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PEROXYL RADICAL SCAVENGING ACTIVITY OF *GINKGO BILOBA* EXTRACT EGb 761

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Abctract—Antioxidant mechanisms have been proposed to underlie the beneficial pharmacological effects of EGb 761, an extract from *Ginkgo biloba* leaves used for treating peripheral vascular diseases and cerebrovascular insufficiency in the elderly. *In vitro* evidence has been reported that EGb 761 scavenges various reactive oxygen species, i.e. nitric oxide, and the superoxide, hydroxyl, and oxo-ferryl radicals. However, the ability of EGb 761 to scavenge peroxyl radicals (reactive species mainly involved in the propagation step of lipid peroxidation) has not been investigated. To characterize further the antioxidant action of EGb 761, we measured the protective effects of EGb 761 during: (1) the oxidation of B-phycoerythrin by peroxyl radicals generated in aqueous solution by 2,2′-azobis (2-amidinopropane) hydrochloride (AAPH); and (2) the reaction of luminol or *cis*-parinaric acid with peroxyl radicals generated from 2,2′-azobis (2,4-dimethylvaleronitrile) (AMVN) in liposomes or in human low density lipoprotein (LDL), respectively. To evaluate the peroxyl radical scavenging activity of EGb 761 in a more physiologically relevant model of damage to lipid-containing systems, we also analyzed the effect of the extract on the oxidation of human LDL exposed to the azo-initiators in terms of: (1) accumulation of cholesterol linoleate ester hydroperoxides, (2) depletion of α-tocopherol and β-carotene, and (3) changes in intrinsic tryptophan fluorescence. EGb 761 afforded protection against oxidative damage in all the systems we analyzed; thus, it is an efficient scavenger of peroxyl radicals. This result extends the oxygen radical scavenging properties of the extract and supports the hypothesis of an antioxidant therapeutic action of EGb 761.

Key words: Ginkgo biloba; peroxyl radicals; azo-initiators; low density lipoprotein; antioxidants; free radicals

Extracts from the leaves of *Ginkgo biloba* trees have been used therapeutically for centuries in traditional Chinese medicine. In modern Chinese pharmacopeias, both the leaves and fruit are recommended for treating problems of heart and lungs. In western countries, EGb 761, prepared from *G. biloba* leaves according to a well-defined procedure, is used for the treatment of peripheral vascular disease and cerebrovascular insufficiency in the elderly; in particular, intermittent claudication and symptoms thought to be due to cerebral insufficiency (difficulties of concentration and memory, confusion, dizziness, tinnitus) are reported to be relieved by the extract, without side-effects [see Refs 1 and 2 for reviews].

Among the molecular mechanisms proposed to underlie the beneficial effects of EGb 761, an antioxidant action has been claimed [3]. Recent studies have provided considerable support for the occurrence of free radical and lipid peroxidation reactions in peripheral arterial and venous diseases, and in injury to the central nervous system [4–8]. In addition, evidence for antioxidant activities of EGb 761 is accumulating: EGb 761 is protective in model systems of oxidative stress, including cardiac [9–12] and retinal [13, 14] ischemia–reperfusion injury, as well as retinal damage in diabetic rats [15, 16].

However, to characterize a substance as an antioxidant, its interaction against a wide range of species more directly responsible for oxidative damage should be assessed. In particular, a complete screen of antioxidant ability should include an assessment of the capacity of a putative antioxidant to scavenge superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, and heme-associated ferryl species [17]. EGb 761 has been reported to scavenge superoxide and hydroxyl radicals [18, 19], to inhibit the production of reactive oxygen species in stimulated leukocytes [20] and to interact with nitric oxide [21]. However, a study on the scavenging effects of EGb 761 against peroxyl radicals has not been reported. Peroxyl radicals formed in both hydrophobic and hydrophilic environments upon the reaction of carbon-centered radicals with oxygen are biologically relevant active species because of their likelihood to damage cellular constituents. For example, peroxyl radicals inactivate both alcohol dehydrogenase [22] and α-antiproteinase [23]. Moreover, formation of peroxyl radicals is the major chain-propagation step in lipid peroxidation.

