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Inhibition of plasma lipid peroxidation by anti-atherogenic antioxidant BO-653, 2,3-dihydro-5-hydroxy-4,6-di-tert-butyl-2,2-dipentylbenzofuran

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Abstract

BO-653, 2,3-dihydro-5-hydroxy-4,6-di-tert-butyl-2,2-dipentylbenzofuran, is a synthetic antioxidant which is now being developed as an anti-atherogenic drug. The antioxidant action of BO-653 against lipid peroxidation in rat plasma was studied and compared with its analogue BO-653M, 2,3-dihydro-5-hydroxy-4,6-di-methyl-2,2-dipentylbenzofuran, and vitamin E. BO-653 was readily incorporated into plasma by oral administration and it inhibited plasma lipid peroxidation more efficiently than vitamin E independent of the presence or absence of vitamin C. On the other hand, its analogue BO-653M having two methyl substituents in place of tert-butyl groups of BO-653 did not inhibit the lipid peroxidation in plasma as efficiently as BO-653, demonstrating clearly that the tert-butyl groups at the ortho-position play a key role in determining the antioxidant efficacy.

Keywords: Antioxidant; Atherosclerosis; BO-653; Vitamin C; Vitamin E; Lipid peroxidation

1. Introduction

It is widely accepted that the oxidative modification of low density lipoprotein (LDL) plays an important role in the pathogenesis of atherosclerosis and consequently the inhibition of LDL oxidation has been the subject of extensive studies [1]. Many natural and synthetic antioxidants have been studied from basic, clinical and epidemiological viewpoints. The results of in vitro studies, animal models, clinical trials, and epidemiologic and intervention studies do not consistently show the beneficial effects of antioxidants [2,3]. For example, the supplementation of α-tocopherol (α-T), a major form of vitamin E present in LDL and plasma, shows either beneficial or no effect against atherosclerosis and risk of cardiovascular diseases [4,5]. It has been also pointed out that α-T may act as a prooxidant in the oxidation of LDL [6]. It is noteworthy, however, that the oxidation of LDL in vivo must be induced and mediated by several oxidants by different mechanisms and that the radical-scavenging antioxidants such as vitamin E may be effective against free radical-mediated oxidation, but not against non-radical oxidation [7]. It has been also suggested that vitamin E may exert anti-atherogenic effects by non-antioxidant function such as inhibition of cell proliferation, platelet adhesion and aggregation, and scavenger receptor expression [8].

2,3-Dihydro-5-hydroxy-2,2-dipentyl-4,6-di-tert-butyl-benzofuran (BO-653) was designed and synthesized as a novel anti-atherogenic antioxidant [9–12]. It has been shown that BO-653 exerts a potent antioxidant capacity against the lipid peroxidation [9,13] and LDL oxidative modification in vitro [14,15] and also an anti-atherogenic effect in animal models [16]. The anti-atherogenic function of BO-653 other than direct antioxidant effect has been also studied by genomic analyses [17,18]. It has been found that BO-653 and α-T exert similar reactivity toward

Abbreviations: BO-653, 2,3-dihydro-5-hydroxy-4,6-di-tert-butyl-2,2-dipentylbenzofuran; BO-653M, 2,3-dihydro-5-hydroxy-4,6-dimethyl-2,2-dipentylbenzofuran; CE, cholesteryl ester; FC, free cholesterol; LDL, low density lipoprotein; MeO-AMVN, 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile); OxFC, oxidized cholesterol; PC, phosphatidylcholine; T, tocopherol

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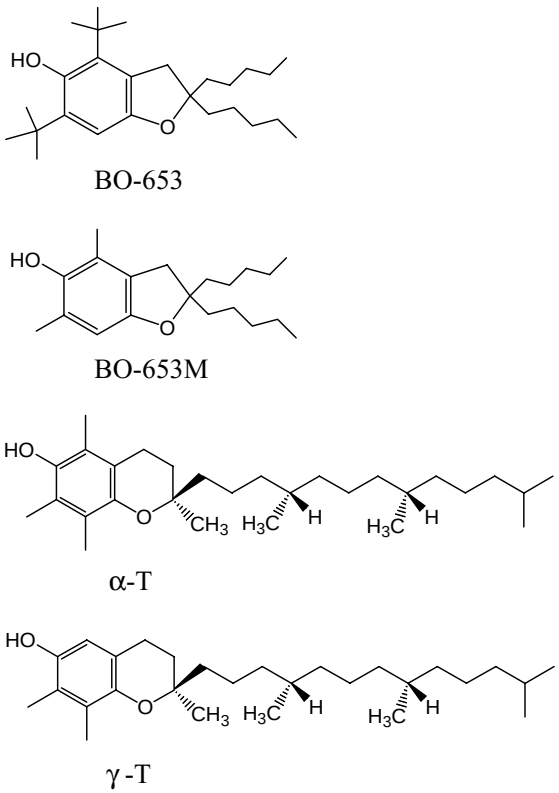


