Mitoxantrone and Amtantrone Inhibit Hydroperoxide-dependent 
Initiation and Propagation Reactions in Fatty Acid Peroxidation*

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Peroxidation of polyunsaturated membrane lipids by oxygen free radicals is stimulated by several redox-cycling compounds and has been implicated in the cellular toxicity of these agents (1, 2). The production of superoxide anion (O2-)

appears to be the primary event in the peroxidation process (3–8). Superoxide anion is not sufficiently reactive to abstract hydrogen from unsaturated lipids to initiate peroxidation (9, 10); rather, secondary reactive oxygen species derived from O2- or H2O2, such as 'OH, are thought to ultimately initiate lipid peroxidation (6, 7, 11–13). At present, the most generally accepted mechanism for reactive oxygen production from O2- is an iron-dependent Haber-Weiss reaction in which H2O2, formed by O2- dismutation, reacts with chelated ferrous iron (ferrous iron reduced by O2- to form OH (14–16). Although other reaction schemes for the production of oxidizing species have been proposed and include intermediates such as the perferryl ion (ferrous-dioxygen) or the ferryl ion, they nonetheless represent iron-dependent production of a strong oxidant species capable of initiating lipid peroxidation (11, 17, 18).

Fatty acid peroxidation consists of initiation and propagation reactions. Initiation occurs following hydrogen abstraction by hydroxyl radicals to form alkyl radicals (L'). Lipid radicals formed during initiation propagate peroxidation by the concerted effects of two reactions.

\[
\begin{align*}
\text{L}^- + \text{O}_2 &\rightarrow \text{LOO}^- \quad (1) \\
\text{LOO}^- + \text{LH} &\rightarrow \text{LOOH} + \text{L}^- \quad (2)
\end{align*}
\]

A second initiation pathway involves iron-dependent decomposition of lipid hydroperoxides formed during propagation.

\[
\begin{align*}
\text{Fe}^{3+} + \text{LOOH} &\rightarrow \text{Fe}^{2+} + \text{LO} + \text{OH}^- \quad (3) \\
\text{Fe}^{2+} + \text{LOOH} &\rightarrow \text{Fe}^{3+} + \text{LOO}^- + \text{H}^+ \quad (4)
\end{align*}
\]

Decomposition of lipid hydroperoxides is facilitated by both ferric and ferrous iron, although the rate is considerably more rapid when catalyzed by the ferrous form (19–22). Ferrous iron-promoted hydroperoxide-dependent initiation appears not to involve O2- or 'OH, but is markedly attenuated by iron chelators such as EDTA (22). Hydroperoxide-dependent initiation reactions require the presence of LOOH formed initially via hydroxyl radical-dependent pathways (23). Once a critical concentration of LOOH is achieved, the predominant mechanism of initiation appears to be lipid hydroperoxide-dependent rather than hydroxyl radical-dependent (23).

We have recently described the inhibition of lipid peroxidation by mitoxantrone (NSC 301739) and ametantrone (NSC 287513), two new bisalylaminanthracenedione antineoplastic agents (24, 25). These compounds diminished endogenous rabbit and rat liver microsomal, cardiac sarcosomal, and cardiac mitochondria malondialdehyde production, as well as that stimulated by redox-cycling compounds such as

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parasquat, nitrofurantoin, and doxorubicin (24-26). Although mitoxantrone and ametantrone are effective antioxidants, the mechanisms by which these agents inhibit basal and drug-stimulated lipid peroxidation remains unclear. The present work reports the effects of mitoxantrone and ametantrone on conjugated diene formation from linoleic acid as catalyzed by NADPH-cytochrome P-450 reductase, xanthine oxidase, and several chemical oxidant systems. The data implicate a termination mechanism resulting from an inhibitory effect of the anthracenediones on fatty acid hydroperoxide-dependent initiation and propagation reactions.

