Effect of polyunsaturated fatty acids and antioxidants on lipid peroxidation in tissue cultures

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Abstract Lipid peroxidation was measured by the thiobarbituric assay for malondialdehyde (MDA). A small amount of MDA was formed when medial cells from guinea pig aorta were grown in tissue culture. The polyunsaturated fatty acids 8,11,14-eicosatrienoic acid, 5,8,11,14-eicosatetraenoic acid, and 7,10,13,16-docosatetraenoic acid generated significant amounts of MDA in a time-dependent manner when they were added to cultures of medial cells and fibroblasts. MDA or its precursor remained within the cell and did not accumulate in the media. Indomethacin enhanced MDA formation from polyunsaturated fatty acid. α-Tocopherol, α-tocopherolquinone, and 2,6-di-tert-butyl-4-methylphenol (BHT) inhibited MDA formation when a polyunsaturated fatty acid was incubated with the prooxidant cumene hydroperoxide. Menadione had no effect on MDA formation in the cumene hydroperoxide system. α-Tocopherol and α-tocopherolquinone inhibited MDA formation when they were added to cells in culture. Menadione had no effect on MDA formation in tissue culture. Anti-oxidant effects which were time-dependent showed that intracellular MDA was generated from a lipid peroxide precursor during the thiobarbituric acid assay. Relative plating efficiency was measured in medial cells and fibroblasts. α-Tocopherolquinone and α-tocopherol enhanced the extent of cell proliferation. α-Tocopherolquinone overcame the inhibitory effect of a polyunsaturated fatty acid on the extent of cell proliferation. Menadione was cytotoxic. Thus antioxidant data support the hypothesis that the extent of cell proliferation is controlled in part by lipid peroxidation.—**Gavino, V. C., J. S. Miller, S. O. Ikharebha, G. E. Milo, and D. G. Cornwell.** Effect of polyunsaturated fatty acids and antioxidants on lipid peroxidation in tissue cultures. *J. Lipid Res.* 1981. **22:** 763–769.

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Polyunsaturated fatty acids such as 8,11,14-eicosatrienoic acid (8,11,14–20:3), 5,8,11,14-eicosatetraenoic acid (5,8,11,14–20:4), and 7,10,13,16-docosatetraenoic acid (7,10,13,16–22:4) decrease the extent of cell proliferation (number of colonies arising from individual cells) in cultures of smooth muscle or medial cells (1–5) and fibroblasts (2, 5). The monounsaturated fatty acid 9-octadecenoic acid enhances the extent of cell proliferation in cultures of medial cells (2). Cells treated with the polyunsaturated fatty acid accumulate large amounts of lipid as triglyceride (4–6) and show large increases in the number of both lipid droplets and lysosomes (4). α-Tocopherol (vitamin E) restores cell proliferation even though it has no effect on triglyceride accumulation, lipid droplets, and lysosomal enzyme activity (4). Cells treated with the polyunsaturated fatty acid synthesize large amounts of prostaglandins E_1 and E_2 (1, 3) and these polyunsaturated fatty acid metabolites inhibit the extent of cell proliferation (7). α-Tocopherol restores cell proliferation even though it does not inhibit prostaglandin biosynthesis (3). These studies with α-tocopherol show that neither triglyceride biosynthesis and the related changes in cell morphology nor prostaglandin biosynthesis explain the effect that polyunsaturated fatty acids have on the extent of cell proliferation.

Polyunsaturated fatty acids readily undergo lipid peroxidation (8–12). Since α-tocopherol is an antioxidant (8–12), it could restore cell proliferation through the inhibition of lipid peroxidation. We have examined this hypothesis by measuring lipid peroxidation in cells treated with polyunsaturated fatty acids and with α-tocopherol.