Scheme 1. Antioxidants studied in this work.

peroxyl radicals, a chain carrying species of lipid peroxidation, and similar capacity for the inhibition of lipid peroxidation in homogeneous solution [11]. It was also found that the aryloxyl radical derived from BO-653 was much more stable than α-tocopheroxyl radical toward lipids, lipid hydroperoxide and ascorbate [19]. This suggests that BO-653 may act as a more potent antioxidant against LDL oxidation than α-T but that it is less efficiently spared by ascorbate [20].

The present study was carried out to elucidate the antioxidant action of BO-653 against the oxidation of plasma which is apparently more relevant to biological environment than the suspensions of isolated LDL. The oxidation of isolated LDL and its inhibition by antioxidants have been studied extensively, but those for plasma have received much less attention. In the present study, the antioxidant action of BO-653 against plasma oxidation was compared with those of its analogue 2,3-dihydro-5-hydroxy-4,6-dimethyl-2,2-dipentylbenzofuran (BO-653M) (Scheme 1) and α-T.

2. Materials and methods

2.1. Antioxidants and reagents

BO-653 and BO-653M were prepared as described previously (10). Natural α-T and 2,2,5,7,8-pentamethyl-6-chromanol (PMC) were kindly supplied by Eisai Co. A lipophilic radical initiator 2,2′-azobis-(4-methoxy-2,4-dimethylvaleronitrile) (MeO-AMVN) was obtained from Wako Pure Chemical Ind. and used as received. Other chemicals were those of the highest quality available commercially.

2.2. Animals

Male Sprague–Dawley strain rats (weighing 200–220 g) were purchased from Japan SLC Inc. The animals housed in a room maintained at 23 ± 3 °C with a 12 h/12 h light/dark cycle were fed a commercial diet (CE-2, Nippon Clea Co.) and water ad lib. The experimental protocols were approved by the Animal Welfare, Care and Use Committee in AIST Kansai. The rats were allowed to fast for 16 h with water freely available, and then administered BO-653 (225 mg/kg) dissolved in corn oil orally. The control rats received vitamin E stripped corn oil. After 4 h, the rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and sacrificed by collecting the blood from the abdominal aorta, using a syringe containing sodium heparin as an anticoagulant. Blood was centrifuged at 2400 g for 10 min to obtain plasma.

2.3. Oxidation of plasma

When required, plasma was dialyzed using dialysis membrane for 18 h at 4 °C in saline to remove vitamin C and other water-soluble antioxidants. When required, BO-653M or PMC was added as a methanol solution to the plasma and incubated for 10 min at 37 °C before oxidation. The oxidation of plasma (plasma/saline = 1/1 by volume) was carried out at 37 °C under air. The oxidation was initiated by the addition of MeO-AMVN dissolved in acetonitrile or $CuSO_4$ dissolved in water. The oxidation was analyzed as follows: oxygen absorption in the oxidation of plasma was also measured using an oxygen monitor equipped with a Clark-type oxygen electrode (YSI model 5300).

2.4. HPLC analysis

The mixture of oxidized samples was extracted with chloroform/methanol (2/1) by twice as volume as the sample and chloroform layer was analyzed as follows: vitamin E, BO-653 and BO-653M were measured with an HPLC by an amperometric electrochemical detector (NANOSPACE SI-1, Shiseido) set at 800 mV, with an ODS column (LC-18, 5 μm, 250 mm × 4.6 mm, Supelco) and methanol/tert-butyl alcohol (95/5 by volume) as eluent at 1 ml/min. The accumulation of free cholesteryl hydroperoxide (FCOOH) and phosphatidylcholine hydroperoxides (PCOOH) was followed with an HPLC using post-column chemiluminescence detector (CLD-10A, Shimadzu). ODS-2 column (5 μm, 250 mm × 4.6 mm, GL Science) was used and after passage through the UV

