Palm oil antioxidant effects in patients with hyperlipidaemia and carotid stenosis-2 year experience

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Antioxidants appear to play a role in the prevention of atherosclerosis. Here, we investigated the antioxidant properties of a γ-tocotrienol and α-tocopherol enriched fraction of palm oil, in patients with carotid atherosclerosis. Serum lipids, fatty acid peroxides, platelet aggregation, and carotid artery stenosis were measured over a 24-month period in 50 patients with cerebrovascular disease. Change in stenosis was measured with bilateral duplex ultrasonography. These studies revealed apparent carotid atherosclerotic regression in eight and progression in two of the 25 antioxidant patients, while none of the control group exhibited regression and ten of 25 showed progression ($P<0.01$). Serum α-tocopherol doubled while tocotrienols were undetectable throughout the study. Serum thiobarbituric acid reactive substances, decreased in the treatment group from 1.08 ± 0.14 to 0.80 ± 0.14 μM ($P<0.05$) after 24 months, and in the placebo group, they increased nonsignificantly from 0.99 ± 0.16 to 1.06 ± 0.17 μM. Both antioxidant and placebo groups displayed significantly increased collagen-induced platelet aggregation responses ($P<0.05$) as compared with entry values. Serum total cholesterol, low density lipoprotein cholesterol, and triglyceride values remained unchanged in both groups, as did the plasma high density lipoprotein cholesterol values. Palm oil tocols appear to benefit the course of carotid atherosclerosis.

Introduction

In recent years, evidence has linked processes involving oxygen-derived free radicals with the initiation and propagation of atherosclerosis. In particular, the oxidative modification of low density lipoproteins (LDL), with its ensuing sequelae of cytotoxic, thrombogenic, and chemotactic events, has emerged as a key step in promoting atherosclerosis[1-3]. Antioxidants, especially vitamin E, have received considerable attention as potential anti-atherogenic agents for over 40 years. A number of recent studies have demonstrated the ability of antioxidants to prevent *ex vivo* and *in vitro* LDL oxidation, and to potentially be able to ameliorate the development of atherosclerotic lesions[4-6]. Epidemiological studies have linked the dietary intake of vitamin E and other antioxidants with a reduced risk of coronary heart disease[7,8], ischaemic heart disease[9], ischaemic stroke[10], coronary mortality[11], and with a decrease in carotid artery wall thickness[12]. As the vitamin-rich distillate of palm oil [Palm Oil Research Institute of Malaysia (PORIM), Kuala Lumpur, Malaysia] is enriched with several antioxidant members of the vitamin E family (tocopherols and tocotrienols), it was felt worthwhile to investigate its antioxidant effects in patients with cerebrovascular disease.

Materials and methods

The study cohort consisted of 50 subjects (23 males and 27 females), ranging from 49-83 years of age, with carotid artery atherosclerosis as determined by duplex carotid ultrasonography. Eligibility criteria included subjects less that 85 years of age who had either had a hemispheric transient ischaemic attack, monocular blindness for less than 24 hours, or a nondisabling hemispheric stroke within the previous year. Subjects had carotid artery stenosis ranging from 15-79%. Subjects having stenosis beyond 79% were referred to surgery. Forty-four percent of placebo and 60% of test subjects had more than 49% stenosis of the carotid artery. All patients continued to receive their current medical care, including antihypertensive, antidiabetic, and anti-platelet (usually aspirin) treatment, except that 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors were discontinued.

At baseline, patients underwent standardised history, physical, and neurological examinations, an assessment of functional status, standardised laboratory tests, 12-lead electrocardiography, a chest X-ray, and duplex carotid ultrasonography. A synopsis of subject characteristics appear at baseline showing their similarity (Table 1; collagen-induced aggregation in ohms of resistance (Ω) was 16.9 for the antioxidant group, 17.7 for the placebo group; thrombin ATP release (μM) was 0.99 ± 0.46 and 1.02 ± 0.39 for the antioxidant and placebo groups, respectively).

Ultrasonography of the carotid arteries was done baseline and repeated at six and twelve months, and annually thereafter. Degree of stenosis was graded as: 0-15%; 16-49%; 50-79%, and 80-99%. Ultrasound duplex measurements were done with an Acuson 128 XP ultrasonograph (Acuson Co, Mountain View, CA) equipped with a 5 or 7 MHz transducer. The transducer aperture was 38 mm. Subjects were examined in the supine position. Change from one category to the next was reckoned regression or progression; a change of two categories was considered marked regression or progression. All ultrasound studies were done by the same person, who was unaware of treatment assignments and results of earlier measurements. The artery affecting neurological involvement was used for study in an attempt to more easily correlate the pathological and clinical changes.