MATERIALS AND METHODS

NADPH, hypoxanthine, Na₂EDTA, Na₃EDTA, Lubrol PX, xanthine oxidase (1.1 units/mg), and soybean lipoxidase Type 1 (130,000 units/mg) were purchased from Sigma. Linoleic acid (99.9%) was obtained from Calbiochem-Behring. Acetaldehyde (Eastman) was freshly distilled for each experiment. Mitoxantrone and ametantrone were supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. All other reagents were of the highest purity available. Buffers were passed through Chelex 100 cation exchange resin (Bio-Rad) to remove contaminating metal ions. Hydroperoxide concentrations were determined by titration with certified standard potassium permanganate solution (Fisher). Pyrophosphate-extracted microsomes were prepared from livers of phenobarbital-induced rabbits as described previously (25). Liver microsomal NADPH-cytochrome P-450 reductase was purified to electrophoretic homogeneity with an average specific activity of 47 pmol of cytochrome c reduced per min/mg of protein (30 °C, in 0.3 M potassium phosphate buffer, pH 7.7) according to previously published methods (27). Linoleic acid hydroperoxide was prepared from linoleic acid by the action of soybean lipoxidase (26) and quantitated using an extinction coefficient of 25,000 M⁻¹ cm⁻¹ (11).

Stock solutions of linoleic acid micelles (10 mM) were prepared daily by adding 2.8 mg/ml linoleic acid to 30 mM NaCl, pH 11, and slowly titrating to pH 7.0 with HCl. The opaque micellar suspension was made 0.5% (v/v) in Lubrol PX to provide an optically clear dispersion (29). Stock solutions of ferric EDTA chelates (0.1 mM FeCl₃, 1.1 mM EDTA) were prepared daily by addition of solid FeCl₃ to EDTA in 30 mM NaCl and adjusting the solution to pH 7.0. Ferrous EDTA chelates were prepared directly in the incubation mixtures already containing EDTA by addition of a neutral FeCl₃ solution which had been continuously bubbled with nitrogen. This procedure limited autoxidation of Fe²⁺ prior to its introduction into the reaction mixture (29). Fatty acid peroxidation reaction mixtures typically contained 30 mM NaCl, pH 7.0, 1 mM linoleic acid, 0.08% Lubrol, chelated iron (0.1 mM FeCl₃ or 0.1 mM FeCl₃, 0.11 mM EDTA), and 30 mM acetaldehyde/xanthine oxidase (0.037 units) or 100 μM NADPH/NADPH-cytochrome P-450 reductase (72 pmol). Exact reaction conditions are indicated in the text and figure legends. Conjugated diene formation was monitored at 234 nm, as described by Richmond et al. (31).

RESULTS

Enzyme-dependent Oxidation of Linoleic Acid—A system consisting of cofactor, linoleic acid micelles, molecular oxygen, and either P-450 reductase or xanthine oxidase was employed to study the effects of mitoxantrone and ametantrone on enzyme-dependent peroxidation. Detergent-solubilized NADPH-cytochrome P-450 reductase catalyzed the oxidation of linoleic acid micelles, monitored by the increase in absorbance at 234 nm. The correspondence between increased absorbance at 234 nm and the formation of conjugated diene hydroperoxides has been established by high performance liquid chromatography and thin layer chromatography (8, 11). Linoleic acid oxidation was linear with respect to time (2-10 min) and showed an obligatory requirement for NADPH and P-450 reductase.

The anthracenediones mitoxantrone and ametantrone potently inhibited P-450 reductase-dependent linoleic acid oxidation, as shown in Fig. 1. Both anthracenediones markedly diminished the rate of linoleic acid oxidation at concentrations as low as 0.25 μM, with half-maximal inhibition achieved at approximately 0.4 and 0.7 μM mitoxantrone and ametantrone, respectively.

Since superoxide is generally regarded as the initial reduction product of molecular oxygen by P-450 reductase, the effects of mitoxantrone and ametantrone on P-450 reductase activity were determined. The oxidation of NADPH by P-450 reductase was monitored at 340 nm in a reaction mixture identical to that employed in the assay of linoleic acid oxidation (see legend to Fig. 1). The rate of cofactor oxidation, approximately 23 nmol/min, was unaffected by either 1 μM mitoxantrone or ametantrone, while higher concentrations of