In biological systems, the accessibility of a radical to its scavenger can be an important factor in limiting

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the scavenging action of a compound. To define the antioxidant action of EGb 761 with respect to the site of peroxyl radical generation, we tested the ability of solutions of EGb 761 to scavenge peroxyl radicals generated in hydrophilic or hydrophobic environments. We used the hydrophilic azo-initiator AAPH* and the hydrophobic azo-initiator AMVN to generate peroxyl radicals at a constant rate in aqueous and hydrophobic regions, respectively [24]. The peroxyl radical scavenging activity of EGb 761 was assayed by measuring: (1) the oxidation of B-phycoerythrin by peroxyl radicals generated in aqueous solution by AAPH; and (2) the reaction of luminol or *cis*-parinaric acid with peroxyl radicals generated from AMVN in liposomes or in human LDL, respectively.

Oxidative damage to biological lipids is involved not only in the pathophysiology of cerebral or peripheral vascular diseases but also in a variety of other chronic diseases such as cancer, cataracts, and atherosclerosis [25]. Thus, to evaluate the peroxyl scavenging activity of EGb 761 in a more physiologically relevant model of damage to lipid systems, we also analyzed the effect of the extract on oxidative damage to human LDL. Lipoproteins were exposed to azo-initiators, and the effect of the extract was analyzed in terms of: (1) accumulation of CEOOHs; (2) depletion of α-tocopherol and β-carotene; and (3) changes in intrinsic tryptophan fluorescence.

MATERIALS AND METHODS

Materials. *G. biloba* extract (EGb 761) was a gift from IPSEN Laboratories (France). AMVN and AAPH were purchased from Polysciences, Inc. (Warrington, PA); *cis*-parinaric acid was purchased from Molecular Probes (Junction City, OR); DOPC, luminol, B-phycoerythrin, bovine albumin and EDTA were from the Sigma Chemical Co. (St. Louis, MO). Tocol, α-tocopherol and β-carotene were gifts from Hoffmann-La Roche (Nutley, NJ). Sephadex G-25 was from Pharmacia (Uppsala, Sweden). HPLC grade solvents were purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals were of the highest analytical grade available.

Preparation of liposomes. DOPC (50 mg) in chloroform was dried with a stream of nitrogen, resuspended in 5 mL of 20 mM Tris–HCl, pH 7.4, by vortexing, sonicated for 20 min with a probe sonicator, and then used after dilution to a final concentration of 2 mg lipid/mL.

Isolation of human LDL. Fresh blood, collected in EDTA from normolipidemic volunteers, was centrifuged at 1000 *g* for 15 min at 4° to separate plasma from erythrocytes. Additional EDTA (3 mM) was added to the plasma, and LDL was isolated and separated from contaminating plasma proteins as reported by Chung *et al.* [26]. LDL was then stored up to 7 days at 4° in the presence of 3 mM EDTA in sealed vials flushed with argon. Immediately before use, LDL was passed over a Sephadex G-25 gel filtration column and eluted with PBS (150 mM NaCl, 20 mM phosphate, pH 7.4) to remove salt solutions.

PBS suspensions of LDL at a final concentration of 1 mg protein/mL were used in all experiments. Protein concentrations were determined by the Markwell modification of the Lowry method [27] using bovine albumin as a standard.

Oxidation of B-phycoerythrin by AAPH-generated peroxyl radicals. To assess the activity of EGb 761 as a scavenger of peroxyl radicals generated in aqueous solutions, we used the fluorometric method of Glazer [28] with slight modifications. A solution of B-phycoerythrin (17 nM in PBS) was incubated at 37° for 10 min with continuous stirring in a quartz cuvette. AAPH (5 mM final concentration) was added, and the loss of B-phycoerythrin fluorescence was monitored for 1 hr in a SPEX fluorometer (excitation at 540 nm, emission at 575 nm). In the experiments with EGb 761, different concentrations of the extract were added 10 min after the initiation of AAPH-induced B-phycoerythrin oxidation, and the fluorescence of the protein was monitored. Solutions of AAPH and EGb 761 were prepared fresh in PBS before use.

