Inhibition of Malonaldehyde Formation by Antioxidants from ω3 Polyunsaturated Fatty Acids

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ABSTRACT: The inhibitory effect of α-tocopherol, β-carotene, 2″-*O*-glycosyl isovitexin (2″-*O*-GIV), and butylated hydroxytoluene (BHT) on malonaldehyde (MA) formation from ω3 polyunsaturated fatty acids (PUFA) was determined by gas chromatography. The levels of MA formed from 1 mg each of octadecatetraenoic acid (ODTA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) upon oxidation with Fenton's reagent were 29.8 ± 1.5, 17.2 ± 1.5, and 22.0 ± 0.7 nmol, respectively. BHT was most effective toward protecting all three ω3 PUFA, whereas β-carotene did not exhibit any inhibitory effect. 2″-*O*-GIV inhibited MA formation from EPA and DHA by 56 and 43%, respectively, showing the second greatest inhibitory activity after BHT. α-Tocopherol inhibited MA formation from ODTA and DHA by 67 and 28%, respectively, but it did not show any activity toward EPA oxidation. The naturally occurring antioxidant, 2″-*O*-GIV, may be useful to prevent oxidation of ω3 PUFA.

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KEY WORDS: Antioxidant, β-carotene, ω3 fatty acids, 2″-*O*-glycosyl isovitexin, malonaldehyde, α-tocopherol.

A number of authors have published comprehensive reviews on ω3 polyunsaturated fatty acids (PUFA) in health and disease, including the effects on plasma lipids and lipoproteins (1), the reduction of coronary heart disease (2), and the effect on hypertension (3). Epidemiological and laboratory evidence indicates that consumption of fish oils rich in ω3 PUFA is associated with a decreased incidence of cardiovascular disease (4). Consequently, fish oil tablets have been recommended to increase serum PUFA levels that decrease the risk of coronary artery diseases (5). Recently, it has been reported that fish oil also interrupts vascular thrombus formation, suggesting inhibitory effects of ω3 PUFA from fish oil upon thrombosis (6).

On the other hand, the highly unsaturated fatty acids, such as ω3 PUFA, render fish tissue and fish oil extremely susceptible to autooxidation and rapid deterioration, followed by the production of toxic aldehydes (7). Lipid content in edible flesh ranges from a minimum of 0.5% to a maximum of 25%. The major ω3 PUFA in fish oils are eicosapentaenoic acid (EPA) (10%) and docosahexaenoic acid (DHA) (30–33%) (8). Among the lipid peroxidation products, β-dicarbonyl compounds, such as malonaldehyde (MA), and α,β-unsaturated aldehydes, such as acrolein and 4-hydroxynonenal, have received much attention as biologically active agents (9). For example, MA reacts with biological nucleophiles, such as DNA (10).

Consequently, measurement of the formation of these toxic aldehydes from ω3 PUFA upon oxidation may provide additional information regarding the link between these compounds and the toxic effects of lipid peroxidation. In the present study, lipid peroxidation of ω3 PUFA was determined by measuring the levels of MA formation. The inhibitory effect of antioxidants toward MA formation from these lipids upon oxidation also was investigated.

MATERIALS AND METHODS

Materials. Octadecatetraenoic acid (ODTA), EPA, DHA, Trizma base, Trizma hydrochloride, butylated hydroxytoluene (BHT), hydrogen peroxide, and β-carotene were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-Methylhydrazine, 2-methylpyrazine, sodium dodecyl sulfate (SDS), and α-tocopherol were bought from Aldrich Chemical Co. (Milwaukee, WI). Ferrous chloride was purchased from Fisher Scientific Co. (Fair Lawn, NM).