A number of controversial studies beginning shortly after the discovery of vitamin E (13, 14) and continuing to the present time (15–17) have described the antioxidant properties not only of α-tocopherol but also of its metabolite α-tocopherolquinone. We have re-investigated the role of α-tocopherolquinone as an antioxidant. Since 1,4-benzoquinones and 1,4-naphthoquinones are reduced to semi-quinones at different rates by free radicals (12, 18), we have com-

Abbreviations: MDA, malondialdehyde; BHT, 2,6-di-tert-butyl-4-methylphenol; TBA, thiobarbituric acid. Unsaturated fatty acids are designated as follows: numerical position of each double bond-number of C atoms:number of double bonds.

pared the antioxidant properties of the 1,4-benzoquinone, α-tocopherolquinone, with the antioxidant properties of the 1,4-naphthoquinone, menadione. Finally, we have measured the effect of different quinones on the extent of cell proliferation in cultures of medial cells and fibroblasts.

MATERIALS AND METHODS

Materials

Fatty acids were purchased from Nu Chek (Elysian, MN). Fatty acids were checked for purity by gas–liquid chromatography and were shown to be peroxide-free by thin-layer chromatography (1). α-Tocopherol and α-tocopherolquinone were purchased from Eastman Organic Chemicals (Rochester, NY). Menadione was purchased from Sigma Chemical Co. (St. Louis, MO). 2,6-Di-tert-butyl-4-methylphenol (BHT) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Cumene hydroperoxide was purchased from Matheson, Coleman and Bell (Norwood, OH).

Tissue culture

Primary cultures of fibroblasts were established from neonatal foreskin (2, 6) and primary cultures of smooth muscle cells (3, 4, 6, 19) were established from the dissected medial layer of guinea pig aorta from prepubertal males. Each one to two split grown to confluency was counted as one passage number. Cells were harvested and used from two to six passages. The medium for growing cells to confluency (Growth Medium) was prepared from 1× Eagle's minimum essential medium containing Hank's salts and 25 mM HEPES buffer (GIBCO, Grand Island, NY) supplemented with 50 μg per ml gentamycin sulfate (Schering, Kenilworth, NJ), 2 mM glutamine (GIBCO), 1× nonessential amino acids (Microbiological Associates, Walkersville, MD), 1 mM sodium pyruvate (Microbiological Associates), and 1.3 mg of sodium bicarbonate. This medium was supplemented with 5% fetal bovine serum (Reheis, Phoenix, AZ, Lot P 34112 and Lot R 32112) for medial cells and 10% fetal bovine serum for fibroblasts.

The medium used in cell proliferation and lipid peroxidation experiments (Experimental Medium) consisted of Growth Medium supplemented with 1× essential amino acids, 1× essential vitamins, and 20% fetal bovine serum. Experimental Medium was used with both cell lines.

Fatty acids were dissolved in 95% ethanol and diluted 1.6:100 then 1:8 to a final 1:500 dilution with Experimental Medium. Antioxidants were dissolved in 95% ethanol and diluted 1.6:100 then 1:40 to a final 1:2500 dilution with Experimental Medium. Control cultures were treated with Experimental Medium containing the same amount of 95% ethanol.

Lipid peroxidation

The antioxidant properties of quinones were first measured in a non-enzymatic system. Cumene hydroperoxide, an agent which initiates lipid peroxidation in tissue homogenates and in whole animals (20), was used to generate malondialdehyde (MDA) from 5,8,11-octadecatrienoic acid (5,8,11–18:3). The reaction mixture contained 1.0 μM 5,8,11–18:3 and 0.25 μM ferric chloride in 1 ml of 0.1 M phosphate buffer (pH 7.4) with or without a known antioxidant or a quinone. To this was added 100 μl of a cumene hydroperoxide solution (2.2 μl of cumene hydroperoxide dissolved in 1 ml of distilled water). The mixture was allowed to stand for 10 min at room temperature and then 2 ml of a thiobarbituric acid (TBA) reagent (15% trichloroacetic acid and 0.375% thiobarbituric acid in 0.25 N hydrochloric acid, w/v) (21) were added to the mixture. This mixture was heated for 15 min in boiling water, cooled in ice, and then extracted with 3 ml of chloroform–acetic acid 2:1 (v/v) (22). The mixture was centrifuged briefly to separate the phases and the absorbance of the upper phase was then measured at 532 nm. Absorbance was converted to nmol MDA from a standard curve generated with 1,1,3,3-tetramethoxypropane.