Using a table of random numbers, patients were assigned to receive 300 mg capsules containing *either* 16 mg α-tocopherol, 40 mg γ- and α-tocotrienols, and 240 mg palm superolein, *or* placebo (300 mg palm superolein). Palm superolein is a mixture of triglycerides with the predominant fatty acids being oleic, palmitic, and linoleic (6:4:1.51 ratio). The dosage of capsules

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containing placebo or palm oil antioxidants was 4 capsules daily. At the three- and six-month follow-up visits, the dosage was increased to 5 and 6 capsules daily, respectively, in an attempt to lower serum lipids. The dosage then remained at six capsules/day (96 mg α-tocopherol and 240 mg tocotrienol) for the remainder of the study. Patients were blinded throughout the trial; none in either group reported side effects. Investigators also were blinded about treatment groups, except for the one who distributed the capsules.

Table 1. Comparison of patient characteristics at study entry by treatment group.

Patient Characteristic	Palm Vitee (n=25)	Placebo (n=25)	P
Age	66.4	66.7	ns
Gender (F/M)	13/12	14/11	ns
Antiplatelet (aspirin) use (no.)	15	11	ns
Anticoagulant use (no.)	21	24	ns
Hypertensives (no.)	9	14	ns
Diabetics (no.)	5	2	ns
Abnormal EKG[b] (no.)	16	15	ns
Abnormal chest X-ray[b] (no.)	7	12	ns
Cigarette use (no.)	5	4	ns
Height, inches (m)	64.8±4.2 (1.65±0.1)	64.4±3.5 (1.63±0.1)	ns
Weight, lbs, (kg)	152.8±39.5 (69.4±17.9)	150.8±26.9 (68.6±12.2)	ns
Systolic BP (mm Hg)	138.6±14.4	147.8±20.0	ns
Diastolic BP (mm Hg)	82.4±9.7	81.2±9.6	ns
No. > 49% carotid stenosis	15	11	ns

Data expressed as means ± SEM; ns, not significant; (b) Abnormal for cardiovascular pathology; BP = blood pressure

During the baseline, 3-, 6-, 9-, 12-, 18-, and 24-month visits venous blood was drawn after overnight fasting into EDTA and Vacutainer SST tubes (Becton Dickinson Co., Rutherford, NJ) with a sterile activator, stored at ambient temperature for five minutes, spun at ambient temperature at 3400 rpm, and either analysed immediately or stored frozen overnight before analysis. Though samples were identified during analysis, the analysis were done in a lab separate from the outpatient clinic. A lipid profile was measured in each subject to control for dietary changes or medicinal usage compliance which might have affected stenosis. It included serum total cholesterol, LDL cholesterol (by difference)[13], and triglycerides for each visit using Reflotron methodology, which uses cholesterol esterase, oxidase, horseradish peroxidase, and 3,3' 5,5'-tetra-methylbenzidine dye, which is quantitated by reflectance (Boehringer-Mannheim, Indianapolis, IN) and high density lipoprotein (HDL) cholesterol using the same chemistry with EDTA-treated plasma. Triglycerides were quantitated by reaction with an esterase, glycerol kinase, glycerol phosphate oxidase, and peroxidase in the presence of a dye 4-(4-di-methylaminophenyl)-5-methly-2-(4-hydroxy-3,5-dimethyl-oxy-phenol)-imidazole-dihydrochloride. All analyses were done under amber light to reduce photooxidation. Fatty acid hydroperoxides were assayed according to Ohishi *et al.*[14]. Cumene hydroperoxide was used as the standard. The serum level of thiobarbituric acid reactive substances (TBARS) was measured to quantitate malon-dialdehyde equivalent materials by the method of Mihara *et al.*[15]. Serum levels of a-tocopherol were measured during baseline and at the 24-month visits by a high-performance liquid chromatography method of Bieri *et al.*[16] to monitor compliance. This method was not able to separate and detect measurable tocotrienols. Platelet aggregation in whole blood was measured with an impedance aggregometer (Chronolog Corp, Havertown, PA), using thrombin and collagen as agonists to initiate aggregation[17], measuring only the primary wave of aggregation after the reaction had proceeded for six minutes. These measurements were made on a background of aspirin, as all subjects were taking aspirin during the trial.

