

An hydroalcoholic extract of *Curcuma longa* lowers the apo B/apo A ratio Implications for atherogenesis prevention

Ana Ramírez-Boscá^{a,*}, Alfonso Soler^b, Miguel A. Carrión^a,
Joaquín Díaz-Alperi^a, August Bernd^c, Cesar Quintanilla^a,
Eliseo Quintanilla Almagro^a, Jaime Miquel^d,

^a A.S.A.C. Pharmaceutical International A.I.E., C/ Sagitario 14, 03006 Alicante, Spain

^b Ibermútua, Alicante, Spain

^c Department of Dermatology, J.W. Goethe University, Germany

^d Department of Biotechnology, University of Alicante, San Vicente, Alicante, Spain

Received 10 March 2000; received in revised form 17 July 2000; accepted 19 July 2000

Abstract

It is generally accepted that free-radical induced blood lipid peroxidation and especially peroxidized LDL play a central role in the pathogenesis of atherosclerosis and related cardiovascular disease. Moreover, recent research highlights the key contribution of apolipoprotein B (apo B) to atherogenesis as the main inductor of one of its earlier steps, i.e. macrophage proliferation. This has led us to investigate the apo B response to a very effective phenolic lipid-antioxidant, namely an hydroalcoholic extract of *Curcuma longa*, which according to our previous work does not show any toxic effects and decreases the levels of blood lipid peroxides, oxidized lipoproteins and fibrinogen. The present study shows that a daily oral administration of the extract decreases significantly the LDL and apo B and increases the HDL and apo A of healthy subjects. This and recent data on the increased anti-atherogenic action of the physiological antioxidant tocopherol in the presence of phenolic co-antioxidants (which eliminate the tocopheroxyl radical), justifies planned clinical research to test the usefulness of the curcuma extract as a co-antioxidant complement to standard treatments to prevent or retard atherosclerosis. © 2000 Published by Elsevier Science Ireland Ltd.

Keywords: *Curcuma longa*; Antioxidant; Apolipoprotein B/A

* Corresponding author.

1. Introduction

Although the mortality due to atherosclerosis and related cardiovascular diseases (CVDs) has decreased in the last decade, these diseases are still the main cause of death in the industrialized countries. Thus, according to the American Heart Association (1995), ~40% of the American population dies of some form of CVD, a fact that has prompted intensive research on the altered oxidative, inflammatory and cellular-proliferation mechanisms responsible for the atherosclerotic process.

There is an increasing acceptance of the concept that oxidized LDL (oxLDL) is an essential contributor to the formation of atherosclerotic lesions (Haberland et al., 1988; Palinski et al., 1989; Witzum, 1994; Tangirala et al., 1995). This agrees with the finding that LDL from those lesions shows the physical and biological properties of oxLDL (Ylä-Herttua et al., 1989), that there are circulating autoantibodies against oxLDL (Palinski et al., 1990), and that administration of some antioxidants protects against atherosclerosis in animal models (Kita et al., 1987; Bjorkham et al., 1991; Sparrow et al., 1992).

Presently, much work is focused on the relation of atherogenesis with the apolipoproteins A and B (Apo A, Apo B). Apo A plays a key role in the metabolism of HDL-cholesterol, which is esterified in the bloodstream by lecithin cholesterol acetyltransferase, using Apo A as a cofactor, and then returns to the liver for excretion as bile acids or redistribution to other tissues. Since high levels of Apo A are accompanied by high concentrations of the oxidation-resistant HDL, Apo A is thought to be a marker of adequate anti-atherogenic defense. By contrast, apo B is associated with the LDL, which plays a central role in the uptake of cholesterol-rich LDL particles by peripheral tissues and liver. A high concentration of LDL (and therefore of apo B), is atherogenic, since it is ingested by macrophages, thus producing foamy cells (Chaput et al., 1999; Hashimoto et al., 2000). LDL is also involved in other pathological processes such as up-regulation of adhesion molecule expression, attachment to endothelial cells, migration and subendothelial localization of macrophages, recruitment of smooth muscle cells and platelet activation, with resulting risk of thrombosis (Ross, 1995; Witting et al., 1999). As pointed out by Martens et al. (1999), oxidatively modified apo B plays a central role in the above mechanisms, since it is the main macrophage-proliferation inducing factor.

