). Intermediate supplemental vitamin E concentrations have intermediate effects. Thus, vitamin E induces a concentration-dependent slowing of the rate of iron/ascorbate-stimulated L$^{\bullet}$ production by cells as well as the total amount of L$^{\bullet}$ observed. No discernible lag phase was observed in these experiments.

Vitamin E Slows O_2 Consumption of Intact Cells during Lipid Peroxidation

Measurement of oxygen consumption has often been used by investigators to estimate oxidative stress of both lipid solutions and cells; it is useful as a complementary method because it measures the utilization of a reactant. We monitored the utilization of oxygen during iron/ascorbate-induced oxidative stress by 22:6-modified cells enriched with vitamin E in order to allow comparison with the lipid free radical observations. In the absence of supplemental vitamin E, oxygen is consumed rapidly with 50% being lost in less than 3 min (Fig. 2). The presence of vitamin E during oxidative stress results in a slower initial rate of oxidation at all vitamin E concentrations; no lag phase could be detected. These observations of the effect of vitamin E on the uptake of oxygen, a reactant, and the generation of the lipid radical product are, in general, parallel.

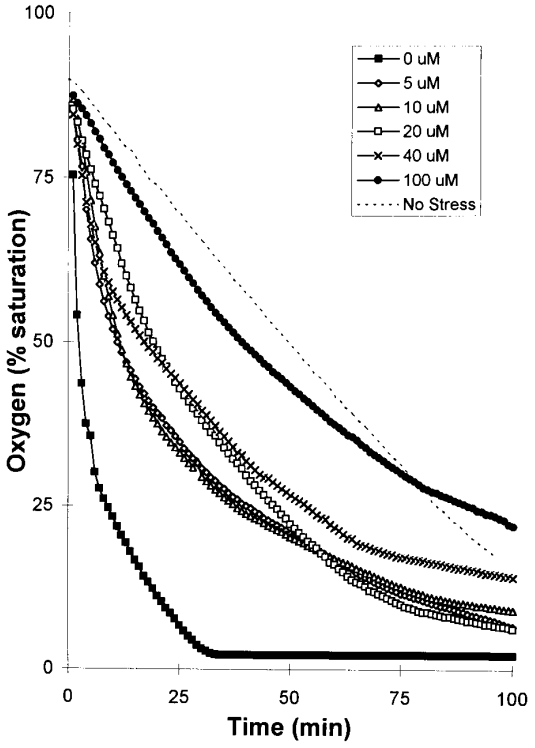

FIG. 2. Vitamin E slows the rate of oxygen consumption. Cellular incorporation of α-tocopherol slows the rate of oxygen consumption during cellular lipid peroxidation, but there is no apparent lag phase. L1210 cells (5×10^6 cells/ml) enriched with 22:6ω3 and supplemented with various concentrations of α-tocopherol acetate were subjected to the oxidative stress presented by 20 μM Fe^{2+} and 100 μM ascorbic acid in the presence of 50 mM 4-POBN. Each point is the mean of 5–7 experiments from oxygen probe recordings. These conditions of the incubations were identical to those described in the legend of Fig. 1. The incubations were initially air-saturated which implies that dissolved $[O_2] \approx 250$ μM.

Oxidative Events Correlate With Membrane Damage

Trypan blue is excluded from cells when the plasma membranes are intact and functioning normally. However, when these membranes are damaged, cells then take up the dye. The number of cells capable of excluding trypan blue, a measure of cell membrane integrity, after an oxidative stress (an exposure to 20 μM Fe^{2+} and 100 μM ascorbate) increased as the vitamin E level increased (Fig. 3). The experimental conditions were designed to be similar to those used for both the EPR and oxygen uptake experiments. In the absence of supplemental vitamin E, the number of intact cells decreased rapidly and less than 10% remained able to exclude dye at ≈25 min. When media levels of vitamin E were increased to 100 μM, the dye penetration decreased markedly; even after 120 min of exposure to the oxidative stress, up to 20% of the cells were still able to exclude dye. This apparent rescuing of the cells was less at 5 μM vitamin E media supplementation. Because vitamin E is a membrane antioxidant, it seems likely that the oxidative stress disrupts the integrity of cellular membranes, including plasma membranes, leading to cell death.