Reaction of luminol with peroxyl radical generated in DOPC liposomes from AMVN. To evaluate the protective effect of EGb 761 against peroxyl radicals generated in membranes, we used a slight modification of the method reported by Kagan *et al.* [29]. DOPC liposomes (2 mg lipid/mL), luminol (150 μM) and various concentrations of EGb 761 were incubated at 40° for 15 min in a chemiluminescence tube of an LKB 1250 luminometer. AMVN (final concentration 12 mM) was then added, and the induction of chemiluminescence at 450 nm was recorded for at least 15 min. AMVN was used as a freshly prepared ethanol solution.

Oxidation of cis-parinaric acid by AMVN-generated peroxyl radicals in the presence of LDL. To assess the scavenging properties of EGb 761 against peroxyl radicals generated in a lipophilic environment, an adaptation of the method reported by Laranjinha *et al.* [30] was used. LDL was incubated at 37° for 5 min in a quartz cuvette of a SPEX fluorometer. *cis*-Parinaric acid, in ethanol, was added to a final concentration of 2 μM, and its fluorescence was monitored (excitation at 328 nm, emission at 415 nm). AMVN (final concentration 1 mM) was then added, and the fluorescence was monitored for 30 min. To evaluate the effect of EGb 761, the extract was added, at a final concentration of 100 μg/mL, 5 min after the beginning of AMVN-induced *cis*-parinaric acid oxidation.

Intrinsic tryptophan fluorescence. LDL was oxidized as described below. Aliquots were collected at regular intervals, and the fluorescence spectra were recorded at room temperature in a SPEX fluorometer after excitation at 290 nm [31].

The absorbance of solutions of EGb 761 (100 μg/mL) is negligible at all wavelengths used in the photometric methods described above; thus, direct interference of the extract can be excluded.

Peroxyl radical-induced LDL oxidation. LDL suspensions, in the presence or absence of EGb 761,

* Abbreviations: AAPH, 2,2'-azobis (2-amidinopropane) hydrochloride; AMVN, 2,2'-azobis (2,4-dimethylvaleronitrile); CEOOH, cholesterol linoleate ester hydroperoxide; DOPC, L-α-phosphatidylcholine, dioleolyl; and LDL, low density lipoprotein.

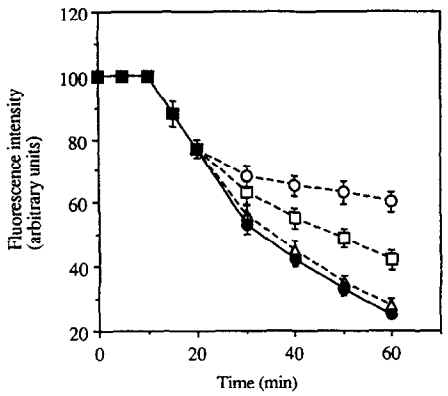

Fig. 1. Scavenging effect of EGb 761 on AAPH-induced peroxyl radicals as measured by fluorescence decay of B-phycoerythrin. B-phycoerythrin (17 nM in PBS) (solid line, closed circles) was exposed at 37° in PBS to AAPH (5 mM); then EGb 761 was added (broken lines) to a final concentration of 10 (open triangles), 50 (open squares) or 100 μg/mL (open circles). Fluorescence intensity was measured with 540 nm excitation and 575 nm emission. Means ± SD were obtained from three separate experiments; in some cases, error bars are smaller than the symbols.

