α-Tocopherol Prooxidant Effect and Malondialdehyde Production

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α-Tocopherol at high concentration (1.25×10^{-4}M) exhibited a prooxidant effect during autoxidation of linolenic and arachidonic acids. This prooxidant activity involved a significant increase of the conjugated diene level, especially with linolenic acid. High performance liquid chromatographic evaluation of malondialdehyde, a by-product of the hydroperoxides of polyunsaturated fatty acids, showed that malondialdehyde was not increased during prooxidant effect of α-tocopherol. Thus, malondialdehyde does not seem to be a good indicator for the manifestation of the prooxidant activity of α-tocopherol.

The measurement of malondialdehyde (MDA), a degradation product of polyunsaturated fatty acids (three or more double bonds), is often used to estimate the level of lipid peroxidation, especially in biological materials (1-4).

Some workers have shown a good correlation between MDA formation, conjugated diene formation and peroxide value (5,6). The most common method used for the quantitation of MDA is the thiobarbituric acid (TBA) reaction which leads to the formation of red complex with an absorption maxima at 532 nm (7,8). Nevertheless, TBA reacts with other MDA-like substances (9-11) and is increased by iron salts. It has not been made clear whether the TBA reaction used for biological sample is demonstrating principally MDA or MDA-like substance, or decomposing product that arises during the heating stage of the reaction of tissue samples with TBA (9).

Recently, HPLC has been found to be faster, more sensitive and less affected by side reactions for the direct analysis of free MDA (12-14).

Previously, we investigated the prooxidant activity of α-tocopherol during the autoxidation of polyunsaturated fatty acids in aqueous media (15,16). This activity leads to an increase of the level of hydroperoxides having conjugated diene structure (17).

This study has been carried out with the aim of evaluating the MDA production during the prooxidant effect of α-tocopherol on linolenic and arachidonic acids.

MATERIALS AND METHODS

Materials. Linolenic (99%) and arachidonic (99%) acids were purchased from Sigma Chemical Co., St. Louis, Missouri. These acids were dispersed with 0.5% Tween 20 (Merck) in 0.025 phosphate-buffered aqueous solution (pH 9.0) under nitrogen and in the dark. Linolenic and arachidonic acid concentrations were 10^{-2} M, and these stock dispersions were adjusted to pH 6.9 just before use. α-Tocopherol was synthesized and supplied by Hoffmann-La Roche, France. It was dispersed with Tween 20 in phosphate buffer (pH 7.0) in the same procedure as described above (under nitrogen and in the dark). The concentration of the stock dispersion of α-tocopherol was 5×10^{-4} M, and it was stored at 4 C.