Subject compliance was monitored by pill counts and measurement of serum vitamin levels, as indicated with each data panel. Pill counts showed 95% compliance for antioxidants.

Statistics were performed using Student's t-test, the paired t-test, or the Mann-Whitney test. Available 3-, 6-, 9-, 12-, 18-, or 24-month data are presented.

Results

Those patients being supplemented with tocopherol and tocotrienols for 24 months approximately doubled their serum α-tocopherol levels compared to baseline (Table 2). Serum levels of tocotrienol were below the detection limits of our high-performance liquid chromatography system; hence, no tocotrienol data are presented.

Data of the effect of the palm oil antioxidants vs. placebo in lowering serum lipid oxidation products are shown (in Table 2). Patients receiving antioxidants attenuated their generation of TBARS after 24 months, with values decreasing from (mean ± SEM) 1.08 ± 0.14 to 0.80 ± 0.14 μM ($P<0.05$); whereas placebo subjects' values showed no significant change (0.99 ± 0.16 to 1.06 ± 0.17 μM). At baseline, a difference was seen in hydroperoxide levels, possibly attributed to cigarette smoking (Table 1). Although not significant, the fatty acid hydroperoxides increased in both groups, rising from 1.73 ± 0.15 at baseline to 2.85 ± 0.55 μM after 24 months in the test group and 2.27 ± 0.23 to 3.02 ± 0.62 μM in the placebo group.

After 24 months, both antioxidant supplement (16.9 ± 2.4 Ω to 25.5 ± 3.0 Ω; $P<0.03$) and placebo (17.7 ±2.5 Ω to 28.8 ± 3.0 Ω; $P<0.01$) were associated with increase of collagen-induced platelet aggregation when compared with baseline. The platelet

Table 2. Serum TBARS, LOPS, and α-tocopherol values and whole blood platelet responses of subjects given tocotrienols and tocopherol or placebo for 24 mon[a].

Palm oil fraction (tocotrienols)							
Analyte	**Baseline**	**3 mon**	**6 mon**	**9 mon**	**12 mon**	**18 mon**	**24 mon**
Collagen(Ω)	16.9±2.4	14.6±2.6	9.52±1.7	15.6±2.2	12.0±1.8	13.2±2.9	25.5±3.0[c]
ATP(μM)	0.99±0.09	1.02±0.08	0.92±0.09	0.92±0.09	1.24±0.15	1.24±0.09	1.24±0.09
TBARS(μM)	1.08±0.14	0.66±0.10	0.68±0.11	0.68±0.11	0.80±0.11	1.24±0.22	0.80±0.14[d]
LOPS(μM)	1.73±0.15	1.58±0.20	4.52±1.3	4.1±1.1	3.6±1.5	2.6±0.60	2.85±0.55
Vit E(μM)	1.90±0.21	-	-	-	-	-	3.22±0.31[e]
Placebo							
Collagen(Ω)	17.7±2.5	12.6±1.7	14.9±2.0	15.4±1.7	12.0±1.6	14.1±1.4	28.8±3.0[f]
ATP(μM)	1.02±0.08	0.97±0.10	0.99±0.14	1.00±0.13	1.04±0.15	0.91±0.08	0.91±0.08
TBARS(μM)	0.99±0.16	1.02±0.15	1.01±0.14	1.21±0.15	1.26±0.11	1.42±0.14	1.06±0.07
LOPS(μM)	2.27±0.23	1.58±0.20	2.1±0.32	6.7±3.14	3.6±1.90	2.8±0.70	3.02±0.62
Vit E(μM)	1.64±0.14	-	-	-	-		1.19±0.14[g]

TBARS, thiobarbituric reactive substances; LOPS, fatty acid hydroperoxides. a, Data expressed as mean ± SEM. b, Thrombin agonist challenge, 1 NIH unit/mL. c, $P<0.03$ vs. respective baseline value. d, $P<0.05$ vs. respective baseline value. e, $P<0.01$ vs. respective baseline value. f, $P<0.01$ vs. respective baseline value. g, $P<0.05$ vs. respective baseline value.