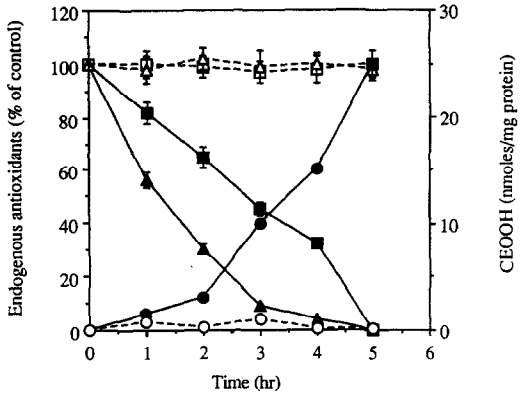

Fig. 2. Effect of EGb 761 on oxidative modification of human LDL induced by AAPH. Human LDL (1 mg protein/mL) was incubated in PBS at 37° with AAPH (5 mM) in the absence (solid lines, closed symbols) and in the presence (broken lines, open symbols) of EGb 761 (100 μg/mL). Key: cholesterol linoleate ester hydroperoxide (circles); α-tocopherol, initial concentration: 2.5 nmol/mg protein (triangles); and β-carotene, initial concentration: 0.27 nmol/mg protein (squares). Endogenous antioxidants are expressed as a percentage of the level measured in control samples incubated in the absence of the azo-initiator. In control samples, formation of cholesterol linoleate ester hydroperoxide and depletion of α-tocopherol or β-carotene were not observed. Means ± SD, obtained from triplicate assays within one representative experiment out of three, are reported; in some cases, error bars are smaller than the symbols.

were preincubated at 37° for 10 min. AAPH or AMVN (5 or 1 mM final concentration, respectively) was added and incubated for 5 hr with agitation in a water bath under air. At timed intervals, aliquots of the incubation mixture were collected and analyzed as described below.

HPLC assay of CEOOHs, α-tocopherol and β-carotene. LDL oxidation was stopped by the addition of EDTA (0.1 mM final concentration) and freezing at −80°. Lipids were extracted from LDL with hexane [32], using tocol as internal standard. α-Tocopherol, β-carotene and CEOOH were separated simultaneously by HPLC on an Alltech C18 Adsorbosphere-HS column, 3 μm particle size, 15 cm × 0.46 cm with a mobile phase of 60% acetonitrile, 30% isopropanol, 10% methanol and 0.1% ammonium acetate (v/v) at a flow rate of 1 mL/min. Measurements were made with a Hewlett Packard model 1050 diode array detector with signal channels set at 234, 295 and 450 nm. Spectra were collected simultaneously for the identification of the major components. The identification and quantitation of the individual components were achieved by referring to appropriate standards. CEOOH was prepared as reported [33].

RESULTS

Thermal decomposition of the hydrophilic azo-initiator AAPH generates peroxyl radicals at a defined rate in aqueous solution [24] causing a decrease of B-phycoerythrin fluorescence [28]. To assess the peroxyl radical scavenging activity of EGb 761 in solution, we evaluated the ability of EGb 761 to protect the hydrophilic protein B-phycoerythrin from degradation (Fig. 1). When B-phycoerythrin (17 mM) was exposed at 37° to AAPH (5 mM) in PBS, there was a loss of the protein fluorescence intensity, whose rate decreased upon the addition of EGb 761. The effect of the extract was concentration dependent. The rate of the AAPH-dependent decrease of phycoerythrin fluorescence was not changed by the addition of 10 μg/mL EGb 761, but 100 μg/mL of EGb 761 virtually stopped the degradation of phycoerythrin.

The efficacy of EGb 761 in scavenging peroxyl radicals in solution was also evaluated by analyzing the effect of the extract on the oxidation of LDL exposed to AAPH (Fig. 2). Treatment of human LDL with peroxyl radicals generated at 37° from AAPH (5 mM) for 5 hr caused a progressive accumulation of CEOOH and a concurrent loss of α-tocopherol and β-carotene. Although different LDL samples had different concentrations of endogenous antioxidants and showed different rates of oxidation, they all showed similar trends of oxidative modification: the rate of depletion of α-tocopherol was higher than that of β-carotene. In all LDL preparations, EGb 761 (100 μg/mL) completely protected LDL from oxidative modification: no decreases in α-tocopherol or β-carotene were observed, nor was there an accumulation of CEOOHs.