2″-*O*-Glycosylisovitexin (2″-*O*-GIV) was isolated from young green barley leaves (*Hordium vulgare* L. var. *nudum* Hook), harvested two weeks after germination, by a method previously reported (11) by using column chromatography with Amberlite XAD-2 nonionic polymeric absorbent (Aldrich Chemical Co.). After column chromatography, the 2″-*O*-GIV fraction was further purified to 100% with a preparative high-performance liquid chromatography equipped with a 25 × 1 cm i.d. Develosil ODS-5 column (Nomura Chemical Co., Ltd., Aichi, Japan) and a ultraviolet detector at λ = 280 nm. The structure of 2″-*O*-GIV is shown in Scheme 1.

Oxidation of ω3 PUFA, ODTA, EPA, and DHA by Fe_2Cl_2/H_2O_2. An aqueous solution (5 mL), containing 5 mg of an ω3 PUFA, 0.05 mol Trizma buffer (pH 7.4), 200 μL of 3.8 M potassium chloride, and 200 μL of 5% SDS, was stirred with or without 20 μL of 0.1 M $FeCl_2$ and 55 μL of 30% H_2O_2 at 37°C for 16 h. The oxidation reaction was stopped by adding 50 μL of 4% BHT (in dichloromethane). These samples were prepared for use as controls.

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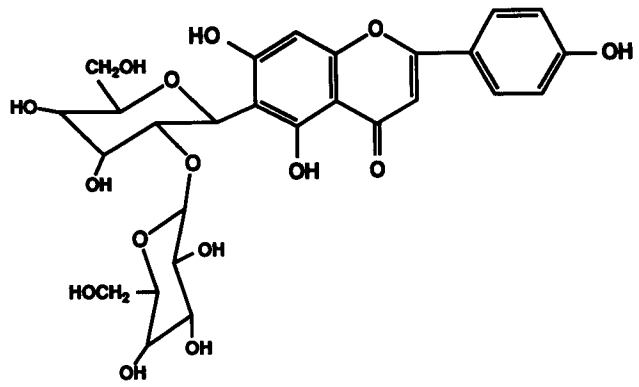

SCHEME 1

Oxidation of ω3 PUFA in the presence of antioxidants. Various amounts (0, 5.0, 10.0, and 15.0 μmol) of α-tocopherol, BHT, β-carotene, or 2″-*O*-GIV were added to the above ω3 PUFA samples before addition of Fe_2Cl_2, and then the samples were treated as in the procedures described above.

Analysis of MA formed from ω3 PUFA. The gas-chromatographic method for MA analysis reported previously (12,13) was used. The MA formed from PUFA was reacted with *N*-methylhydrazine, and the resulting derivative, 1-methylpyrazole, was analyzed with 2-methylpyrazine as an internal standard by a gas chromatograph (Hewlett-Packard Model 5880; Hewlett Packard, Avondale, PA), equipped with a fused-silica capillary column (DBWAX 30 m × 0.25 mm i.d.) and a nitrogen–phosphorus detector (NPD).

Each experiment was repeated three times, and the results are reported in mean ± standard deviation.

RESULTS AND DISCUSSION

Formation of MA from PUFA has been reported many times in the literature (10). Direct measurement of MA, however, is extremely difficult due to its reactivity. Therefore, a stable derivative must be prepared to determine accurate MA levels. The thiobarbituric acid (TBA) assay, in which MA and TBA are reacted and the resulting red complex is measured colorimetrically, has been most commonly and widely used to measure the degree of oxidation or MA levels in a lipid peroxidation system. The method, however, is not specific for MA, and its sensitivity is not sufficient for trace analysis of MA (13). Recently, we satisfactorily analyzed, by using gas chromatography, trace amounts of MA in lipids (14) and in liver microsomes (12) as the *N*-methylhydrazine derivative 1-methylpyrazole. The lowest detection level of 1-methylpyrazole by an NPD was 8.9 pg, equivalent to 7.8 pg of MA in the present study.