Lipid peroxidation in tissue cultures were measured with cells that were grown to confluency in Falcon T-25 flasks containing 4 ml of Experimental Medium. Cells were treated with 120 μM fatty acid with or without 10 μM vitamin E or a quinone. After a specified time interval, cells were killed and disrupted by the addition of 2 ml of 20% trichloroacetic acid to the medium in the flask. Four ml of 0.67% TBA was added to the flask and this mixture was incubated for 20 min at 97°C. The flask contents were decanted and centrifuged at 12,000 *g* for 10 min at 4°C. The absorbance of the supernatant was measured at 532 nm. Tissue culture media contains a pH indicator, phenol red, that contributes a background absorbance at 532 nm. The TBA absorbance from the media was subtracted from the TBA absorbance from the culture. Absorbance was converted to nmol MDA from a standard curve generated with 1,1,3,3-tetramethoxypropane. Lipid peroxidation was reported as nmol MDA per culture. Lipid peroxidation was not observed when the media with or without added fatty acid was incubated without cells.

Extent of cell proliferation (colony formation)

Medial cells were seeded at 200 cells per cm^2 in Falcon single-well (60 by 15 mm) plates. Cells were allowed to attach to the plastic for 1 day before initi-

ating treatments. The medium was changed at the initial treatment and the cells were retreated at day 5. The medium was removed after 8 to 10 days and the cultures were fixed in 3% phosphate-buffered formalin, stained with filtered Giemsa, and the stained area was measured with an Optomax Visual Analysis System (Optomax, Inc., Wallis, NH). The stained area, which varied directly with the number of colonies in the Falcon well, was used to estimate the plating efficiency or percentage of seeded cells that gave rise to colonies (23). The extent of cell proliferation was reported as relative plating efficiency or the ratio in percent of plating efficiencies for treatment and control groups (1–5).

RESULTS

MDA is a product of both lipid peroxidation (8–12, 20, 21, 22) and of prostaglandin biosynthesis (24). A small amount of MDA was found when medial cells were incubated with Experimental Medium alone (**Table 1**). Large amounts of MDA were found when these cells were incubated with either 8,11,14–20:3, 5,8,11,14–20:4, or 7,10,13,16–22:4 (Table 1). When media and cells were separated before the TBA reaction, 90% of the MDA was found in cells and 10% was found in media. The incubation was repeated in the presence of sufficient indomethacin to block prostaglandin biosynthesis in a smooth muscle cell culture (1). More MDA was formed when 8,11,14–20:3 and 5,8,11,14–20:4 were incubated with indomethacin than without indomethacin (Table 1). Indomethacin had no effect on MDA formation in cells treated with 7,10,13,16–22:4 (Table 1).

MDA formation was time-dependent in both fibroblast and medial cell cultures (**Table 2**). Fibroblasts generated small amounts of MDA at 6 hr while medial cells generated relatively large amounts of MDA at this time interval. In fact, medial cells formed significantly more MDA than fibroblasts at every time interval studied. The MDA content of fibroblast cultures increased from 24 to 48 hours while the MDA content of medial cell cultures reached a maximum in 24 hr and did not change when the incubation period was extended to 52 hr. These data are consistent with fatty acid uptake data (6) which showed that confluent medial cells accumulated 83% of added 120 μM fatty acid in 24 hr while confluent fibroblasts accumulated only 25% of added 120 μM fatty acid in 24 hr.

TABLE 1. Lipid peroxidation in confluent medial cells incubated for 52 hr with a polyunsaturated fatty acid, with or without indomethacin

Treatment	Indomethacin	
	0	10 μM
	nmol MDA/culture	
Control[a]	0.7 ± 0.5[b]	
Control + 8, 11, 14–20:3 (120 μM)	9.9 ± 1.8	12.1 ± 0.8[c]
Control + 5, 8, 11, 14–20:4 (120 μM)	9.9 ± 0.4	14.5 ± 0.9[d]
Control + 7, 10, 13, 16–22:4 (120 μM)	11.1 ± 1.6	11.4 ± 3.0

[a] Confluent medial cells were incubated with Experimental Medium for 52 hr, then analyzed for MDA.
[b] Mean ± S.D.
[c] Significant difference ($P < 0.05$) from incubation with fatty acid alone.
[d] Significant difference ($P < 0.005$) from incubation with fatty acid alone.