The protective effect of EGb 761 on LDL oxidative modification was concentration dependent (Fig. 3). EGb 761 (10 μg/mL) efficiently protected LDL from the accumulation of CEOOHs and from the depletion

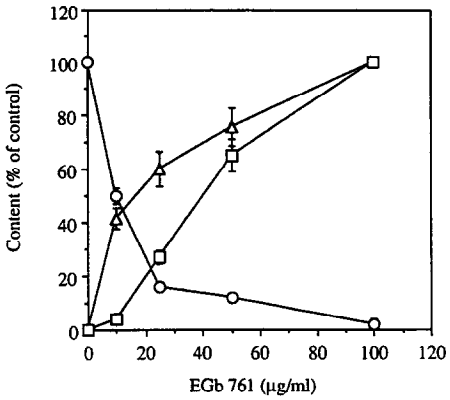

Fig. 3. Effect of EGb 761 concentration on oxidative modification of human LDL induced by AAPH. Human LDL (1 mg protein/mL) was incubated for 5 hr in PBS at 37° with AAPH (5 mM) in the presence of various concentrations of EGb 761. Cholesterol linoleate ester hydroperoxide (circles) is expressed as a percentage of the level measured in control samples incubated in the absence of EGb 761 (i.e. 65, 25, and 93 nmol/mg protein for LDL isolated from three different donors). The contents of α-tocopherol (initial concentration for LDL isolated from three different donors: 6.5, 2.5, and 10 nmol/mg protein) (triangles) and of β-carotene (initial concentration for LDL isolated from three different donors: 0.18, 0.27, and 0.16 nmol/mg protein) (squares) are expressed as a percentage of the levels measured in control samples incubated in the absence of the azo-initiator. Means ± SD were obtained by using the three different LDL preparations; in some cases, error bars are smaller than the symbols.

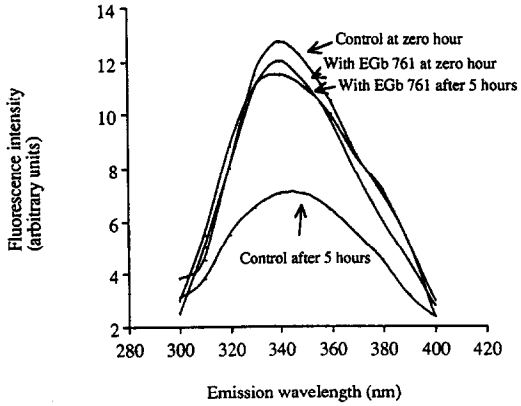

Fig. 4. Effect of EGb 761 on tryptophan fluorescence of AAPH-treated human LDL. Human LDL (1 mg protein/mL) was incubated in PBS at 37° for 5 hr with AAPH (5 mM) in the absence or the presence of EGb 761 (100 μg/mL). Emission spectra (excitation wavelength 290 nm) were recorded at the beginning and after 5 hr of incubation with AAPH. One experiment, representative of three, is shown.

of α-tocopherol, but the extract at this concentration did not protect LDL from β-carotene depletion.