detector, the eluent was mixed with a luminescent reagent in the post-column mixing joint in the chemiluminescence detector at 40 °C. The luminescence reagent containing cytochrome *c* (10 mg) and luminol (2 mg) in 1 L alkaline borate buffer (pH 10) was loaded at the flow rate of 0.5 mL/min. The total sum of hydroperoxides and hydroxides of PC, PCO(O)H, was determined by HPLC spectrophotometrically (SPD-10AV, Shimadzu) at 245 nm. 7-Ketocholesterol (FCCO) and 7-hydroxycholesterol (FCOH) were measured with an HPLC at 245 and 210 nm, respectively. The accumulation of cholesteryl ester hydroperoxide (CEOOH) and CE hydroxide (CEOH) was also followed with an HPLC by spectrophotometric detector at 234 nm. ODS column (Wakosil-II 5C18RS, 5 μm, 250 mm × 4.6 mm, Wako) was used and acetonitrile/isopropyl alcohol/water (44/54/2 by volume) was eluted at 1 mL/min. The hydroperoxides were measured specifically by the HPLC-chemiluminescence analysis, while HPLC-UV analysis gave the sum of hydroperoxide and alcohol.

2.5. Reproducibility

The experiments of antioxidant administration were repeated three times. The absolute concentrations of antioxidants in plasma varied considerably with rats, but the pattern of the oxidation and its inhibition by antioxidants were essentially the same and satisfactorily (±10%) reproducible.

3. Results

BO-653 was given to rats orally and blood was obtained 4 h after administration. Plasma was separated and the antioxidants were analyzed. It was found that the plasma level of BO-653 was increased to a similar level of α-T when BO-653 was administered orally. The oxidation of plasma thus obtained was induced by the addition of lipophilic azo initiator, MeO-AMVN, or $CuSO_4$ in the presence and absence of vitamin C. When required, vitamin C was removed from the plasma by dialysis as described in Section 2. It was confirmed that the concentrations of lipids and lipophilic antioxidants such as vitamin E and BO-653 did not change appreciably before and after dialysis.

Typical examples of the oxidation of rat plasma induced by MeO-AMVN in the presence and absence of vitamin C are shown in Fig. 1. The antioxidant concentrations in the plasma varied with the dosages and subjects, but essentially the same pattern was observed independent of the absolute concentrations of the antioxidants. Following messages are obtained from these results. (1) In the presence of vitamin C, BO-653 and vitamin C were consumed concomitantly from the beginning, while α-T and γ-T (results not shown) were spared. Little lipid peroxidation proceeded in the presence of vitamin C. After depletion of

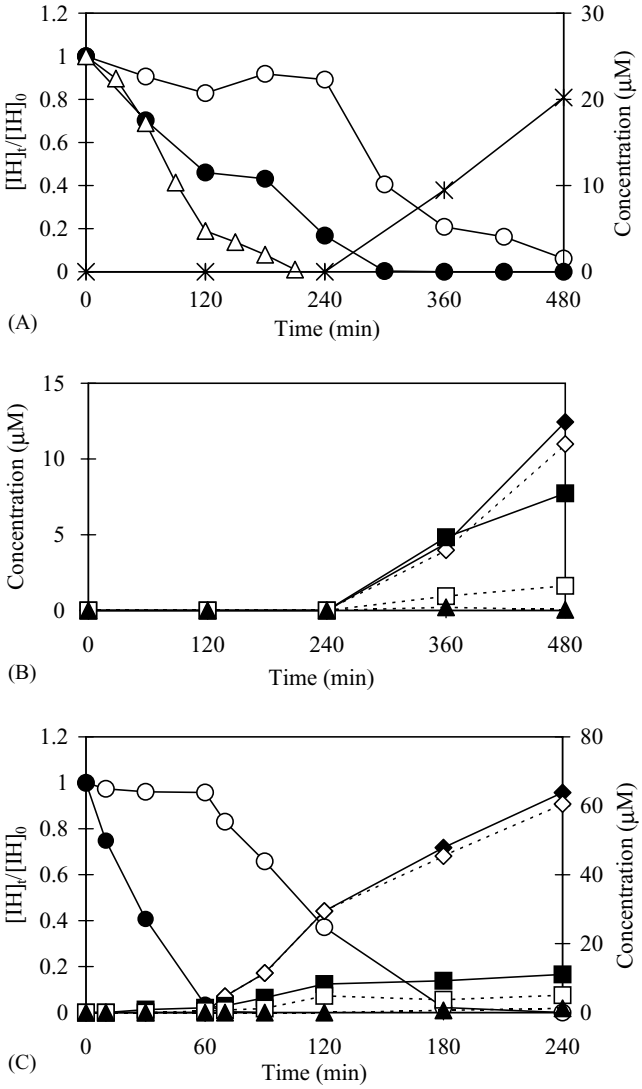
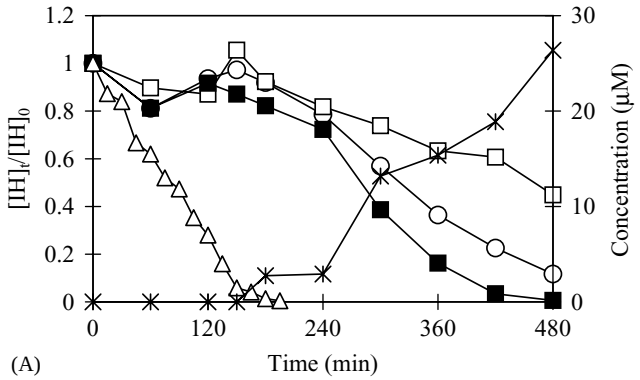