Antioxidant and quinone effects on lipid peroxidation initiated with cumene hydroperoxide

The synthetic antioxidant, BHT, and the naturally occurring antioxidant, α-tocopherol, both inhibited MDA formation from 5,8,11–18:3 through lipid peroxidation initiated with cumene hydroperoxide (**Table 3**). The substituted 1,4-benzoquinone, α-tocopherolquinone, also inhibited MDA formation in

TABLE 2. Lipid peroxidation in confluent fibroblasts and medial cells incubated for specific times with a polyunsaturated fatty acid

Treatment	Incubation Time (hr)			
	2	6	24	52
	nmol MDA/culture			
8, 11, 14–20:3 (120 μM)				
Fibroblasts	0	0.2 ± 0.4[a]	2.7 ± 0.8	5.9 ± 1.6
Medial cells	0.7 ± 0.3[b]	3.9 ± 1.1[b]	10.5 ± 0.8[b]	9.9 ± 1.8[b]
5, 8, 11, 14–20:4 (120 μM)				
Fibroblasts	0	0.2 ± 0.4	3.5 ± 1.6	6.9 ± 0.3
7, 10, 13, 16–22:4 (120 μM)				
Fibroblasts	0	0.9 ± 0.6	3.9 ± 1.0	8.4 ± 1.0

[a] Mean ± S.D.
[b] Significant difference ($P < 0.005$) between fibroblasts and medial cells.

TABLE 3. Effect of antioxidants and quinones on lipid peroxidation initiated with cumene hydroperoxide

Reactant System	Mean ± S.D.
	nmoles MDA
5, 8, 11–18:3 (1 μM)	3.8 ± 0.4
5, 8, 11–18:3 (1 μM) + BHT (10 μM)	1.6 ± 0.3[a]
5, 8, 11–18:3 (1 μM) + α-tocopherol (10 μM)	1.1 ± 0.1[a]
5, 8, 11–18:3 (1 μM) + α-tocopherolquinone (10 μM)	1.9 ± 0.4[a]
5, 8, 11–18:3 (1 μM) + menadione (10 μM)	4.3 ± 0.8

[a] Significant difference ($P < 0.001$) from reaction with fatty acid alone.

this aqueous system while the substituted 1,4-naphthoquinone, menadione, had no effect on MDA formation in this system (Table 3). These data show that both α-tocopherol and α-tocopherolquinone function as antioxidants in the inhibition of MDA generation from a polyunsaturated fatty acid.

Antioxidant and quinone effects on lipid peroxidation in tissue culture

α-Tocopherol and α-tocopherolquinone significantly decreased MDA formation when they were added to confluent monolayers at the same time as a polyunsaturated fatty acid and the cells were incubated for 24 to 52 hr (concurrent addition data in **Table 4**). Menadione had no effect on lipid peroxidation with this incubation system (concurrent addition in Table 4). These data showed that antioxidants and quinones behaved similarly in tissue culture as well as cumene hydroperoxide systems for the generation of MDA through lipid peroxidation.

The initial studies involving the concurrent addition of a polyunsaturated fatty acid and either α-tocopherol or α-tocopherolquinone showed that these antioxidants had little effect on MDA formation at short incubation time intervals (Table 4). For example, antioxidants had no effect on the increment in MDA that was found after a 6-hr incubation period. These data suggested that the cellular uptake of the antioxidant was not rapid enough for inhibition at short incubation time intervals. Cells were, therefore, preincubated for 24 hr with media containing α-tocopherol or α-tocopherolquinone. 8,11,14–20:3 was then dissolved in alcohol and added to the preincubated media. MDA formation was inhibited at 2 and 6 hr when cultures were treated in this way (pre-addition data in Table 4). The relatively slow cellular uptake of the antioxidant apparently masked antioxidant effects at 6 hr when fatty acid and antioxidant were added concurrently. Finally, the small amount of MDA generated when cells were incubated with media alone, 0.7 ± 0.5 nmol MDA/culture (Table 1), was diminished significantly ($P < 0.005$) when cells were pre-incubated with 10 μM α-tocopherolquinone and then incubated with only Experimental Media, −0.2 ± 0.5 nmol MDA/culture.