The peroxidation of lipoproteins has been reported to be associated with a decrease of tryptophan

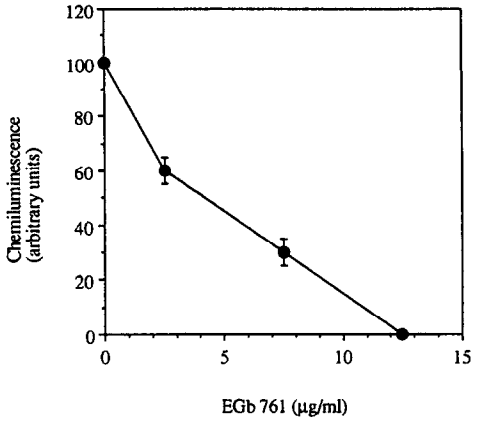

Fig. 5. Scavenging effects of EGb 761 on AMVN-induced peroxyl radicals generated in the presence of liposomes as measured by chemiluminescence. Luminol (150 μM), added to a suspension of liposomes (2 mg DOPC/mL), was exposed for 15 min at 40° to AMVN (12 mM) in 20 mM Tris–HCl, pH 7.4, in the presence of the indicated concentrations of EGb 761. Means ± SD were obtained from three different experiments.

fluorescence due to the degradation of the amino acid residues of the protein component [31]. Therefore, the ability of EGb 761 to maintain the fluorescence of LDL exposed to AAPH-generated peroxyl radicals was also analyzed. Incubation of LDL with AAPH (5 mM) in the absence of EGb 761 for 5 hr caused a marked decrease in tryptophan fluorescence. In all LDL samples, the addition of EGb 761 (100 μg/mL) prevented any appreciable change in LDL fluorescence intensity (a representative example is shown in Fig. 4).

To characterize further the peroxyl radical scavenging properties of EGb 761, we evaluated the ability of the extract to scavenge peroxyl radicals generated within lipid domains. We used the hydrophobic azo-initiator AMVN as a source of radicals within the hydrophobic region of two different lipid models, DOPC liposomes and human LDL. To ensure that the results were independent of the assay system, we used two different probes: luminol for the liposomes and *cis*-parinaric acid for LDL. In addition, we evaluated the efficacy of the extract to protect human LDL from AMVN-induced oxidative damage.

Luminol reacts with peroxyl radicals generated within lipid domains and produces a chemiluminescent signal [29, 34]. When AMVN (12 mM) was added in a luminol solution (150 μM) in the presence of DOPC liposomes at 40°, we observed the induction of a chemiluminescent signal that reached a maximum in 3–4 min and then was stable for at least 10 min (data not shown). No chemiluminescent signal was observed in control samples incubated without liposomes. In the presence of EGb 761, the maximum chemiluminescent signal was lower and there was a longer lag phase prior to the appearance of a signal (data not shown). The effect of the extract was concentration dependent:

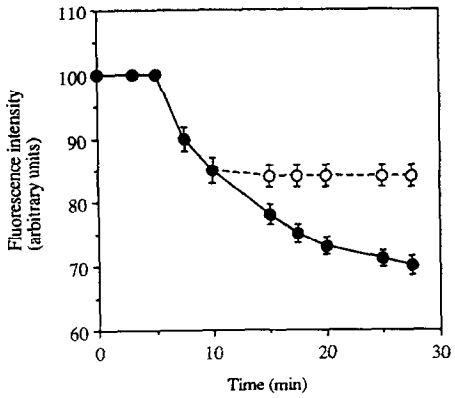

Fig. 6. Scavenging effects of EGb 761 on AMVN-induced peroxyl radicals generated in the presence of human LDL as measured by fluorescence decay of *cis*-parinaric acid. *cis*-Parinaric acid (2 μM) added to a suspension of human LDL (1 mg protein/mL) was exposed at 37° to AMVN (1 mM) (solid line, closed circles) with subsequent addition (broken line, open circles) of EGb 761 (100 μg/mL). Fluorescence intensity was measured (328 nm excitation and 415 nm emission). Means ± SD, obtained from triplicate assays within one representative experiment out of three, are reported; in some cases, error bars are smaller than the symbols.