Cellular Vitamin E Uptake Limits Peroxidation and Membrane Damage

The experiments described above demonstrate that supplementation of media with vitamin E protects cells from the free radical oxidation initiated by iron/ascorbate. We reasoned that the ability to maintain membrane integrity, as measured by trypan blue exclusion, should correlate directly with cellular levels of vitamin E and that the initial rates of oxygen consumption and POBN/L$^{\bullet}$ formation should correlate inversely with cellular vitamin E. Indeed when we compare cellular vita-

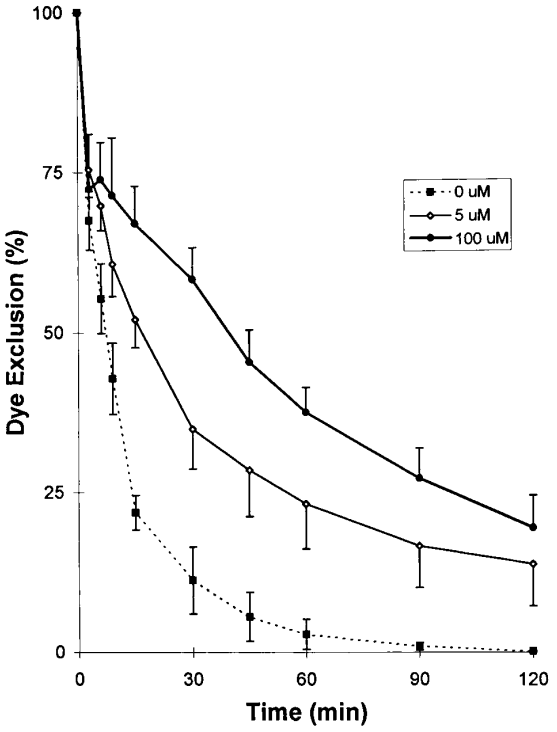

FIG. 3. α-Tocopherol preserves membrane integrity during lipid peroxidation as measured by trypan blue dye exclusion. L1210 cells fatty acid modified with 22:6ω3 and supplemented with various concentrations of α-tocopherol acetate were subjected to the oxidative stress presented by 20 μM Fe^{2+} and 100 μM ascorbic acid in the presence of 50 mM 4-POBN.

min E uptake,[4] we see amazingly parallel curves for dye exclusion and cellular α-tocopherol levels (Fig. 4). In addition, the rates of oxygen uptake and lipid-derived free radical formation are also parallel and show an inverse relationship to plasma membrane damage and tocopherol levels. An interesting aspect of these data is the apparent saturation behavior seen in the curves. In these experiments, maximum vitamin E uptake is achieved with only about 10 μM supplemental tocopherol acetate.[5] That membrane damage follows antioxidant levels so closely suggests that it is indeed the free radical oxidation events that cause the membrane leakiness.

Vitamin E content of cells incubated in media with 10 μM vitamin E was only 12% higher than those cells incubated in media with 5 μM vitamin E (Fig. 4). This 12% higher content seemed to have a >12% inhibitory effect on both lipid radical intensity (Fig. 1) and its initial rate (Fig. 4). This seeming quantitative discordance could be due to disporportionally greater intracellular localization of vitamin E when added to the medium at the higher concentration—that is, an ability of the higher concentration to penetrate to a metabolic site.

Ether Lipid Enhances Oxidative Stress

We have previously observed that the ether lipid drug edelfosine enhances Fe^{2+}-induced oxidative processes in cells (12). When the spin trapping experiments to monitor $L^{\bullet}$ of the current study were repeated in the presence of pharmacological levels of edelfosine to augment oxidative stress, vitamin E had an even greater effect on peroxidation (Fig. 5). In the presence of edelfosine and supplemental vitamin E of above 20 μM, a lag phase is clearly observed. There was little or no effect of vitamin E on the rate of $L^{\bullet}$ production in the presence of edelfosine except at high concentrations of 100 μM or greater.

