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EFFECTS OF THE ANTIOXIDANT TURMERIC ON LIPOPROTEIN PEROXIDES: IMPLICATIONS FOR THE PREVENTION OF ATHEROSCLEROSIS

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ABSTRACT

Extracts from the rhyzome of *Curcuma longa* are widely used as food additives in India and other Asiatic and Central American countries. It has been shown that these extracts ("turmeric"), as well as "curcumin" and related phenolic compounds isolated from *Curcuma*, have a powerful antioxidant action when tested in *in vitro* systems. Moreover, previous research from our laboratories has shown significant decreases in the levels of lipid peroxides in the blood of both mice and human subjects administered "turmeric." Our present research complements the previous data, showing that a daily intake of turmeric equivalent to 20 mg of the phenolic antioxidant curcumin for 60 days decreases the high levels of peroxidation of both the HDL and the LDL, *in vivo*, in 30 healthy volunteers ranging in age from 40 to 90 years. The effect was quite striking in the persons with high baseline values of peroxidized compounds in these lipoproteins, while no apparent change took place in the persons having low baseline values.

In view of current concepts on the atherogenic role played by peroxidized HDL, and especially by peroxidized LDL, as inducers of foam and smooth cell proliferation in the arterial wall, this preliminary experiment suggests that the *Curcuma* phenolic antioxidants, because of their high antioxidant activity and lack of toxicity, might be a useful complement to standard hypo-lipidemic drugs in the prevention and treatment of atherosclerosis.

KEYWORDS:

Atherosclerosis, atherogenesis, lipoperoxides, LDL, turmeric, curcuma.

INTRODUCTION

The progressive increase in the number of aged persons (1) has become a great burden for the health systems of the developed countries, because advanced age is often accompanied by degenerative diseases such as hypertension, atherosclerosis, dementia and certain types of cancer, which require long-term medical care and drug administration. Unfortunately, present day emphasis on the prevention and treatment of specific syndromes is not conducive to a successful control of the above disorders. By contrast, progress in the understanding of the cellular and molecular mechanisms of senescence will set the basis for prevention of "accelerated" or "pathological" aging, thus leading to significant increase in the health and quality of life of the aged.

Oxygen radical reactions and peroxidative damage to membranes (2-4) and mitochondria (5-8) seem to play a key role in cellular aging. On the other hand, dietary supplementation with antioxidants and free radical scavengers, such as vitamins C and E, thiol substances and a variety of plant products like nordihidroguaiaretic acid (a phenolic substance isolated from the creosote bush that was used in North American Indian popular medicine) may result in moderate gains in mean lifespan and some degree of protection against "oxidative degenerative disease" (9-11).

As previously reported (12), phenolic antioxidant products are attracting the attention of gerontologists, pharmacologists and other health scientists. The dried rhyzome of the ginger *Curcuma longa* (turmeric) and its principal phenolic compound curcumin are some with potential application. As noted elsewhere (13), *C. longa* (Zingiberaceae) is an herb widely cultivated in tropical areas of Asia and Central America. Its rhyzome, in powder form, is widely used as a food additive (for its flavor and for its yellow color) and has also been very popular in Asian folk medicine for treatment of conditions such as hepatic disorders and rheumathism. Curcuma extracts have a high anti-inflammatory activity after parenteral application in standard animal models of inflammation (12). In addition, turmeric and curcumin have been shown to protect against experimental tumorogenesis in mice (14) and reversed aflatoxin-induced liver damage (15).

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Relevant to the present work, products isolated from *C. longa* have been shown to have a powerful antioxidant effect when tested on the following models: oxidation of linoleic acid in air (16) and water-alcohol (17-18) and in vitro lipid peroxidation of rat brain (19).