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**Fig. 1.** Antracenedione inhibition of NADPH-cytochrome P-450 reductase-catalyzed conjugated diene formation. Reaction mixtures (1.0 ml) contained 30 mM NaCl, pH 7.0; 1 mM linoleic acid; 0.08% Lubrol; 0.1 mM FeCl₃, 0.11 mM EDTA; 72 pmol of P-450 reductase; 0.1 mM NADPH (added to start the reaction); and mitoxantrone (closed symbols) or ametantrone (open symbols) as indicated. Two P-450 reductase/fatty acid suspensions, exhibiting slightly different rates of reductase-dependent peroxidation, were employed. The rate of conjugated diene formation (circles and solid lines) was determined from the linear region of the absorbance change at 234 nm. Per cent inhibition is indicated by squares and dotted lines.
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Effects on Activated Oxygen and Iron Binding—Reactive oxygen species such as HO·, derived from O₂-, are the more proximate initiators of peroxidation. Since HO· may attack aromatic systems, (32, 33), the ability of the anthracenediones to function as hydroxyl radical scavengers was examined. Hydroxyl radical formation in a xanthine oxidase system may be detected directly by the hydroxylation of aromatic compounds. Salicylic acid is hydroxylated by HO· to form primarily 2,3-dihydroxybenzoic acid, which may be detected spectrophotometrically at 510 nm (34). We employed the improved colorimetric assay of Richmond et al. (31) to determine the effects of mitoxantrone and amantantrone on HO· formation from O₂- and H₂O₂ in a hypoxanthine-xanthine oxidase system. The results obtained demonstrated that neither anthracenedione had any significant effect on 2,3-dihydroxybenzoic acid production at concentrations as high as 25 μM. For example, the absorbance value at 510 nm decreased from a control value of 0.600 ± 0.083 in the absence of drug to 0.549 ± 0.050 and 0.574 ± 0.068 in the presence of 25 μM mitoxantrone or amantantrone, respectively. In contrast the

OH scavengers, mannitol and thiourea did diminish dihydroxybenzoic acid production in this system (31) and effectively inhibit the oxidation of fatty acids (4, 6, 11, 13).

Metal ion chelators such as diethylenetriaminepentaacetic acid prevent the iron-dependent production of potent oxidizing species from O₂-, thereby inhibiting the initiation of fatty acid oxidation (11). Chelators also inhibit iron-promoted decomposition of lipid hydroperoxides (22). We therefore examined the iron-binding properties of mitoxantrone and amantantrone. The absolute spectrum of mitoxantrone displays absorption maxima at 609 and 661 nm. Titration with ferric iron resulted in the appearance of a new absorbance band at 729 nm, attributable to the formation of a mitoxantrone-iron complex (Fig. 3A). This change was accompanied by a decrease in the absorbance at 609 and 664 nm, corresponding to the absorption maxima of free mitoxantrone. The appearance of two distinct isosbestic points at 523 and 676
FIG. 4. UV-visible spectroscopic studies on iron-ametantrone interaction. A, titration of ametantrone with ferric iron. Experimental conditions were identical to those described in the legend to Fig. 3. Spectra a–m represent addition of 10–600 μM ferrous ammonium sulfate. B, effect of EDTA on the ametantrone-iron spectrum. To the sample cuvette containing 100 μM ametantrone plus 600 μM ferrous ammonium sulfate, EDTA was added at a final concentration of 1 mM (---) and 3 mM (-----).

nm implies the presence of two discrete absorbing species in solution, mitoxantrone and the mitoxantrone-iron complex. Na₂EDTA addition to solutions of mitoxantrone reversed the spectroscopic changes in both the absolute and difference spectra produced by iron (Fig. 3B). This suggests that EDTA competes favorably for iron bound in the mitoxantrone-iron complex, consistent with the greater stability constant for Fe²⁺-EDTA (approximately 10⁶⁻) (35) relative to that for Fe²⁺-mitoxantrone.

The absolute spectrum of ametantrone displays absorption maxima at 583 and 627 nm (36). Ferric iron addition to 100 μM ametantrone resulted in a small absorption decrease in the longer wavelength band of the ametantrone chromophore (not shown). In contrast, ferric iron addition altered almost exclusively the lower wavelength band of mitoxantrone. Furthermore, unlike mitoxantrone, EDTA failed to reverse these spectral changes, producing only a slight decrease in the absorbance of the chromophore, consistent with simple dilution of ametantrone. Difference spectroscopy demonstrated that titration of ametantrone with ferric iron leads to an attenuation of the absorbance at 651 nm, which corresponds to the longer wavelength maximum of ametantrone (Fig. 4A). No change was observed at 583 nm, the shorter wavelength maximum of ametantrone, although the absorbance at the isosbestic point for free ametantrone (648 nm) was decreased. Increases in the absorbance at 554 and 598 nm were produced by ferric iron. Disodium EDTA failed, however, to reverse the spectral change at 651 nm and produced only a slight reversal of the absorbance increase at 554 and 598 nm (Fig. 4B). In addition, increasing the ionic strength of the ametantrone-containing solution by ammonium sulfate addition resulted in spectral changes similar to those produced by ferric iron. Thus, the results of spectroscopic studies suggest that ametantrone fails to complex iron.