Recent studies (25–27) on the mechanism of lipid peroxidation suggest that MDA and other peroxida-

TABLE 4. Lipid peroxidation in confluent medial cells incubated with 8, 11, 14–20:3 and antioxidants or menadione

Treatment	Incubation Time (hr)			
	2	6	24	52
	nmol MDA/culture			
Concurrent addition[a]				
8, 11, 14–20:3 (120 μM)	0.7 ± 0.3[b]	3.9 ± 1.1	10.5 ± 0.8	9.9 ± 1.8
8, 11, 14–20:3 (120 μM) + α-tocopherol (10 μM)	0.4 ± 0.6	3.4 ± 0.4		2.4 ± 3.3[e]
8, 11, 14–20:3 (120 μM) + α-tocopherolquinone (10 μM)	0.8 ± 0.1	2.7 ± 1.3	1.8 ± 0.2[e]	1.2 ± 0.6[e]
8, 11, 14–20:3 (120 μM) + menadione (10 μM)	0.5 ± 0.3	3.8 ± 1.2		9.5 ± 1.0
Pre-addition[a]				
8, 11, 14–20:3 (120 μM)	2.6 ± 1.0	7.3 ± 0.4	9.2 ± 0.8	
8, 11, 14–20:3 (120 μM) + α-tocopherol (10 μM)	0.6 ± 0.3[c]	1.9 ± 1.6[e]	0.3 ± 0.3[e]	
8, 11, 14–20:3 (120 μM) + α-tocopherolquinone (10 μM)	0.2 ± 0.4[c]	0.6 ± 0.7[e]	0.3 ± 0.1[e]	
Post-addition[a]				
8, 11, 14–20:3 (120 μM)	0.7 ± 0.3	3.9 ± 1.1	10.5 ± 0.8	9.9 ± 1.8
8, 11, 14–20:3 (120 μM) + α-tocopherol (10 μM)			11.3 ± 1.5	7.5 ± 0.8[d]
8, 11, 14–20:3 (120 μM) + α-tocopherolquinone (10 μM)			10.8 ± 0.5	1.1 ± 0.4[e]
8, 11, 14–20:3 (120 μM) + BHT (10 μM)			1.9 ± 0.2[e]	1.5 ± 0.5[e]

[a] Addition sequences: fatty acid and other agent were added to the incubation flask at the same time (concurrent addition); other agent was added to the incubation flask 24 hr before the fatty acid (pre-addition); other agent was added to the incubation flask 24 hr after the fatty acid (post-addition).

[b] Mean ± S.D.

[c] Significant difference ($P < 0.05$) from incubation with fatty acid alone.

[d] Significant difference ($P < 0.01$) from incubation with fatty acid alone.

[e] Significant difference ($P < 0.005$) from incubation with fatty acid alone.

tion products are formed in two sequential steps involving first the formation of peroxy radicals or lipid hydroperoxides and then the formation and breakdown of cyclic endoperoxides. The concurrent addition of a polyunsaturated fatty acid and α-tocopherolquinone resulted in MDA levels at 24 and 52 hr that were lower than the MDA level at 6 hr (Table 4). These data suggested that α-tocopherolquinone may have interrupted the breakdown of a cyclic endoperoxide to MDA. We have attempted to separate the initial peroxidation step and the ultimate breakdown step by preincubating cells with 8,11,14–20:3 for 24 hr, then adding an antioxidant and continuing the incubation for an additional period of time (post-addition data in Table 4). Other studies have suggested that BHT inhibits both the initiation and breakdown steps (27). BHT had no effect on absorbance when it was added directly to 1,1,3,3-tetramethoxypropane in the generation of a standard curve. However, the post-addition of BHT had an immediate effect on MDA formation in tissue cultures. Neither α-tocopherol nor α-tocopherolquinone had an immediate effect on MDA formation. The post-addition of these compounds inhibited MDA formation when incubations were continued for an additional 28 hr. Thus post-addition data showed that antioxidants blocked the breakdown of a precursor involved in the formation of MDA and post-addition data confirmed the observation that the cellular uptake of α-tocopherol and α-tocopherolquinone was not immediate.