The above justifies our work to investigate further the antioxidant action of curcuma extracts and their probable protective effect on the peroxidation of blood lipids that, according to current views, may play a key role in the pathogenesis of atherosclerosis (20,21). Previously, we have reported that a four-week treatment of mice with turmeric resulted in decreased levels of both plasma and liver lipid peroxides (12). Moreover, we have shown that a 45-day intake by healthy individuals of curcuma hydroalcoholic extract results in a significant decrease in the levels of serum lipid peroxides (13). The present research shows that, in addition to the above, the curcuma extract also has a marked lowering effect on the atherogenic peroxidation of HDL- and LDL-cholesterol of human subjects.

MATERIALS AND METHODS

The subjects were 30 volunteers (18 men and 12 women), ranging in age from 40 to 90 years, in apparent good health. After obtaining their informed consent, blood was taken from the anti-cubital vein and used for routine cytological evaluation (using the H-1 Technicon analyzer), and analysis of biochemical parameters was performed using the Boehringer-Mannheim standard methods and reagents and an autoanalyzer Hitachi 717. In addition, a biochemical evaluation of the liver function (determination of plasma levels of gamma-GT, GOT, GPT, alkaline phosphatase and bilirubin) and kidney function (urea and creatinine levels in plasma) was carried out in blood samples of subjects before and at the end of the turmeric treatment. The separation of the LDL and HDL fractions was performed by precipitation with phosphotungstic acid (22), and determination of lipoprotein peroxide levels was carried out according to the thiobarbituric method of Ohkawa et al. (23), acidifying with trichloroacetic acid and extracting the reaction products with N-butanol/pyridine. Then, the treatment was started by daily intake of two tablets of hydro-alcoholic extract of rhyzome of *C. longa*, containing approximately 10 mg of curcumin per tablet (supplied by A.S.A.C. Pharmaceutical International A.I.E., Alicante, Spain). The treatment lasted 60 days and the blood tests were repeated at day 60 after starting the treatment. Statistical analysis of the data was performed using a Student's t-test.

RESULTS AND DISCUSSION

The treatment did not result in any toxic response such as nausea, diarrhea or constipation. Further, there was no apparent detrimental effect on the liver, as shown by the lack of significant changes in the levels of total bilirubin (direct and indirect) nor in the activity of the enzymes GOT, GPT, GGT and alkaline phosphatase. All these parameters remained in the normal range at the completion of the treatment.

Both men and women with high initial levels of HDL- and LDL-peroxides showed a 25-50% significant decrease in these peroxides on the 60th day of treatment (HDL: 0.69 ± 0.82 vs. 0.29 ± 0.12; LDL: 0.50 ± 0.79 vs. 0.19 ± 0.12 [mean ± standard deviation; $p<0.05$]; Fig. 1). It appears that the peroxide-lowering effect of the treatment is more marked in those subjects showing high pre-treatment levels than in those with low levels at the start of the study. In these last subjects, the peroxides were not apparently affected by the treatment.

These preliminary clinical findings are interesting in relation to the above-mentioned recent research on the pathogenesis of atherosclerosis. As pointed out by Harman (24), this process, which sets the basis for many cardiovascular diseases that are the main cause of death in the developed countries, may be linked to the presence of lipid peroxides in the blood that injure the endothelium and trigger an inflammatory process in the arterial wall, in agreement with the fact that the intravenous injection of hydroperoxides of linoleic acid causes lesions in the intima of the rabbit aorta (25). Further support for the lipid peroxidation hypothesis of atherogenesis can be found in the finding of high levels of lipid peroxide in the blood of patients of ischemic heart disease and of subjects with such cardiovascular risk factors as smoking, stress, diastolic hypertension and advanced age (26,27). More specifically, it has been proposed that atherosclerosis is linked to the oxidation of LDL particles (particularly their polyunsaturated cholesterol esters) by the macrophages present in atheromatous plaques (21,28-30). Further, the oxidized LDL can contribute to atherogenesis through an increased production of foam and smooth muscle cells in the arterial wall and increased platelet aggregation that contributes to thrombus formation (29).