Linoleic Acid Oxidation in Model Systems—To examine the effects of anthracenedione on peroxidation reactions subsequent to generation of O₂⁻ and H₂O₂, two chemical systems were employed (Figs. 5 and 6). Free ferrous iron reacts with H₂O₂ to produce OH⁺ by the Fenton reaction. Formation of OH⁺ caused the rapid oxidation of linoleic acid as evidenced by the rapid increase in absorbance at 234 nm (Fig. 5) which proceeded linearly with time for 10–30 s. The initial rate of Fenton reagent-dependent conjugated diene formation was not affected by 25 μM mitoxantrone or ametantrone or by 100 μM ametantrone. These agents did, however, markedly diminish the rate of oxidation following a short delay period (~10 s). High concentrations of mitoxantrone (100 μM) produced an immediate decrease in the rate of conjugated diene formation.

Ferrous-EDTA complexes autoxidize in neutral to alkaline pH solutions, accompanied by oxygen consumption (presumably O₂⁻ formation) and the oxidation of linoleic acid (29). Autoxidation of ferrous-EDTA (80 μM each) in the presence of 1 mM linoleic acid caused the rapid formation of conjugated diene products (approximately 140 nmol/min), as shown in Fig. 6. The initial rate of peroxidation was diminished only minimally by addition of 100 μM mitoxantrone or ametantrone to 100 and 110 nmol/min, respectively. After a 10-s delay, however, both agents markedly inhibited linoleic acid oxidation, slowing the rate of conjugated diene formation to
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The absence of EDTA, there was only a negligible rate of change in produced by the autoxidation of ferrous iron in the presence of EDTA, the presence of 100 pM mitoxantrone added to initiate the reaction. Reactions were carried out at 37 °C, A, no additions; B, 5 μM amemantrone; C, 5 μM mitoxantrone; D, 100 μM amemantrone; E, 100 μM mitoxantrone. The identical reaction mixture from which preformed linoleic acid hydroperoxide was absent and employed as a control showed no detectable increase in the absorbance at 234 nm following iron addition. Thus, conjugated diene formation in the complete system was entirely hydroperoxide-dependent.

The requirement for substantially higher anthracenedione concentrations to inhibit linoleic acid oxidation in chemical systems, as opposed to enzymatic systems, was attributed to the more rapid initial rate of the chemical reaction. The basal rate of conjugated diene formation initiated by Fenton's reagent (150 nmol/min) and Fe²⁺ autoxidation (140 nmol/min) was significantly greater than that catalyzed by either P-450 reductase or by xanthine oxidase (2–4 nmol/min). The concentration of mitoxantrone and amemantrone required to inhibit peroxidation promoted by EDTA, Fe²⁺ autoxidation could be drastically diminished when the reaction rate was decreased (Fig. 7). Decreasing the temperature of the system from 37 to 10 °C and using 10 μM EDTA, 20 μM Fe²⁺ resulted in a decrease in the rate of linoleic acid oxidation from 140 nmol/min (Fig. 6) to 10 nmol/min (Fig. 7). Under these conditions, conjugated diene formation was diminished by 5 μM mitoxantrone or amemantrone and was essentially abolished at 25 μM anthracenedione, comparable to the concentration dependence for inhibition of fatty acid peroxidation observed with P-450 reductase or xanthine oxidase. By comparison, 100 μM drug was needed to inhibit completely the reaction at 37 °C using 80 μM EDTA-Fe²⁺ (Fig. 6).

Lipid Hydroperoxide-dependent Linoleic Acid Oxidation—While initiation reactions are oxygen radical-mediated, the propagation phase of peroxidation is fatty acid hydroperoxide-dependent, requiring hydroperoxide formation via initiation pathways. The effects of mitoxantrone and amemantrone on linoleic acid hydroperoxide-dependent peroxidation were therefore studied, and the results are presented in Fig. 8.
Unchelated ferrous iron decomposes lipid hydroperoxides to species capable of initiating peroxidation of unsaturated lipids (Reaction 3) (19–22). Addition of 60 μM Fe^{2+} to a suspension of 1 mM linoleic acid, which contained 20 μM linoleic acid hydroperoxide, caused a rapid increase in the absorbance at 234 nm. Control reaction mixtures from which preformed hydroperoxide was omitted exhibited no detectable conjugated diene formation. Thus, the ferrous ion-stimulated initiation of linoleic acid oxidation in the presence of 20 μM linoleic acid hydroperoxide was entirely dependent on lipid oxy radicals formed by iron-dependent decomposition of preformed hydroperoxides (Reactions 3 and 4). Mitoxantrone or ametantrone, when present in this hydroperoxide-dependent system, caused a decrease in the rate of conjugated diene formation. In contrast to oxygen radical-dependent systems (Figs. 1, 2, and 5–7), no delay period was observed prior to the onset of inhibition. Mitoxantrone was a more effective inhibitor than ametantrone as was observed previously (Fig. 5). The anthracenedione concentrations which diminished hydroperoxide-dependent oxidation were lower than those required to inhibit Fenton reagent-dependent (Fig. 5) and EDTA-Fe^{2+}-dependent (Fig. 6) peroxidation, even though the uninhibited rates of the three reactions were comparable.