Although the differences were not statistically significant, concurrent addition and pre-addition data both suggested that α-tocopherolquinone was a more effective antioxidant than α-tocopherol in our tissue culture system (Table 4). This suggestion was supported by the post-addition study where a statistically significant difference ($P < 0.005$) was found between the inhibitory effects of a α-tocopherolquinone and α-tocopherol (Table 4).

Effect of antioxidants on the extent of cell proliferation (relative plating efficiency)

Our previous studies (3, 4) showed that α-tocopherol restored the extent of cell proliferation in medial cells treated with polyunsaturated fatty acids. If lipid peroxidation modulates cell proliferation, then a more effective antioxidant in tissue culture, such as α-tocopherolquinone (Table 4), should also restore the extent of cell proliferation in cells treated with a polyunsaturated fatty acid. The concurrent addition of α-tocopherolquinone restored the relative plating efficiency of medial cells treated with 80 μM 5,8,11,14–20:4 (**Fig. 1**). Moreover, 0.1 μM α-tocopherolquinone (Fig. 1) had the same effect as 1.0 μM α-tocopherol (3) in restoring the relative plating efficiency of medial cells treated with a polyunsaturated fatty acid.

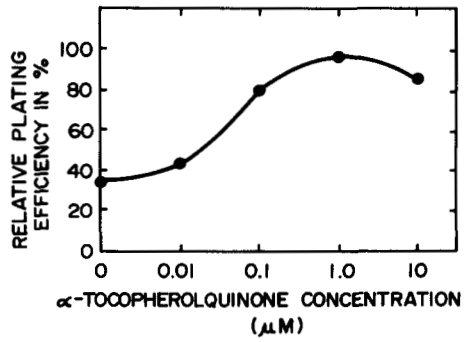

Fig. 1. Extent of cell proliferation (relative plating efficiency) in medial cells treated with 80 μM 5,8,11,14–20:4 and increasing concentrations of α-tocopherolquinone.

Lipid peroxidation is found not only in cells treated with a polyunsaturated fatty acid but also in cells grown only in Experimental Medium (Table 1). Our previous studies showed that α-tocopherol enhanced the extent of cell proliferation in these cells (3, 4). In the present study, these results with α-tocopherol were confirmed with both medial cells (**Fig. 2**) and fibroblasts (**Fig. 3**). Furthermore, α-tocopherolquinone at all concentrations had an even greater effect than α-tocopherol on relative plating efficiency (Figs. 2 and 3). Menadione did not function as a quinone antioxidant (Tables 3 and 4), and menadione did not enhance the extent of cell proliferation (Fig. 2). Thus α-tocopherol, α-tocopherolquinone, and menadione data were consistent with the hypothesis that the extent of cell proliferation was controlled in part by lipid peroxidation. Finally, α-tocopherol and menadione were cytotoxic at high concentrations (Figs. 2 and 3). α-Tocopherolquinone was only cytotoxic at high concentrations in the more sensitive fibroblast line (Figs. 2 and 3).

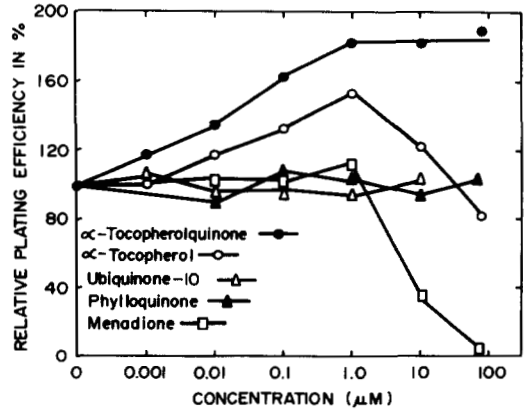

Fig. 2. Extent of cell proliferation (relative plating efficiency) in medial cells treated with increasing concentrations of α-tocopherol or specified quinones.