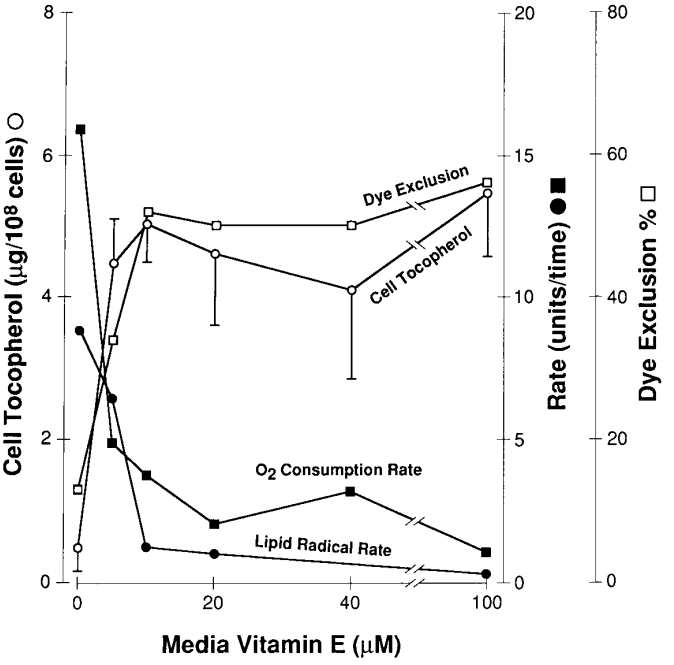

FIG. 4. Effect of vitamin E concentration on inhibition of peroxidative events, oxidative membrane damage, and cellular uptake of the antioxidant to compare saturation levels. The vertical scale shows initial rate of generation of POBN/$L^{\bullet}$ (units/s derived from the linear portion of the curve in Fig. 1), negative initial rate of oxygen consumption (%/min derived from the linear portion of the curve in Fig. 2), protection against membrane damage (percentage of cells excluding dye at 30 min derived from Fig. 3), and cellular uptake of vitamin E (μg/10^8 cells). The saturating concentration for the biologic events occurs at about the same concentration as cellular uptake.

[4] α-Tocopherol acetate is not an antioxidant. To be an effective cellular antioxidant, it must first be de-esterified to form free α-tocopherol. Our HPLC assay determines free α-tocopherol.

[5] In our previous study of α-tocopherol uptake by cells, we did not observe cellular tocopherol saturation until the media had been supplemented to a level of $\approx$100 μM (13). In that study the media contained 10% serum, while here only 5% was used in the cell culture incubations. Clearly, media serum levels influence the uptake of vitamin E by cells.

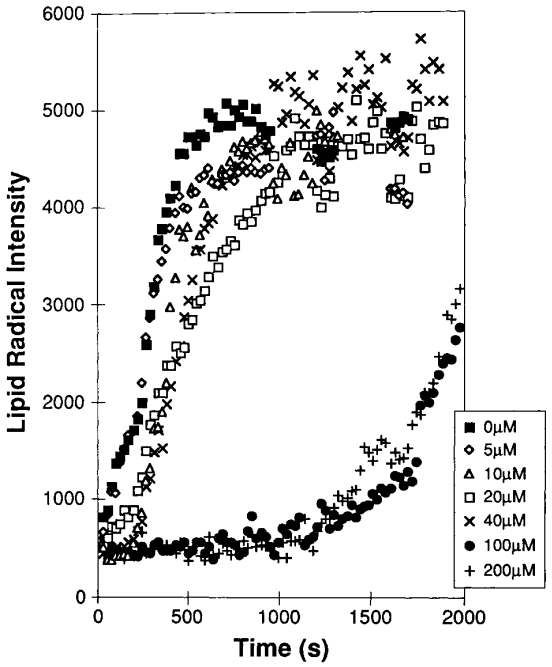

FIG. 5. Vitamin E delays ether lipid-enhanced lipid peroxidation. Cellular incorporation of α-tocopherol delays lipid radical formation remarkably during lipid peroxidation in the presence of edelfosine as determined by EPR detectable POBN/L$^{\bullet}$ adducts. In addition, an apparent lag phase was introduced. 22:6ω3 fatty acid-modified cells were subjected to the oxidative stress of 20 μM Fe^{2+} and 100 μM ascorbic acid in the presence of 50 mM 4-POBN. At 218 s, after the introduction of Fe^{2+} and ascorbic acid, 40 μM edelfosine was added to enhance lipid peroxidation. Each point is the mean of 5–7 experiments and represents the EPR signal height of the low field peak.