ATP response to thrombin did not change in the placebo group (1.02 ± 0.39 to 0.91 ± 0.42), but changed from 0.99 ± 0.46 to 1.24 ± 0.46 (nonsignificant) in the antioxidant group. Because both groups used aspirin, and aspirin usage was monitored carefully during the study, it appears that the increase seen in platelet aggregation resulted from a decreased adherence to prescribed aspirin intake.

Table 3. Comparison of change in carotid stenosis in groups receiving tocotrienols and tocopherol or placebo for six and twenty-four months[a].

		Antioxidant	Placebo
6 months	MR	1	0
	NC	18	20
	MP	0	3
	Total number	25	25
24 months	MR	1	0
	NC	15	14
	MP	0	4
	Total number	25	25

a: Data expressed as number of subjects per category. MR, marked regression; K, regression; NC, no change; P, progression; MP, marked progression.

The percentage change of carotid stenosis in patients by category differed in the groups receiving palm oil antioxidants and placebo after 24 months of treatment. The data indicated (Table 3) that seven (28%) of the subjects supplemented with antioxidants for 24 months moved one category to a lesser apparent degree of stenosis; one (4%) improved two categories, suggesting marked regression. Only two from this group (8%) exhibited progression of carotid atherosclerosis. The remaining 60% manifested no change in disease state. In contrast, 28%, of the placebo group appeared to progress, and 16% markedly progressed in carotid stenosis ($P < 0.002$; Mann-Whitney), while 56% of Doppler studies from the placebo group remained unchanged. No one receiving the placebo showed carotid plaque regression.

Treatment with the palm oil fraction (including 240 mg tocotrienols daily for 24 months) had no measurable effect on serum lipids (Table 4). Compared to baseline, serum total, LDL, HDL cholesterol, and triglyceride values remained virtually unchanged (non-significant) in test and in control groups over the observation periods.

Discussion

The purpose of the present clinical study was to test the effect of palm oil antioxidants in patients with carotid atherosclerosis. After extending the duration of the antioxidant supplementation study to 24 months, we found no measurable improvement in blood lipids, although we did observe an apparent improvement in blood flow in the carotid arteries of eight patients out of 25. Over a one-year period, in a population of hyper-cholesterolaemic Eastern Finnish men, an inverse correlation was observed between plasma anti-oxidant levels (vitamin E, β- carotene, selenium) and the increase in carotid intimal-media thickness[18]. Similarly, in both men and women, an inverse relationship was found between dietary antioxidant intake (α-tocopherol, ascorbic acid, β-carotene) and carotid artery wall thickness[12].

To date, the precise mechanism(s) responsible for plaque regression have not been fully elucidated and appear to be complex and multifactorial. Earlier work from this laboratory documented the effective inhibition of platelet aggregation by antioxidants in hyperlipaemic patients[19]. After taking into account that one measure of the thrombogenicity of the blood was equally altered in both groups, and that the serum lipid profiles remained stable, it appears that the generation of serum TBARS data may provide a clue about the mode of action of palm oil antioxidants. Elegant studies aptly support the theory that antioxidants curtail the levels of blood lipid peroxides, and blunt the formation of atherosclerotic lesions. Esterbauer *et al*[4] demonstrated vitamin E to be very effective in preventing *in vitro* oxidation of LDL, and Carew *et al*[5] showed that the antioxidant probucol inhibited the progression of atherosclerotic lesions in LDL receptor-deficient rabbits.

Throughout this trial, we have observed no change in serum lipid levels. To the contrary, Qureshi *et al*[20,21] have reported a reduction in both serum total and LDL cholesterol in human hyperlipidaemics and pigs supplemented with tocotrienol. Wahlqvist *et al*[22], however, observed serum responses to both tocopherol and, to a lesser effect, tocotrienol supplementation in humans without any change in serum lipids. In preliminary trials with a rat model, we had observed a hypolipidemic effect with γ-tocotrienol[23]; however, our results in rats were not confirmed by the outcome in patients on uncontrolled diets who were taking a variety of medications. From the present data, it appears that a decrease in serum cholesterol was not a prerequisite in the eight patients suggested to have reduced carotid atherosclerosis. This result is in contrast to coronary atherosclerosis, as we reported earlier[24]. Cholesterol is, indeed, a poor predictor of carotid, compared to coronary, plaque thickness. Miettenen *et al*[25] have reported that although no significant decrease in coronary risk was measured when clofibrate was given with probucol, no stroke cases were observed in the 1200-subject study. Mao *et al*[6] demonstrated that the attenuation of atherosclerotic lesions in Watanabe rabbits by a probucol analogue was not contingent on a concomitant reduction in the serum cholesterol levels. Similarly, probucol reduced the incidence of atherosclerotic lesions in a group of Japanese hypercholesterolaemic quail without altering the plasma total or lipoprotein cholesterol levels[26]. These investigators attributed this effect to probucol's antioxidant properties. However, in view of the recent finding that probucol results in lowered HDL_{2b} levels in hypercholesterolemics[27], probucol use in atherosclerosis must be viewed with caution.