Procedure. Samples (100 ml each) were prepared by

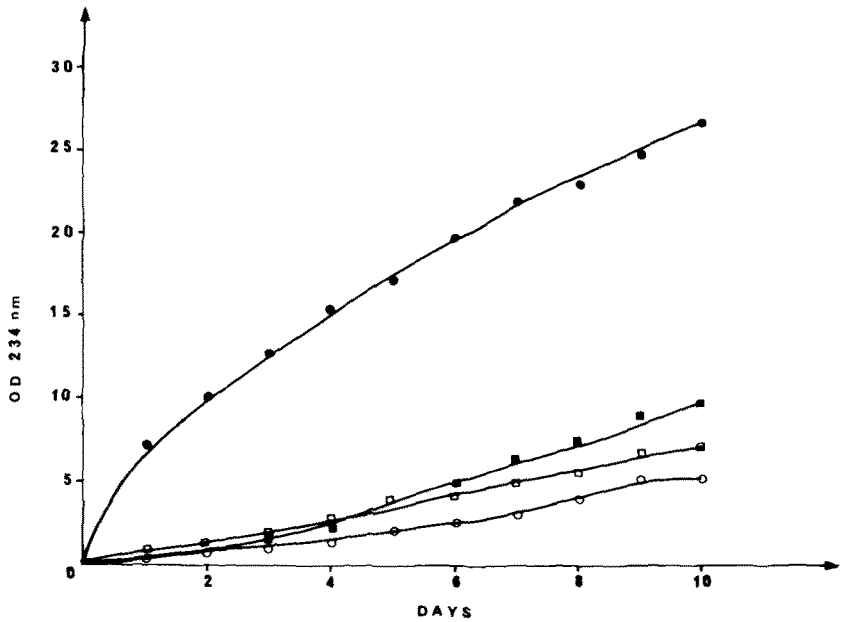

FIG. 1. Measurement of conjugated dienes during linolenic and arachidonic acids oxidation with and without α-tocopherol. Linolenic acid, (○); linolenic acid + α-T, (●); arachidonic acid, (□); arachidonic acid + α-T, (■).

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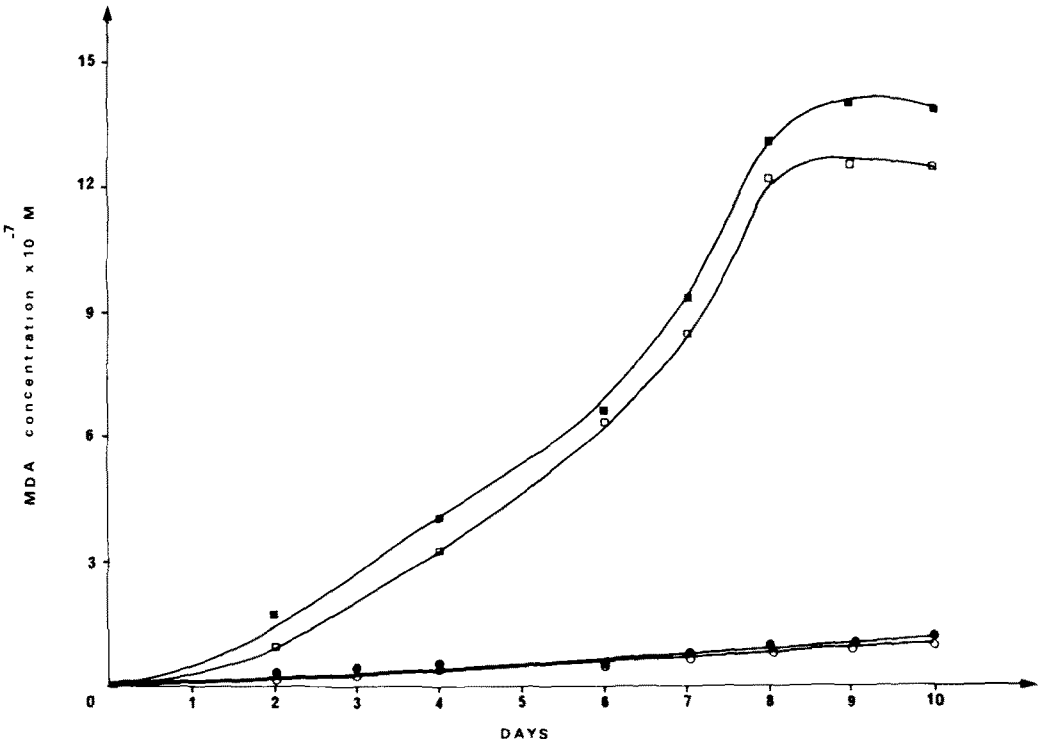

FIG. 2. HPLC measurement of MDA concentration during linolenic and arachidonic acids oxidation with and without α-tocopherol. Legends are the same as for FIG. 1.

mixing aliquots of the stock dispersions in glass tubes at time zero. The final concentrations were 2.5×10^{-3} M for fatty acids and 1.25×10^{-4} M for α-tocopherol. The samples were left in dark and under air at room temperature. Controls without linolenic and arachidonic acids were placed in similar conditions.

Conjugated diene measurement. The autoxidation of linolenic and arachidonic acids was accompanied by an increase of absorption at 234 nm because of conjugated diene formation. The absorbance was measured with a Pye Unicam SP 8-400 UV/Vis Spectrophotometer. The samples were diluted so that the absorption was less than or equal to 2.