In view of the above, Johnson (31) concludes that an increased intake of antioxidants may protect against atherosclerosis. Accordingly, a study of the *Harvard School of Public Health* in over 120,000 persons in health-related professions shows that those that ingest high levels of antioxidants reduce their risk of suffering coronary disease up to 40% (32). Likewise, according to the Cambridge Heart Antioxidant Study (33), administration of a daily dose of 400-800 I.U. of vitamin E, a powerful dietary antioxidant, to over 2000 patients with angiographic evidence of coronary atherosclerosis seems to reduce the risk of non-fatal myocardial infarction.

A curcuma product, namely curcumin, may have a higher potential antioxidant activity than tocopherol for prevention of pathological lipid peroxidation, as suggested by recent work in which antioxidant activity has been evaluated by protecting against necrosis in the rat skin flap model (34). This, in addition to the fact that the products extracted from curcuma are approved for human consumption as food additives, justifies further work to investigate their effects on hyperlipidemic pa-

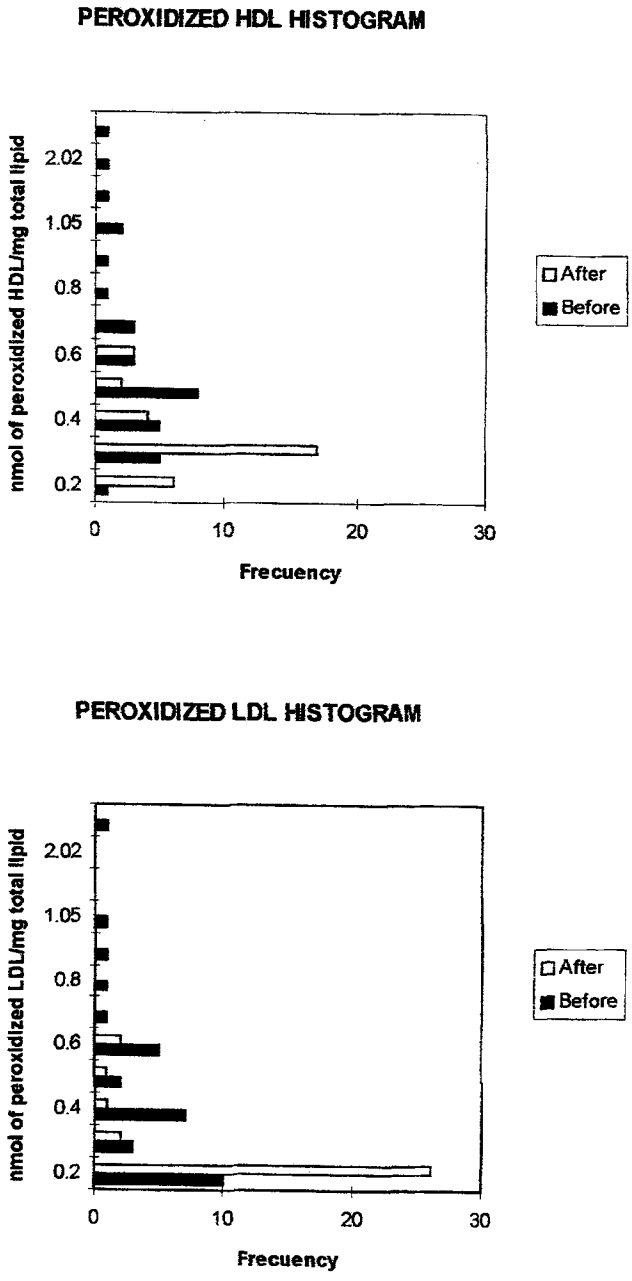

Figure 1. Administration of turmeric at a daily dose equivalent to 20 mg of the antioxidant curcumin to healthy volunteers, during two months, results in a statistically significant decrease in both peroxidized HDL- and LDL-cholesterol. This may protect the blood vessel walls against the oxidant stress that plays a key role in atherogenesis and cardiovascular disease.

tients, as an adjunct to the treatment of these patients with lipid-lowering drugs. The curcuma extracts might be useful anti-atherogenic agents not only in the hyperlipidemias but also in persons showing high levels of lipid peroxidation in their blood as the result of genetic factors or environmental stress.

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