The above as well as recent research showing that there is a correlation between high levels of apo B in serum and cardiovascular disease (Zhang et al., 1992; Yamamoto et al., 1999) justify the determination of this apolipoprotein in order to evaluate more accurately the atherosclerosis-dependent health risk. The apo B analysis is especially useful in subjects with a hyper-LDL lipidemia, an early cardiovascular or cerebrovascular disease or a family history of atherosclerosis. It should be noted that the apo B/apo A ratio is a more adequate predictor of

atherosclerotic risk than the individual values of each of these apolipoproteins, because atherosclerosis is usually accompanied by an increase in apo B and a decrease in apo A. Therefore, minor pathological changes in these apolipoproteins may become more evident by determination of the above ratio.

In our opinion, the above justifies the present work to test the response of apo A and apo B to diet supplementation with a hydroalcoholic extract of *Curcuma longa* (Masuda et al., 1992), an antioxidant product which according to previous studies may have an anti-atherogenic action, as suggested by its lowering effects on blood cholesterol, triglycerides and phospholipids (Suresh Babu and Srinivasan, 1997) as well as on lipid peroxides (Miquel et al., 1995; Ramírez-Boscá et al., 1995), lipoprotein peroxides (Ramírez-Boscá et al., 1997) and fibrinogen (Ramírez-Boscá et al., 2000).

2. Materials and methods

The initial study group was composed of 30 subjects (16 men and 14 women) ranging in age from 24 to 70 years. They were in good health, held managerial or scientific/technical jobs and fulfilled the following inclusion criteria: (a) no recent infection disease in the last 6 weeks; (b) no gastrointestinal disorders resulting in malabsorption; (c) no intake of thyroxine, estrogen, oral contraceptives, hypolipidemic drugs or antioxidant supplements for the past 6 months; (d) normal blood count and hepatic and renal function. Informed consent was obtained and, right before the start of the study, blood was taken from the cubital vein after overnight fasting, and HDL was determined in serum by the method of Kakuyami et al. (1994), LDL using the equation of Fredewald, and apo A and apo B by the nephelometric method of the 'International Federation of Clinical Chemistry'. From the initial group, 12 men ranging in age from 43 to 70 years were chosen for the treatment on the basis of their high values of LDL (i.e. over 150 mg/dl). As in our previous research on the effects of curcuma extract on lipid peroxides, lipoprotein peroxides and fibrinogen (Ramírez-Boscá et al., 1995, 1997, 2000), the treatment consisted in the daily intake of two tablets of a hydro-alcoholic extract of rhizome of *C. longa* containing ~10 mg of curcumin per tablet (supplied by A.S.A.C. Pharmaceutical International AIE, Alicante, Spain). At the end of the 30-day period of treatment the above blood tests were carried out again. The data were expressed as mean \pm SD of the individual values and, because of their non-parametric distribution, their statistical evaluation was performed by the Mann-Whitney paired test, using 0.05 as the level of significance..

3. Results and discussion

The administration of the curcuma extract did not result in any side effects such as nausea, diarrhoea or constipation throughout the course of the treatment. As shown in Table 1, the treatment caused a decrease in the levels of LDL and apo B

accompanied by a rise in HDL and apo A. Further, there was a striking decrease in the mean apo B/apo A ratio after the 30-day curcuma extract administration.