Fig. 1. Oxidation of plasma obtained from rat which was given BO-653 orally as described in Section 2. The oxidation was started by the addition of 0.50 mM MeO-AMVN at 37 °C in air. (A) Consumption of α-T (○), BO-653 (*) and vitamin C (△) and accumulation of total hydroperoxides (✳). The initial concentrations of α-T, γ-T, BO-653 and vitamin C were 6.62, 0.163, 9.37 and 24.0 μM respectively. (B) Accumulation of CEO(O)H (◆), CEOOH (◇), PCO(O)H (■), PCOOH (□), and OxFC(▲), during the oxidation shown in panel A. (C) Oxidation after removal of vitamin C by dialysis. Consumption of α-T (○) and BO-653 (*) and accumulation of CEO(O)H (◆), CEOOH (◇), PCO(O)H (■), PCOOH (□) and OxFC (▲). The initial concentrations of α-T, γ-T and BO-653 were 5.37, 0.205 and 7.55 μM, respectively. The plot of γ-T was omitted for simplicity. CEO(O)H and PCO(O)H denote a sum of hydroperoxide and alcohol of CE and PC, respectively.

vitamin C and BO-653, α-T was consumed and the lipid peroxidation products were accumulated simultaneously. (2) CEOOH was the major product, while PCOOH was formed as a minor product and little oxidation products of FC were observed initially. The reduction of CEOOH to CEOH was minimal, whereas considerable PCOOH was reduced to the corresponding alcohol in the plasma. (3)Vitamin C completely inhibited the α-T consumption and lipid peroxidation. (4) BO-653 spared α-T and γ-T



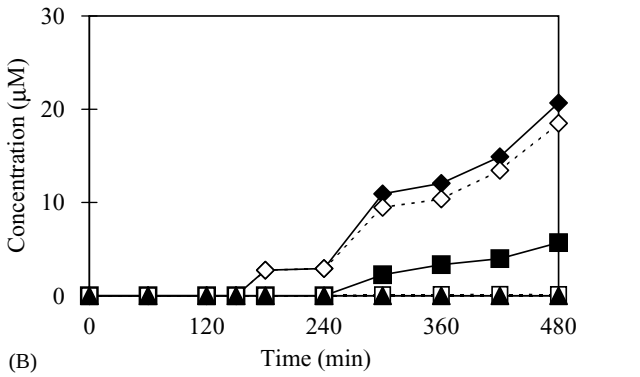
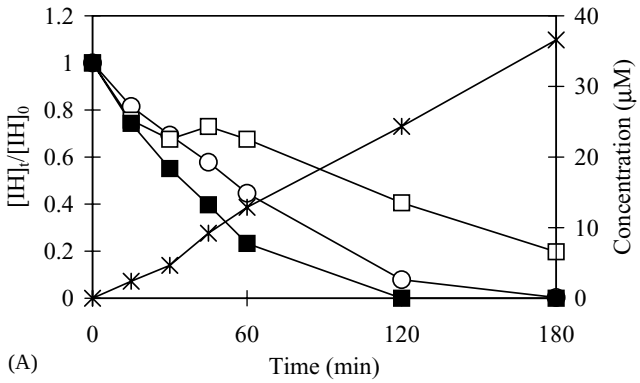