MDA evaluation by HPLC. The formation of MDA in samples was evaluated by HPLC on LDC chromatograph (Sopares, Gentilly, France) equipped with a Constametric III pump, a Valco 7000 psi injector and a Spectromonitor III UV detector set at 270 nm and sensitivity of 0.05 AUFS (absorbance units full scale). Peak areas were measured and printed with a LDC/Milton Roy CI-10 integrator. Samples were injected directly (100 μl loop) into HPLC instrument and separated with an aminophase column (Spherisorb NH-5μ) according to the method developed by Esterbauer and Slater (13).

The eluting solvent was composed of Tris buffer 0.037 M, pH 7.4 acetonitrile (9:1, v/v) at a flow rate of 1.0 ml/min. An ODS precolumn was used to protect the analytical column (18). The column was equilibrated daily with 50 ml of eluent prior to analysis.

The identification and calibration of MDA was done by running a reference chromatogram with a solution of freshly prepared free MDA (12). The concentration of MDA in samples was calculated by using standard curve of free MDA.

RESULTS AND DISCUSSION

Fatty acid autoxidation measured by the formation of conjugated diene at 234 nm was increased by addition of α-tocopherol (1.25×10^{-4} M, Fig. 1) as described in our earlier report (15). The prooxidant effect of α-T was more prominent in the case of linolenic acid than with arachidonic acid.

The concentration of MDA measured by HPLC (18) was almost the same with and without α-tocopherol (Fig. 2). But MDA produced during arachidonic acid autoxidation was comparatively much higher than linolenic acid.

In our experiment, no correlation could be observed between conjugated diene and MDA formation during the autoxidation of linolenic or arachidonic acid with α-tocopherol at a prooxidant level.

Using the extinction coefficient $2.8\ 10^{-4}$ hydroperoxide $mol^{-1}cm^{-1}$, the level of hydroperoxide (conjugated diene) was calculated from the OD value and expressed in percentage of the initial concentration of fatty acids.

During linolenic acid autoxidation, the prooxidant effect of α-tocopherol caused production of higher amounts of conjugated diene (Fig. 1), while it could not alter the MDA formation (Fig. 2). Thus, at 10 days of oxidation, the level of conjugated diene reached ca. 40% and the MDA represented only 0.1% of the conjugated dienes. In the case of arachidonic acid, α-tocopherol did not result in appreciable change in conjugated diene and MDA formation. Neverthe-

less, at 10 days of oxidation the MDA represented 4% of conjugated dienes present in the sample, which corresponded to 40 times more than in the case of linolenic acid.

In previous works, we have shown that during the autoxidation of linoleic acid with and without α-tocopherol, conjugated dienes correlated well with the total hydroperoxide produced and also with the degradation of linoleic acid in the sample, especially during the first 10 days (17). In contrast, we noted a discrepancy between the conjugated dienes present in the sample, which were six times less important than the degradation of arachidonic acid measured by gas liquid chromatography (19).

The absence of correlation between conjugated diene and MDA formation can be explained on the basis that α-tocopherol protected the hydroperoxides from decomposition, especially those of linoleic acid (20). This may be the reason that during autoxidation of linolenic acid with α-tocopherol, diene conjugation continuously increased while MDA concentration remained almost constant.

Linolenic and arachidonic acids without α-tocopherol afforded almost the same amount of conjugated diene (ca. 8–10%) after 10 days of incubation, while MDA content represented ca. 5 mol % of arachidonic acid-derived diene and only 0.6 mol % of linoleic acid-derived diene. These results agreed well with those of previous workers who noted that less than 1% of methyl linolenate hydroperoxides contributed to the formation of MDA (11) and that in biological samples rich in arachidonic acid MDA production accounted for 5–10% of fatty acid oxidation (21–23). In our experiment, the MDA level reached its maximum as early as the ninth day in arachidonic acid but continued to increase linearly for linolenic acid.

From the above results, it can be inferred that MDA measurement does not seem to be a good indicator to determine the prooxidant effect of α-tocopherol in autoxidation of linolenic acid.

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