The selective effects of EDTA-chelated and unchelated Fe^{2+} on oxygen radical-dependent initiation and on lipid hydroperoxide-dependent propagation were exploited to characterize further the anthracenedione inhibition of fatty acid peroxidation (Fig. 9). Peroxidation in a system containing 1 mM linoleic acid micelles and 100 μM anthracenedione was initiated by addition of 80 μM EDTA-Fe^{2+}. The reaction, as observed in Fig. 6, was inhibited following a brief delay, representing formation of 10–15 nmol of lipid hydroperoxide. When there was no further increase in the absorbance at 234 nm, free or chelated Fe^{2+} was added to the reaction mixture.

EDTA chelation of Fe^{2+} causes rapid Fe^{2+} autoxidation and oxygen radical-dependent initiation of linoleic acid oxidation (Fig. 6) but prevents initiation by Fe^{2+}-promoted hydroperoxide decomposition (22). EDTA-chelated Fe^{2+} (80 μM each) added after cessation of peroxidation (light arrows) produced renewed conjugated diene formation which was again inhibited following a 10–15 s delay. Repeated additions of EDTA-Fe^{2+} had a similar effect in the presence of either ametantrone (trace A) or mitoxantrone (trace B). Each aliquot of 80 μM EDTA-Fe^{2+} produced an incremental increase in the absorbance at 234 nm, approximately equivalent to 10–15 μM conjugated diene products. Thus, reactivation of anthracenedione-inhibited peroxidation reflected only oxygen radical-dependent initiation which occurred even in the presence of mitoxantrone or ametantrone during the initial stage of oxidation.

Unchelated ferrous ion causes decomposition of lipid hydroperoxides to radicals which initiate linoleic acid oxidation, but is unable to initiate peroxidation in the absence of hydroperoxides (Fig. 8). Unchelated ferrous iron added after peroxidation ceased (Fig. 9, heavy arrows) was unable to cause further linoleic acid oxidation in the presence of either ametantrone (trace C) or mitoxantrone (trace D), reflecting inhibition of fatty acid hydroperoxide-dependent peroxidation. These results demonstrate a differential effect of the anthracenediones on oxygen radical-versus fatty acid hydroperoxide-dependent initiation and propagation reactions.

**DISCUSSION**

The mechanism by which the anthracenedione antineoplastic agents mitoxantrone and ametantrone inhibit the peroxidation of polyunsaturated fatty acids has been investigated. The experimental approach employed was to examine the effects of mitoxantrone and ametantrone on the individual component reactions comprising the peroxidative sequence utilizing both enzymic and chemical systems.

The anthracenediones were potent (0.25–5 μM) inhibitors of linoleic acid oxidation catalyzed by either cytochrome P-450 reductase or xanthine oxidase. Mitoxantrone and ametantrone fail to stimulate significantly either cofactor consumption or superoxide generation in rabbit hepatic microsomal suspensions, and ametantrone appears to inhibit slightly H2O2 production in this system (37, 38). Since ametantrone has been shown to inhibit the activity of microsomal and purified P-450 reductase toward cytochrome P-450 and artificial electron acceptors (37, 39), potential inhibition of reductase activity in the linoleic acid system was considered. Mitoxantrone and ametantrone had little or no effect on P-450 reductase activity (NADPH oxidation) at concentrations which significantly diminished linoleic acid oxidation. In microsomes, superoxide production was similarly unaffected by anthracenedione concentrations which inhibited lipid peroxidation by greater than 80% (24, 26). Thus, although higher concentrations of ametantrone (0.3–5.0 mM) do diminish the activity of P-450 reductase (39), anthracenedione inhibition of P-450 reductase-catalyzed linoleic acid oxidation was
clearly not the result of enzyme inhibition. Furthermore, neither mitoxantrone nor ametantrone altered hydroxyl radical-dependent hydroxylation of salicylic acid at concentrations as high as 25 μM, suggesting that these agents are not general hydroxyl radical scavengers at the concentrations employed. We therefore conclude that anthracenedione antioxidant effects do not result from inhibition of reactive oxygen formation; inhibition must occur at a subsequent step.