Fig. 2. Oxidation of rat plasma containing α-T, γ-T and BO-653M induced by 0.50 mM MeO-AMVN at 37 °C under air. The initial concentrations of α-T, γ-T, BO-653M and vitamin C were 6.84, 0.14, 6.05, and 97.4 μM, respectively. (A) The consumption of α-T (◯), γ-T (□), BO-653M (■) and vitamin C (△) and the formation of total lipid hydroperoxides and hydroxides (✳) were followed as described in Section 2. (B) Formation of CEO(O)H (◆) and CEOOH (◇), PCO(O)H (■), PCOOH (□) and OxFC (▲). CEO(O)H and PCO(O)H denote a sum of hydroperoxide and alcohol of CE and PC, respectively.



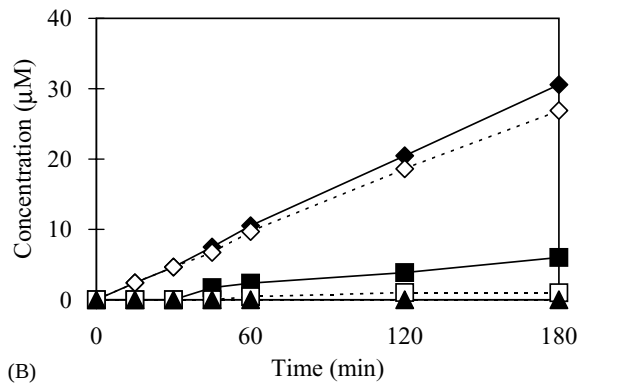


Fig. 3. Oxidation of rat plasma in the absence of vitamin C. The plasma, experimental conditions and symbols are the same as those of Fig. 2, except that vitamin C was removed by dialysis. The initial concentrations of α-T, γ-T and BO-653M were 5.70, 0.14 and 4.31 μM, respectively.

efficiently and it inhibited lipid peroxidation completely even in the absence of vitamin C.

The most striking difference between BO-653 and α-T is that BO-653 was capable of inhibiting lipid peroxidation almost completely even in the absence of vitamin C and that BO-653 was not spared by vitamin C. In contrast, α-T did not inhibit the lipid peroxidation of plasma efficiently in the absence of vitamin C and it was spared efficiently by vitamin C.

In order to elucidate further the action of BO-653 against plasma oxidation, the oxidation of plasma in the presence of BO-653M was studied. BO-653M was added to the plasma directly, not by oral administration. The results of the oxidation of plasma containing BO-653M in the presence and absence of vitamin C are shown in Figs. 2 and 3, respectively. It can be seen that vitamin C spared α-T, γ-T and BO-653M efficiently and that the consumption of these three lipophilic antioxidants began concomitantly after depletion of vitamin C, the rate being BO-653M > α-T > γ-T. The lipid peroxidation was inhibited completely in the presence of vitamin C but started appreciably even in the presence of BO-653M, α-T and γ-T. When the plasma was oxidized after removal of vitamin C by dialysis, the consumption of BO-653M, α-T and γ-T and accumulation of lipid peroxidation products started without lag phase (Fig. 3).

On the other hand, when BO-653 was present together with BO-653M, α-T and γ-T, only BO-653 was consumed predominantly at first, and BO-653M, α-T and γ-T were spared and importantly the lipid peroxidation was completely inhibited in the presence of BO-653 even in the absence of vitamin C (Fig. 4). α-T, γ-T and BO-653M decreased after depletion of BO-653.

The results shown in Figs. 2–4 also support the previous findings that PCOOH is readily reduced in plasma [21], whereas CEOOH is not. This is explained by the higher rate of reduction of PCOOH by glutathione peroxidases and selenoprotein P than CEOOH. The oxidation of free cholesterol was minimal under the present reaction conditions.

The oxidation of plasma diluted by equal volume of PBS was also induced by the addition of 100 μM $CuSO_4$ and the rate of oxidation was measured by following the oxygen uptake. The results are shown in Fig. 5. Vitamin C was removed by dialysis before oxidation and BO-653M and PMC were added to the plasma as a methanol solution. BO-653 inhibited the oxidation completely, whereas α-T, PMC and BO-653M suppressed the oxidation incompletely to a similar extent.