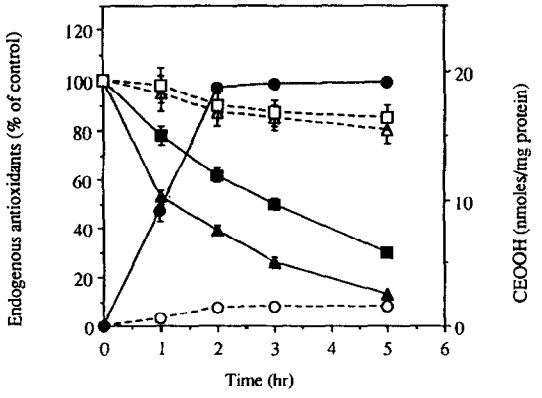

Fig. 7. Effect of EGb 761 on oxidative modification of human LDL induced by AMVN. Human LDL (1 mg protein/mL) was incubated in PBS at 37° with AMVN (1 mM) in the absence (solid lines, closed symbols) and in the presence (broken lines, open symbols) of EGb 761 (100 μg/mL). Key: cholesterol linoleate ester hydroperoxide (circles); α-tocopherol, initial concentration: 3.9 nmol/mg protein (triangles); β-carotene, initial concentration: 0.21 nmol/mg protein (squares). Endogenous antioxidants are expressed as a percentage of the levels measured in control samples incubated in the absence of the azo-initiator. In control samples, the formation of cholesterol linoleate ester hydroperoxide and the depletion of α-tocopherol or β-carotene were not observed. Means ± SD, obtained from triplicate assays within one representative experiment out of three, are reported; in some cases, error bars are smaller than the symbols.

EGb 761 at 12.5 μg/mL completely inhibited the induction of the chemiluminescent signal (Fig. 5.)

To further define EGb 761 as a scavenger of peroxyl radicals generated in a hydrophobic environment, we evaluated its effect on AMVN-mediated oxidation of *cis*-parinaric acid, an oxidative-stress sensitive fluorescent hydrophobic probe [30]. When *cis*-parinaric acid (2 μM) was added to a suspension of human LDL, and exposed to AMVN (1 mM) at 37°, a loss of fluorescence was observed. EGb 761 (100 μg/mL) halted further *cis*-parinaric oxidation completely in all of the LDL preparations that we used (Fig. 6).

EGb 761 was also able to protect LDL against oxidative damage induced by AMVN. Although different LDL samples had different concentrations of endogenous antioxidants and showed different rates of oxidation, they all showed similar trends of oxidative modification. The exposure of suspensions of LDL to AMVN (1 mM) for 5 hr at 37° resulted in an accumulation of CEOOHs, and a depletion of α-tocopherol and β-carotene (Fig. 7, solid lines, closed symbols). The rate of α-tocopherol depletion was higher than that of β-carotene. In the presence of EGb 761 (100 μg/mL), the formation of CEOOH was inhibited almost completely. The extract also protected LDL against depletion of α-tocopherol and β-carotene (Fig. 7, broken lines, open symbols).

DISCUSSION

The accessibility of an antioxidant to the site of radical generation and to the molecular targets of oxidative stress is an important factor in limiting the scavenging action of a compound. Peroxyl radicals can be produced both in solution and in membranes; thus, to evaluate the scavenging properties of EGb 761 against peroxyl radicals, we analyzed the ability of EGb 761 to protect biological targets from oxidative damage induced by peroxyl radicals generated from a hydrophobic source (AMVN) or a hydrophilic source (AAPH).

We demonstrated that EGb 761 is a scavenger of peroxyl radicals generated in both lipid and aqueous environments. EGb 761 (100 μg/mL) afforded complete protection against AAPH-induced degradation of the soluble protein B-phycoerythrin (Fig. 1). It also completely protected the hydrophobic oxidative stress-sensitive probe, *cis*-parinaric acid, from oxidative damage caused by peroxyl radicals generated in LDL by the hydrophobic azo-initiator AMVN (Fig. 6). The ability of EGb 761 to scavenge peroxyl radicals generated in lipid domains and to protect hydrophobic targets was independent of systems used; EGb 761 also protected luminol added to a suspension of DOPC liposomes from AMVN-induced oxidation. In this case, full protection was obtained by using 12.5 μg/mL EGb 761 (Fig. 5).