Our results highlight the potential of antioxidants in preventing, and possibly reversing, the natural course of carotid atherosclerosis.

Finally, two recent meta-analyses of randomised, controlled trials[28,29] found that lowering serum cholesterol through modified diets or medications did not reduce stroke mortality or morbidity in middle-aged men. These results were further substantiated by

Table 4. Response comparison of study groups to 24-mon antioxidant supplementation on serum cholesterol fractions and triglycerides.

Antioxidants							
Serum moiety (mM/L)	Baseline	3mon	6mon	9mon	12mon	18mon	24mon
Cholesterol	6.05±0.25	6.01±0.28	6.15±0.31	6.10±0.26	6.17±0.33	6.20±0.30	5.95±0.26
LDL-chol	4.27±0.21	4.27±0.27	4.39±0.30	4.25±0.22	4.39±0.29	4.42±0.29	4.11±0.21
HDL-chol	0.98±0.06	1.01±0.06	0.92±0.04	0.98±0.05	1.00±0.07	1.08±0.07	1.07±0.10
Triglyceride*	5.67±0.77	5-03±0.62	7.08±1.20	5.96±1.02	5.45±0.66	4.53±0.50	5.60±0.23
Placebo							
Cholesterol	5.90±0.33	5.86±0.21	5.68±0.28	6.03±0.27	5.67±0.26	5.92±0.02	5.77±0.23
LDL-chol	4-19±0.28	4.15±0.23	4.00±0.27	4.14±0.30	4.01±0.27	4.21±0.18	4.03±0.22
HDL-chol	1-10±0.05	1.12±0.07	1.10±0.05	1.10±0.06	1.15±0.06	1.04±0.05	1.15±0.09
Triglyceride*	4.71±0.82	3.70±0.31	4.92±0.90	5.20±1.03	3.85±0.36	4.20±0.36	4.36±0.15

LDL-chol, low density lipoprotein cholesterol; HDL-chol, high density lipoprotein cholesterol. Data expressed as mean ± SEM; ns, not significant.
* Average molecular weight 914 daltons.

an even more recent Swedish report[30] which showed no difference in carotid-intimal-media thickness or plaque status in a group with comprehensive risk reduction (including a 9% LDL cholesterol decrease) after 3.5 years of observation.

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生育三烯酚對高脂血症和頸動脈狹窄病人的抗氧化效果

摘要

本文研究了强化γ-生育三烯酚和α-生育酚棕櫚油對頸動脈硬化病人的抗氧化性能. 用50位腦血管疾病病人爲對象, 測定24個月的血清脂類, 脂肪酸過氧化物, 血小板凝集和頸動脈狹窄的變化, 用雙側超聲波技術測定頸動脈狹窄的改變. 該研究顯示25位給予生育三烯酚的明顯頸動脈硬化病人, 有8位頸動脈硬化消退, 有2位動脈硬化擴展, 但是在對照組則没有一位動脈硬化消退, 同時有10位動脈硬化擴展(P<0.01). 24個月后, 治療組血清中的硫巴比妥酸反應物質從1. 08±0. 14μm降至0. 80±0. 14μm (P,0.05), 對照組則從0. 99±0. 16μm增至1. 06±0. 17μm, 增加無顯著差异. 與實驗前比較, 生育三烯酚組和對照組顯示膠元引起的血小板凝集增加(P<0.05). 兩組的血清總膽固醇, 低密度脂蛋白膽固醇和甘油三酯值和血漿高密度脂蛋白膽固醇維持不變. 作者得出結論, 生育三烯酚似乎有益于頸動脈狹窄的緩解.

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