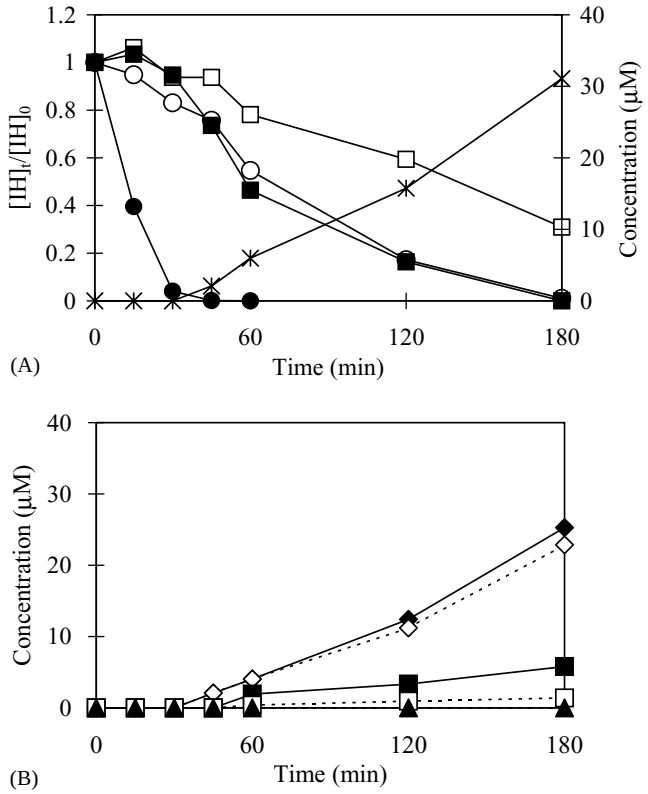


Fig. 4. Oxidation of rat plasma containing α-T, γ-T, BO-653M and BO-653 induced by 0.5 mM MeO-AMVN at 37 °C under air. Vitamin C was removed by dialysis prior to oxidation. (A) Consumption of α-T (○), γ-T (□), BO-653M (■) and BO-653 (*) and accumulation of total lipid hydroperoxides and hydroxides (✳). The initial concentrations of α-T, γ-T, BO-653M, and BO-653 were 6.12, 0.12, 4.13 and 4.57 μM, respectively. (B) Formation of CEO(O)H (◆) and CEOOH (◇), PCO(O)H (■), PCOOH (□) and OxFC (▲). CEO(O)H and PCO(O)H denote a sum of hydroperoxide and alcohol of CE and PC, respectively.

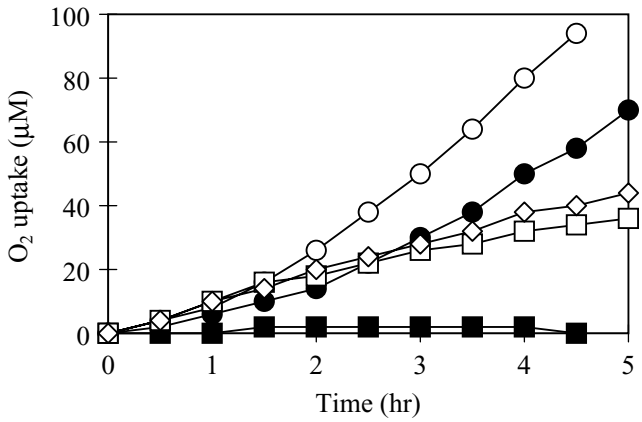


Fig. 5. Oxidation of rat plasma induced by 0.10 mM $CuSO_4$ at 37 °C under air. Vitamin C was removed by dialysis and 5.0 μM antioxidant was added to the plasma prior to oxidation. The plasma contained 5.0 μM α-T endogenously. The antioxidant added: none (○), α-T (*), PMC (◇), BO-653M (□), BO-653 (■).

4. Discussion

The currently prevailing oxidation hypothesis for atherosclerosis suggests that antioxidants, which suppress the oxidative modification of LDL should be effective for prevention of atherosclerosis. In fact, many, if not all, animal and intervention studies show the beneficial effects of antioxidants for prevention of atherosclerosis [1–5]. Consequently the development of anti-atherogenic drug has been the subject of extensive studies [11,12,22,23]. BO-653 has been found to exert potent antioxidant activity against isolated LDL oxidation [14,15] but its antioxidant action in plasma has not been studied yet. It has been assumed that vitamin C is the only antioxidant in plasma that can completely protect endogeneous lipids from oxidative damage [24]. However, the results of the present study on the oxidation of plasma of rats which were administered BO-653 orally demonstrate clearly that BO-653 inhibits plasma lipid peroxidation completely induced by either free radical initiator or copper, more effectively than α-T, the most abundant lipophilic antioxidant in plasma and LDL.