The protective effect of EGb 761 against oxidative modification of human LDL exposed to azo-initiators (Figs. 2 and 7) confirms the scavenging properties of the extract on peroxyl radicals. However, the protective effect of EGb 761 was stronger in LDL exposed to AAPH than to AMVN. The kinetics of AAPH-induced oxidation of the different components in LDL that we observed were similar

to those reported by Noguchi *et al.* [35], as well as to those reported for the oxidation of LDL induced by copper [36, 37]: CEOOH formation occurred only after depletion of most of the α-tocopherol. Loss of β-carotene also occurred, but at a rate lower than that for α-tocopherol (Fig. 2). The rates of oxidation of the different components of LDL are consistent with an initial formation of radicals mostly outside or at the surface of LDL. After depletion of most of the α-tocopherol, peroxidation takes place inside the lipoprotein as a consequence of propagation of lipid peroxidation-chain reactions. The pattern of LDL oxidation is also consistent with a pattern of locations that places cholesterol ester in the core of LDL, α-tocopherol exposed on the surface, and β-carotene less accessible in the core. It is likely that the molecules of EGb 761 in solution scavenge AAPH-generated peroxyl radicals in the aqueous phase or at the surface of LDL, and the molecules of the extract that partition in the lipid domains of LDL can scavenge peroxyl radicals formed both at the surface and in the lipid domain. The ability of EGb 761 to protect α-tocopherol appeared greater than its ability to protect β-carotene; this was especially apparent at low concentrations of EGb 761 (Fig. 3). This could be due to a lower antioxidant activity of the molecules of EGb 761 in the LDL against peroxyl radical generated in the lipid domain, as well as to an unfavorable location of the antioxidant molecules of EGb 761 with respect to β-carotene.

The greater efficacy of EGb 761 in scavenging peroxyl radicals formed in solution or at the surface of LDL compared with the hydrophobic regions of LDL is supported by the data obtained upon treatment of LDL with AMVN. When human LDL was exposed to AMVN, which generates peroxyl radicals primarily in hydrophobic regions, the formation of CEOOH was independent of α-tocopherol depletion, in agreement with results previously reported [35]. Maximum levels of CEOOH formation were observed even in the presence of high levels of α-tocopherol (Fig. 7), and a residual quantity of α-tocopherol was still present after 5 hr of treatment. Even in the presence of EGb 761 (100 μg/mL) there was some depletion of α-tocopherol and β-carotene, as well as a detectable production of CEOOH.

EGb 761 is a standardized mixture of different compounds containing two major groups of substances: flavanoid glycosides and terpenoids. Flavanoids have been reported to be effective scavengers of superoxide [18, 38–40], hydroxyl radicals [41, 42], and inhibitors of lipid peroxidation [43]. Our results do not allow us to distinguish which component of EGb 761 is responsible for its scavenging properties against peroxyl radicals. As EGb 761 is a mixture of different chemical constituents, its scavenging activity could be due to a particular component as well as to the interactions of different antioxidant molecules.

The peroxyl scavenging activity we observed extends the antioxidant properties of EGb 761 and indicates a possible role for the extract in the treatment of diseases involving free radicals and oxidative damage. Specifically, the ability of EGb 761 to protect LDL against oxidative modification suggests a potential therapeutic use in the pathogenesis of atherosclerosis, which has been reported to be due partly to oxygen radical-induced modification of lipid or protein components of human LDL [37, 44]. Accumulation of foam cells is characteristic of atherosclerotic lesions [45, 46], and foam cells may be the result of accumulation of cholesterol, after excessive uptake of oxidized LDL by artery wall macrophages and smooth muscle cells [47, 48]. Formation of oxidized LDL as a result of free radical-initiated reactions in the body has also been hypothesized [49, 50].

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