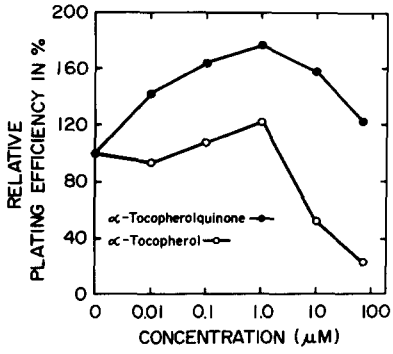

Fig. 3. Extent of cell proliferation (relative plating efficiency) in fibroblasts treated with increasing concentrations of α-tocopherol or α-tocopherolquinone.

DISCUSSION

In 1933, Mason (28) summarized many studies that suggested that the antioxidant α-tocopherol was essential for tissues in which cell proliferation and differentiation were unusually rapid. These studies were later extended by Bernheim (29) who measured MDA and found that tissues with a high mitotic index such as regenerating liver, bone marrow, and intestinal mucosa showed diminished lipid peroxidation when they were compared to other non-regenerating tissues. The role of α-tocopherol in cell proliferation and differentiation has been extended by experiments with the rotifier *Asplanchna sieboldi* (30). Our tissue culture data support these studies. Cells in tissue culture undergo lipid peroxidation and generate an MDA precursor. Cells challenged with a polyunsaturated fatty acid generate increased amounts of an MDA precursor. Antioxidants inhibit MDA formation, enhance the extent of cell proliferation (relative plating efficiency) in control cultures, and restore the extent of cell proliferation in cultures challenged with a polyunsaturated fatty acid. These observations help to explain early (31) and recent (32) studies that noted that α-tocopherol generally enhanced cell growth in tissue cultures.

Antioxidants and other reducing agents may inhibit the formation of peroxy radicals and lipid hydroperoxides, and antioxidants may interfere with the subsequent formation and breakdown of cyclic endoperoxides to MDA (8–12, 25–27). Several observations suggest that antioxidants act at both steps in our tissue culture systems. Pre-addition studies showed that both α-tocopherol and α-tocopherolquinone strongly inhibited MDA formation while post-addition studies showed that α-tocopherolquinone was more effective than α-tocopherol as an inhibitor of MDA formation. These data indicate that α-tocopherol preferentially inhibits the formation of lipid hydroperoxides. The immediate and delayed post-addition inhibitory effects of BHT and α-tocopherolquinone suggest that these antioxidants, like stannous chloride (25), convert lipid peroxides to stable products preventing their breakdown to MDA. Apparently, preformed MDA does not accumulate in the cell. MDA, like ethylene (26), is formed from the breakdown of lipid peroxides during assay.

Several early investigators have suggested that α-tocopherolquinone functions as an antioxidant (13, 15). Our studies both with cumene hydroperoxide and with cells challenged by polyunsaturated fatty acids support this suggestion. Since 1,4-benzoquinones are more rapidly reduced to semi-quinones than 1,4-naphthoquinones (12, 18) and the 1,4-naphthoquinone, menadione, does not function as an antioxidant, α-tocopherolquinone may interrupt free radical chain reactions through its conversion to a semi-quinone. Alternatively, in biological systems, α-tocopherolquinone may be reduced to the hydroquinone (33) which then functions as an antioxidant (15).

α-Tocopherol and α-tocopherolquinone at relatively low concentrations (0.01 to 0.1 μM) enhance the extent of cell proliferation. These antioxidants are cytotoxic in the 100 μM and greater concentration range. Cytotoxicity and the biological effects found at high antioxidant concentrations (17) may be related to the surface properties of α-tocopherol and its derivatives (34) and the ability of these compounds to mimic a non-ionic detergent (35).

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