BO-653 and α-T have the similar reactivities toward lipid peroxyl radicals and inhibit the lipid peroxidation in organic solution by the similar efficiency [9]. On the other hand, the most striking difference against the plasma lipid peroxidation is that BO-653 inhibits it completely even in the absence of ascorbate, whereas α-T does not and considerable lipid peroxidation proceeds in the presence of α-T. Stocker and his colleagues [6] found that α-T might act as a prooxidant against LDL oxidation under certain conditions and interpreted this effect by phase transfer and chain transfer mechanisms: that is, α-T carries the aqueous radicals into LDL particles and then propagates the chain oxidation of LDL lipids. In the present study, a lipophilic radical initiator MeO-AMVN was used and the initiating radicals were generated within lipoproteins. Therefore, the phase transfer mechanism cannot be important in this case and the considerable lipid peroxidation observed during the presence of α-T may be ascribed to a chain transfer effect of α-T. It may be also added that, unlike α-T, BO-653 does not reduce Cu (II) to Cu (I) [13].

The aryloxyl radicals derived from BO-653 is much more stable than α-T radical due to the two bulky tert-butyl groups at the ortho-positions and in fact it has been observed that the rate constant for the hydrogen atom abstraction from polyunsaturated lipids by BO-653 radical is much smaller than that by α-tocopheroxyl radical [19]. The similar effects of substituents on the ortho-positions have been also observed for the related 2,3-dihydro-5-hydroxy-2,2-dimethylbenzofuran derivatives [19]. The striking difference between BO-653 and BO-653M observed in the present study unequivocally demonstrates that the substituents at the ortho-positions play a key role in determining the reactivity of the antioxidant-derived aryloxyl radicals and more importantly the antioxidant capacity. BO-653M behaved similarly with α-T and γ-T, while BO-653 did not. BO-653M, α-T, and γ-T did not inhibit the lipid peroxidation efficiently in the absence of vitamin C

and they were spared by vitamin C and by BO-653. BO-653 was not spared by vitamin C but it inhibited the lipid peroxidation completely by itself. The differences between BO-653 and BO-653M, and also α-T and PMC, in the antioxidant action must stem from the lower reactivity of BO-653-derived radical toward polyunsaturated lipids than BO-653M, which is ascribed to the more significant steric effect of bulky tert-butyl groups on the ortho-positions than methyl substituents. [19] This could be important in vivo at local areas and/or for smokers whose vitamin C concentration is low.

Probucol, 4,4′-(isopropylidenedithio)bis(2,6-di-tert-butylphenol), a hypocholesterolemic and antioxidant drug, also has two tert-butyl substituents on the ortho-positions, but its chemical reactivity toward lipid peroxyl radicals is much smaller than α-T and it cannot reduce α-tocopheroxyl radical [25] and hence it cannot efficiently inhibit the lipid peroxidation of LDL in the presence of α-T and absence of vitamin C.

In conclusion, BO-653 is readily incorporated into plasma by oral administration to rat and inhibits the plasma lipid peroxidation induced by free radicals and copper more efficiently than α-T, independent of presence or absence of vitamin C. These results indicate that BO-653 may act as a potent antioxidant and anti-atherogenic drug.

References

[1] Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Circulation 2002;105:2107–11.

[2] Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomized trials. Lancet 2003;361:2017–23.

[3] Salonen JT. Clinical trials testing cardiovascular benefits of antioxidant supplementation. Free Rad Res 2002;36:1299–306.

[4] Jialal I, Traber M, Devaraj S. Is there a vitamin E paradox? Curr Opin Lipid 2001;12:49–53.

[5] Heinecke JW. Is the emperor wearing clothes? Clinical trials of vitamin E and the LDL oxidation hypothesis. Arterioscler Thromb Vasc Biol 2001;21:1261–4.

[6] Thomas SR, Stocker R. Molecular action of vitamin E in lipoprotein oxidation: implications for atherosclerosis. Free Rad Biol Med 2000;28:1795–805.