The levels of MA formed from 1 mg each of ODTA, EPA, and DHA were 29.8 ± 1.5, 17.2 ± 1.5, and 22.0 ± 0.7 nmol, respectively, under the conditions used in the present study. The values are mean ± standard deviation (n = 3). The most commonly recognized formation mechanism of MA from lipids upon oxidation includes the decomposition of prostaglandin-like endoperoxide intermediates (15). This mechanism requires at least three methylene-interrupted double bonds. However, MA was reportedly formed from fatty acids with less than three double bonds, such as linoleic acid (14,16). A PUFA with conjugated double bonds (ODTA) yielded the highest level of MA in the present study. Therefore, further investigation of the formation mechanisms of MA in lipid peroxidation may be necessary. A recent study suggested that many low-molecular-weight radicals, such as • OH, • CHO, • CH_2CHO, • CH_3, and • $COCH_3$, are formed in an early stage of the oxidative degradation of lipids, and that these radicals combine to form low-molecular-weight carbonyl compounds, including MA (16). Investigation of the formation mechanisms of MA in lipid peroxidation, however, was not within the scope of the present study.

Figures 1–3 show inhibitory activity of β-carotene, BHT, 2″-*O*-GIV, and α-tocopherol toward MA formation from oxidative degradation of ODTA, EPA, and DHA, respectively. Among the antioxidants tested, BHT was most effective, inhibiting MA formation from ODTA (Fig. 1), EPA (Fig. 2), and DHA (Fig. 3) by 80, 93, and 58%, respectively. β-Carotene, however, did not exhibit any inhibitory effects with all three ω3 PUFA. Moreover, β-carotene showed some prooxidative activity with EPA and DHA, even though it has been reported to possess certain antioxidative activity (17,18). The antioxidative activity of β-carotene is reportedly dependent on oxygen concentration. At higher oxygen pressures, such as atmospheric pressure, β-carotene loses its antioxidative activity and shows an autocatalytic prooxidant effect, whereas it exhibits good radical-trapping antioxidative effect at low oxygen concentration (19). The results of the present study are consistent with the above report because the present experiments were performed under atmospheric pressure.

2″-*O*-GIV exhibited the second greatest inhibitory activity toward MA formation after BHT with EPA (56%) and DHA (43%); its effect (60%) was less than that of α-tocopherol

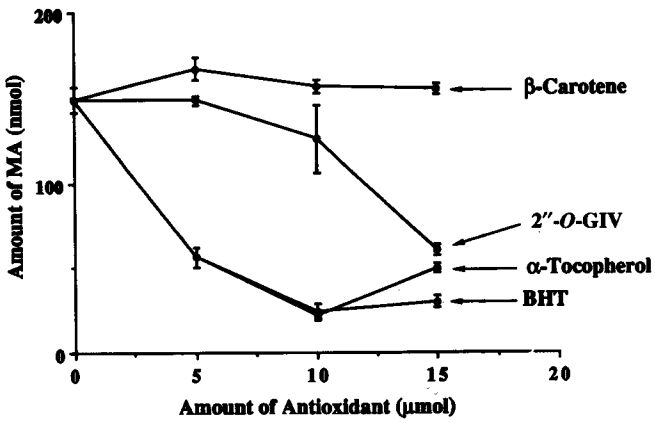

FIG. 1. Effect of α-tocopherol, 2″-*O*-glycosyl isovitexin (2″-*O*-GIV), β-carotene, and butylated hydroxytoluene (BHT) on malonaldehyde (MA) formation from octadecatetraenoic acid oxidized by Fenton's reagent. Values are means ± standard deviations (n = 3).

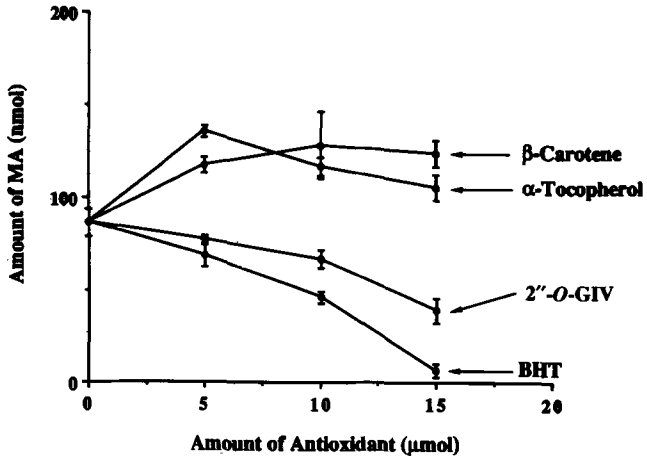

FIG. 2. Effect of α-tocopherol, 2″-*O*-GIV, β-carotene, and BHT on MA formation from eicosapentaenoic acid oxidized by Fenton's reagent. Values are means ± standard deviations (n = 3). See Figure 1 for abbreviations.