α-Tocopherol also affects oxygen consumption of L1210 cells when exposed to Fe^{2+}, ascorbate, and edelfosine (Fig. 6). In the absence of added antioxidant there is rapid uptake of oxygen; dissolved oxygen is completely consumed in only 12 min. In contrast, with 100 μM vitamin E supplementation, there is a definite delay in the onset of the rapid oxidative processes because of the presence of a shoulder on the curve, a lag phase. With intermediate supplemental concentrations of tocopherol, from 5 to 100 μM, there appeared to be some lag as evidenced by the small shoulders on the curves. In the rapid phase of peroxidation, the approximately linear initial rates of oxidation, as determined by oxygen uptake, are nearly the same for all concentrations of supplemental vitamin E; only minor slowing in these rates are observed.

Relationship Between L$^{\bullet}$ and Ascorbate Radical

With careful attention to controls, the ascorbate radical ($Asc^{\bullet -}$) can be used as a marker of oxidative stress (16). In our experiments, an increase in $[Asc^{\bullet -}]_{ss}$ was detected immediately upon the application of an oxidative stress; it then declines and disappears as the lipid radical signal intensity increases (Fig. 7, top).

In the presence of vitamin E, an increase in $[Asc^{\bullet -}]_{ss}$ also occurs initially upon application of an oxidative stress, but at a much lower intensity, presumably due to a blunting effect of vitamin E on the rates of lipid peroxidation and subsequent $Asc^{\bullet -}$ generation (Fig. 7, bottom). In a manner similar to studies in the absence of vitamin E, the [POBN/L$^{\bullet}$] increases as the $[Asc^{\bullet -}]_{ss}$ decreases; however, the rates of change are considerably reduced. Tocopherol delays lipid-derived radical appearance as well as blunts ascorbate radical formation, consistent with an inhibition of the propagation steps of lipid peroxidation.

DISCUSSION

Most studies of chain-breaking antioxidants have been performed on lipid solutions, lipid dispersions, plasma, liposomes, erythrocytes, or erythrocyte ghost membranes. In those studies, oxidation was measured

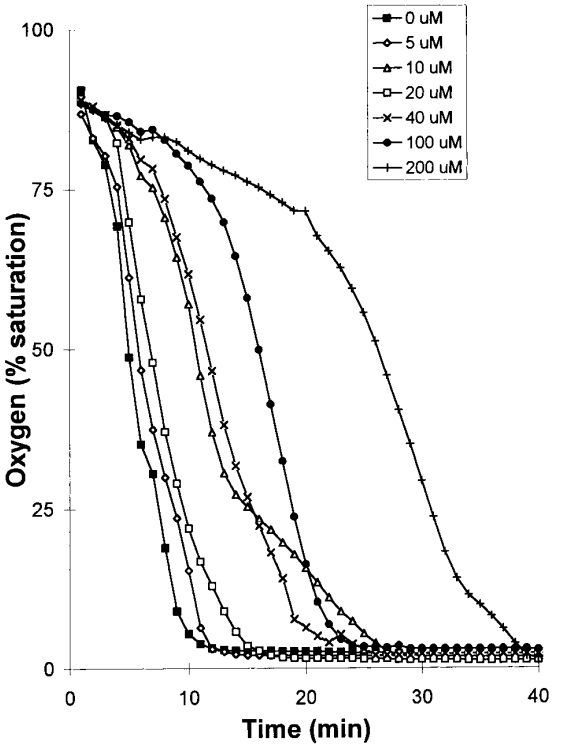

FIG. 6. Vitamin E slows ether lipid-enhanced oxygen consumption. Cellular incorporation of α-tocopherol introduces a lag phase during ether lipid-enhanced lipid peroxidation. L1210 cells fatty acid-modified with 22:6ω3, and supplemented with various concentrations of α-tocopherol acetate, were exposed to the oxidative stress of 20 μM Fe^{2+} and 100 μM ascorbic acid in the presence of 50 mM 4-POBN. At 218 s after the introduction of Fe^{2+} and ascorbic acid, 40 μM edelfosine was added to enhance lipid peroxidation. Values are the mean of 5–7 experiments from oxygen probe recordings. The incubations were initially air-saturated.