[7] Niki E. Antioxidants and atherosclerosis. Biochem Soc Trans 2004;32:156–9.

[8] Ricciarelli R, Zingg JM, Azzi A. The 80th anniversary of vitamin E: beyond its antioxidant properties. Biol Chem 2002;383:457–65.

[9] Noguchi N, Iwaki Y, Takahashi M, Komuro E, Kato Y, Tamura K, et al. 2,3-Dihydro-5-hydroxy-2,2-dipentyl-4,6-di-tert-butylbenzofuran: design and evaluation as a novel radical-scavenging antioxidant against lipid peroxidation. Arch Biochem Biophys 1997;342:236–43.

[10] Tamura K, Kato Y, Ishikawa A, Kato Y, Himori M, Yoshida M, et al. Design and synthesis of 4,6-di-tert-butyl-2,3-dihydro-5-benzofuranols as a novel series of antiatherogenic antioxidants. J Med Chem 2003;46:3083–93.

[11] Noguchi N, Niki E. Phenolic antioxidants: a rationale for design and evaluation of novel antioxidant drug for atherosclerosis. Free Rad Biol Med 2000;28:1538–46.

[12] Meng CQ. BO-653 Chugai. Curr Opin Invest Drugs 2003;4:342–6.

[13] Yoshida Y, Noguchi N, Watanabe A, Iwaki Y, Yanagisawa M, Kodama T, et al. Antioxidant action of 2,2,4,6-tetra-substituted 2,3-dihydro-5-hydroxybenzofuran against lipid peroxidation: Effects of substituents and side chain. Free Rad Res 2002;36:1171–8.

[14] Noguchi N, Okimoto Y, Tsuchiya J, Cynshi O, Kodama T, Niki E. Inhibition of oxidation of low-density lipoprotein by a novel antioxidant, BO-653, prepared by theoretical design. Arch Biochem Biophys 1997;347:141–7.

[15] Muller K, Carpenter KL, Freeman MA, Mitchinson MJ. Antioxidant BO-653 and human macrophage-mediated LDL oxidation. Free Rad Res 1999;30:59–71.

[16] Cynshi O, Kawabe Y, Suzuki T, Takashima Y, Kaise H, Nakamura M, et al. Antiatherogenic effects of the antioxidant BO-653 in three different animal models. Proc Natl Acad Sci USA 1998;95:10123–8.

[17] Takabe W, Kodama T, Hamakubo T, Tanaka K, Suzuki T, Aburatani H, et al. Anti-atherogenic antioxidants regulate the expression and function of proteasome α-type subunits in human endothelial cells. J Biol Chem 2001;276:40497–501.

[18] Noguchi N. Novel insights into the molecular mechanisms of the antiatherosclerotic properties of antioxidants: the alternatives to radical scavenging. Free Rad Biol Med 2002;33:1480–9.

[19] Watanabe A, Noguchi N, Fujisawa A, Kodama T, Tamura K, Cynshi O, et al. Stability and reactivity of aryloxyl radicals derived from a novel antioxidant BO-653 and related compounds. Effects of substituent and side chain in solution and membranes. J Am Chem Soc 2000;122:5438–42.

[20] Niki E, Noguchi N. Dynamics of antioxidant action of vitamin E. Accounts Chem Res 2004;37:45–51.

[21] Yamamoto Y, Takahashi K. Glutathione peroxidase isolated from plasma reduces phospholipid hydroperoxides. Arch Biochem Biophys 1993;305:541–5.

[22] Hach GM. AGI-1067 athero genics. Curr Opin Invest Drugs 2002; 3:433–6.

[23] Campo GM, Cassarelli S, Squadrito F, Altavilla D, Dorigotti L, Caputi AP. Raxofelast (IRFI016): a new hydrophilic vitamin E-like antioxidant agent. Cardiovasc Drug Dev 1997;15:157–73.

[24] Polidori MC, Mecocci P, Levine M, Frei B. Short-term and long-term vitamin C supplementation in humans dose-dependently increases the resistance of plasma to ex vivo lipid peroxidation. Arch Biochem Biophys 2004;423:109–15.

[25] Gotoh N, Shimizu K, Komuro E, Tsuchiya J, Noguchi N, Niki E. Antioxidant activities of probucol against lipid peroxidations. Biochim Biophys Acta 1992;1128:147–54.