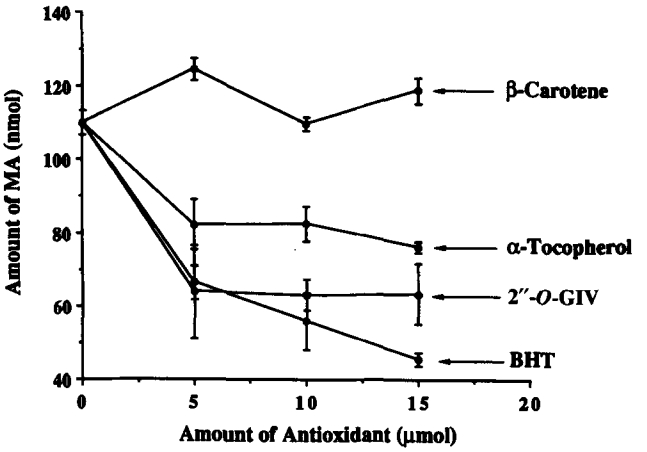

FIG. 3. Effect of α-tocopherol, 2″-*O*-GIV, β-carotene, and BHT on MA formation from docosahexaenoic acid oxidized by Fenton's reagent. See Figure 1 for abbreviations.

(67%) with ODTA. α-Tocopherol did not show any appreciable inhibitory activity toward EPA oxidation. For cod liver oil, consisting of approximately 10% EPA and 30–33% DHA (8), 2″-*O*-GIV showed a higher inhibitory effect than did α-tocopherol toward MA formation, and generally, α-tocopherol inhibited MA formation more effectively from the lipids with lower numbers of double bonds than from those with higher numbers of double bonds (20).

The ω3 PUFA are known to possess some biological activities, including antinflammatory properties, anticancer activity, antiplatelet activity, and hypolipidemic properties (21). Because of these beneficial effects obtained from ω3 PUFA, a fish oil diet supplementation sometimes has been recommended (22). However, recent studies indicate that fish oil breaks down into many toxic carbonyl compounds, including formaldehyde, acrolein, MA, glyoxal, and methyl glyoxal (20,23). Consequently, massive doses of fish oil as a treatment for certain human diseases may cause biological complications. The results of the present study suggest that the use of antioxidants may prevent formation of toxic carbonyl compounds such as MA, both *in vivo* and *in vitro*. On the other hand, use of synthetic chemicals, such as BHT, has been questioned because of suspected toxicity of those chemicals. Therefore, naturally occurring products, such as α-tocopherol and 2″-*O*-GIV, have begun to receive much attention as safe antioxidants.

2″-*O*-GIV was chosen to test with other well-known antioxidants because it showed strong antioxidative activities in various lipid peroxidation systems (20,24,25). For example, antioxidative activity of 2″-*O*-GIV was almost equivalent to that of α-tocopherol in a lipid peroxidation system at the level of 100 μg/1.5 mg of ethyl linoleate (11). 2″-*O*-GIV may be nontoxic to animals because large amounts of barley leaves, which contain approximately 0.50–0.7% of 2″-*O*-GIV (11), have been consumed by livestock since ancient eras. Therefore, if 2″-*O*-GIV is proven not to be hazardous, it may be useful as a food additive to prevent autooxidation of fish oils. Moreover, 2″-*O*-GIV can be obtained in large quantities from a natural source at low cost, in contrast to α-tocopherol, which is expensive.

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