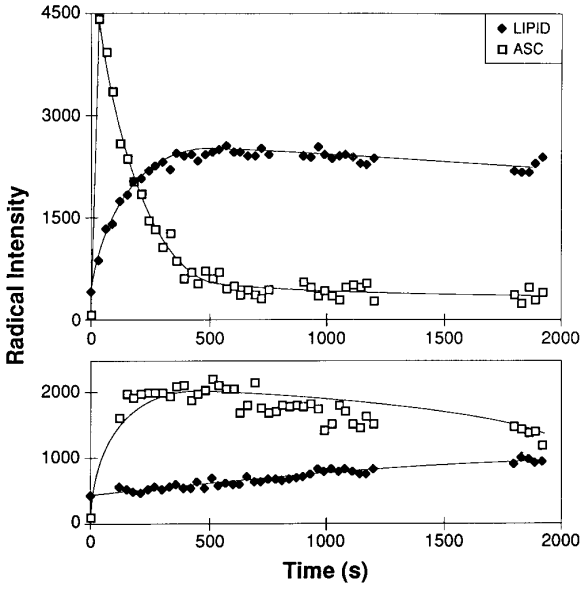

FIG. 7. Comparative kinetics of ascorbate and lipid radical formation during lipid peroxidation without and with vitamin E. L1210 cells enriched with 22:6ω3 were peroxidated with 20 μM Fe^{2+} and 100 μM ascorbic acid in the presence of 50 mM 4-POBN. Ascorbate radical was quantitated directly from EPR spectra, and lipid radical formation was quantitated as POBN/L$^{\bullet}$ adducts from the EPR spectra. Top, No vitamin E supplementation. Bottom, 100 μM vitamin E acetate supplementation. A value of 1000 on the vertical scale corresponds to 46 nM [$Asc^{\bullet -}$].

as oxygen consumption, thiobarbituric acid reactive substances, or consumption of vitamin E. From those studies, it has been concluded that vitamin E suppresses oxidation during an initial lag period, but is consumed in the process. Furthermore, when the antioxidant is depleted, lipid oxidation proceeds at the same rate as it would have if the antioxidant had never been present (2–4). It has been supposed that the same is true for cells exposed to an oxidative stress, since it has been thought that studies of lipid preparations would predict cellular lipid peroxidation.

We have recently developed EPR spin trapping and oxygen uptake techniques for studying the oxidizability of lipids in intact cells. We have applied the techniques in the present study to determine the effect of vitamin E on free radical generation of live cells in real time. We have observed that vitamin E has a major inhibitory effect on free radical-mediated lipid peroxidation as measured by both product generation, L$^{\bullet}$, and oxygen consumption. In the presence of a potent oxidative stress (Fe^{2+} and ascorbate), the rate of cellular lipid oxidation was slowed by vitamin E, and this inhibition correlated with the tocopherol concentration.

This effect of vitamin E on the rate of L$^{\bullet}$ generation by cells differs from observations using lipid solutions and liposomes where a lag phase is observed and the rate of oxidation after the lag phase is unaffected by the antioxidant (2–4). For example, Niki *et al.* studied solutions of methyl linoleate and measured the effect of vitamin E on oxygen consumption induced by azo initiators (2). They found that vitamin E produced a lag phase (referred to as induction period) with a duration that was concentration-dependent. Following that lag phase, the rate of oxidation was similar to the rate in controls without vitamin E, suggesting depletion of the antioxidant. In contrast, in our studies using intact cells, vitamin E inhibited the rate of oxidation, and no lag period could be detected. This difference in studies of solutions versus cells could be due to a cellular localization of vitamin E in cellular sites that are anatomically discrete, thereby resulting in limited accessibility for biochemical reactions. Alternatively, it could be a result of the regeneration of vitamin E by enzyme systems as well as ascorbate present in the cell. In any case, studies of intact cells provide additional insight into the biology of the antioxidant behavior of vitamin E.