The requisite participation of iron in hydroxyl radical formation and hydroperoxide-dependent initiation of fatty acid peroxidation is well-established. Vicinal guinone-hydroquinone moieties, such as are present in mitoxantrone, constitute a favorable environment for electrostatic interaction with iron or other transition metal ions (40–43). We examined whether the anthracenediones might chelate iron to prevent OH formation or hydroperoxide decomposition, thereby inhibiting linoleic acid oxidation. Titration of mitoxantrone with Fe²⁺ caused spectroscopic changes indicative of an mitoxantrone-iron complex which were reversed by EDTA (Fig. 3). There occurred a decrease in absorbance at wavelengths corresponding to free mitoxantrone, two isosbestic points, no change in the wavelength isosbestic for mitoxantrone, and concomitant appearance of an absorbance band at a higher wavelength, as observed with anthracenedione binding to macromolecules (44). Of the two absorbance maxima of mitoxantrone, the shorter wavelength band was affected most by iron complexation. In contrast, ametantrone, which lacks the quinonemethide-hydroquinone structure, appeared not to chelate iron (Fig. 4).

There was no absorbance band at higher wavelength, only one isosbestic point, an absorbance decrease at the wavelength isosbestic for ametantrone, and perturbations only in the longer wavelength band of ametantrone; these changes were not reversed by EDTA. Thus, while both anthracenediones inhibit lipid and fatty acid peroxidation, only mitoxantrone chelates iron. There also exists a stoichiometric argument against inhibition of peroxidation by anthracenedione-mediated iron chelation. P-450 reductase- and xanthine oxidase-dependent systems contained 100 μM Fe²⁺, whereas conjugated diene formation was half-maximally inhibited by 0.5–1 μM mitoxantrone or ametantrone. It is highly unlikely that each anthracenedione molecule is capable of inactivating a 50–100-fold molar excess of ferric iron. Furthermore, anthracenedione inhibition of rabbit hepatic microsomal lipid peroxidation occurred in the presence of a large excess of Fe²⁺. Thus, iron chelation does not constitute a primary factor in the mechanism of inhibition of linoleic acid oxidation by the anthracenediones.

The delay period observed for anthracenedione inhibition of linoleic acid oxidation by the Fenton reagent and EDTA–Fe²⁺ suggested that the anthracenediones might not inhibit the initial reactions in the peroxidative sequence. Rather, inhibition appeared to require an intermediate, such as fatty acid hydroperoxide, not initially present. Peroxidation not initiated by oxygen radicals, such as that initiated by hydroperoxide decomposition, was inhibited without delay. The delay observed for inhibition of oxygen radical-dependent versus hydroperoxide-dependent peroxidation, coupled with the ability of oxygen radicals to reinitiate peroxidation even in the presence of mitoxantrone or ametantrone (Fig. 9), suggests that the anthracenediones do not significantly alter oxygen radical-dependent initiation reactions.

The data presented in Figs. 8 and 9 suggest a selective effect of mitoxantrone and ametantrone on fatty acid hydroperoxide-dependent linoleic acid oxidation. Ferrous ion normally stimulates hydroperoxide-dependent initiation. In the presence of either anthracenedione, Fe²⁺ addition to a linoleic acid solution containing linoleic acid hydroperoxide was unable to promote additional linoleic acid oxidation in the presence of either anthracenedione. In contrast, EDTA–Fe²⁺, which autorozides to stimulate oxygen radical-dependent initiation reactions, enhanced linoleic acid oxidation even in the presence of mitoxantrone and ametantrone. Lipid hydroperoxide-dependent linoleic acid oxidation was much more sensitive to the effects of the anthracenediones (Fig. 8), as compared to oxygen radical-dependent peroxidation produced by the Fenton reagent (Fig. 5) or Fe²⁺ aut oxidation (Fig. 6).

We conclude therefore that the predominant effect of the anthracenediones on fatty acid peroxidation is the inhibition of lipid hydroperoxide-dependent initiation and propagation reactions.

REFERENCES
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