The more effective inhibition of oxidation by vitamin E of cells treated with edelfosine, shown in Figs. 5 and 6 compared to Figs. 1 and 2, is likely due to the augmented oxidative stress contributed by edelfosine. However, in contrast to experiments without edelfosine, vitamin E induces a clear lag phase. In general this result is similar to the studies of oxygen consumption and thiobarbituric acid reactive substance generation on lipid solutions and liposomes in which oxidation was initiated with azo initiators or other compounds (2–4). We suggest that our observation of a lag phase is due to membrane disruption by edelfosine resulting in an experimental preparation of suspended cellular components that behaves in a manner similar to subcellular fractions and homogenous solutions. We have previously reported that edelfosine increases the cytotoxicity of oxidative stress (12). This increased cytotoxicity is thought to be due to the more intense oxidation observed. The membrane alteration produced by edelfosine may result in the oxidative behavior of the cells being more like lipid solutions or liposomes. Our results suggest that the action of vitamin E on subcellular fractions, liposomes, lipid solutions, or damaged cells will have different features as compared to studies on intact viable cells. Investigators should be cautious in extending detailed observations on these preparations to *in vitro* and *in vivo* cellular systems.

The uptake of oxygen as shown in Fig. 2, the generation of the lipid radical product in Fig. 1, and the effect of vitamin E on each are not identical in kinetics and concentration dependence. This difference results from the fact that they are measured with different techniques each of which gives insights into a complicated oxidative process. The oxygen consumption experiments measure the total oxygen consumed. In contrast, the EPR spin trapping experiments record only a small

fraction of the radicals produced in the system. Although the plateau/peak levels of oxygen consumption and radical production in the absence of antioxidant occur at different times, there are similarities: (a) both lipid radical production and oxygen consumption are proceeding at the earliest time point measurable; (b) the highest rate for each occurs during the first 300 s. The addition of vitamin E resulted in an apparent threshold for effect, 0–5 μM for oxygen consumption and 5–10 μM for radical production; these are not dramatically different. The conclusion is that just a small amount of supplemental vitamin E, $\approx$10 μM, will dramatically alter the kinetics of the free radical processes being examined in cell culture experiments by these complementary techniques.

Our studies in the absence of supplemental vitamin E show high $[Asc^{\bullet -}]_{ss}$ immediately after the introduction of an oxidative stress (Fig. 7, top). $[Asc^{\bullet -}]_{ss}$ then declines during the next 600–700 s. When these experiments were repeated with vitamin E-supplemented cells, both $[Asc^{\bullet -}]_{ss}$ and [POBN/L$^{\bullet}$] are markedly reduced. The reduction in $[Asc^{\bullet -}]_{ss}$ at the initial time points is consistent with a lack of a burst of oxidation while the much lower level of [POBN/L$^{\bullet}$] observed indicates an overall lowering of the extent of cellular oxidation. Thus, vitamin E reduces propagation of free radical events in cells.

We hypothesized that free radical-mediated oxidative events can be seminal steps in membrane damage. To examine this proposal, we have compared the kinetics of the oxidative with the dye exclusion events. We postulated that the time at which the [POBN/L$^{\bullet}$] peaks in the EPR studies should correspond to a membrane damage landmark. In addition, there should be a corresponding minimization of $[Asc^{\bullet -}]_{ss}$ signal. In our experiments where an ongoing oxidative stress is applied, the addition of vitamin E should shift these events to a later time point. This is what we observed. First, the time to 50% death in the absence of added vitamin E in our studies is about 420 s (Fig. 3), which corresponds to the approximate time that the $[Asc^{\bullet -}]_{ss}$ is minimized (Fig. 7). This is also approximately the time that the [POBN/L$^{\bullet}$] peaks. It seems likely that these two events, that is the disappearance of $Asc^{\bullet -}$ and the maximizing of [POBN/L$^{\bullet}$], indicate the end of the oxidative episodes that lead to cellular damage. Most importantly, with the addition of 100 μM vitamin E to the media, these events were all delayed.

In summary, we have demonstrated that vitamin E has a profound influence on Fe^{2+}/ascorbate-induced lipid peroxidation in intact mammalian cells. It inhibits the rate of peroxidation as measured by both the generation of a lipid-derived free radical and by the uptake of oxygen. When edelfosine was added in order to augment oxidation similar results were observed; however, a definite delay (lag phase) was observed as seen in previous studies on lipid solutions and dispersions. Although vitamin E is an excellent antioxidant, these *in vitro* studies with intact cells indicate that the kinetic behavior of tocopherol in cells is different from that in model systems.

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