The apparent lack of toxicity of the curcuma extract is in agreement with the result of our previous study on lipoprotein peroxides (Ramírez-Boscá et al., 1997). In that study no toxic reactions to the treatment were found by biochemical evaluation of liver function (determination of plasma levels of gamma-GT, GOT, GPT, alkaline phosphatase and bilirubin) and kidney function (urea and creatine levels in plasma). The lack of toxicity of the extract used in our study is not surprising, since similar curcuma products have been approved for human consumption as widely used food condiments.

The curcuma induced decrease of apo B is interesting in relation to the above mentioned LDL-oxidation hypothesis of atherosclerosis according to which atheroma formation is linked to an increase in circulating apo B (Chaput et al., 1999), as well as to current attempts to prevent or retard atherogenesis by antioxidant supplementation (Johnson, 1993). The clinical use of antioxidants in the prevention of the early stages of atherosclerosis and associated CVDs may be defended on the grounds that oxygen stress appears to be involved not only in atherogenesis but also in a number of related risk factors and diseases such as smoking and stress (Santos et al., 1989), hypercholesterolemia (Lavy et al., 1991), hypertension (Keidar et al., 1994), diabetes (Yagi, 1989; Beaudeau et al., 1995), renal failure (Sutherland et al., 1995) and advanced age (Yagi, 1989; Miquel et al., 1998). The probable beneficial effects of antioxidants in the prevention of oxygen-stress related pathologies have been shown by a study of the Harvard School of Public Health on over 120,000 health-related professionals according to which the ingestion of high levels of antioxidants reduced the risk of suffering coronary disease up to 40% (Rimm et al., 1993). Likewise, according to the Cambridge Heart Antioxidant Study (Stephens et al., 1996), administration of a daily dose of 400–800 I.U. of the lipid-protecting antioxidant vitamin E to over 2000 patients with angiographic evidence of coronary atherosclerosis seemed to reduce the risk of non-fatal myocardial infection.

Table 1

Effects of the treatment with curcuma extract on the HDL, LDL, apolipoprotein A (apo A) and apolipoprotein B (apo B)^{a,b}

Parameter	Before	After	Significance ^c
HDL	45.62 ± 18.38 (8)	78.62 ± 29.63 (8)	<i>P</i> < 0.01
LDL	190.12 ± 23.62 (8)	117.12 ± 73.34 (8)	<i>P</i> < 0.01
Apo A	126.25 ± 28.04 (8)	156.62 ± 45.16 (8)	<i>P</i> < 0.01
Apo B	109.25 ± 22.47 (8)	90.75 ± 59.61(8)	<i>P</i> < 0.05
Apo B/Apo A	93.83 ± 19.14 (8)	62.50 ± 27.56 (8)	<i>P</i> < 0.01

^a Values in mg/dl of serum before and after a 30-day treatment (*n*).

^b Values are expressed as mean ± SD.

^c Statistical significance of the difference between the before and the after treatment values (paired Mann-Whitney U test). The data show a favorable effect of the treatment on all measured parameters.

Although a considerable amount of data supports the key role of tocopherol as the main physiological antioxidant protecting the unsaturated lipids, Witting et al. (1999) pointed out that, surprisingly, oxidized lipids coexist with relatively normal concentrations of alpha-tocopherol, the major endogenous antioxidant associated with LDL. They further state that LDL lipids are most effectively protected from oxidation in the presence of both tocopherol and reducing phenolic compounds, which ‘export’ the radicals from oxidizing LDL particles into the aqueous phase and convert them into non-radical products. Witting et al. (1999) conclude that ‘if arterial LDL causes atherosclerosis, co-antioxidants may be antiatherosclerotic’. This is in agreement with their study showing a protective action of phenolic antioxidants against aortic on lipoprotein peroxidation and atherosclerosis in apolipoprotein E and low density lipoprotein receptor gene double knockout mice. Likewise, the main phenolic antioxidant present in the curcuma extract, namely curcumin, may act as a co-antioxidant that potentiates the effects of vitamin E. In fact, curcumin may have a higher antioxidant power than tocopherol for prevention of pathological lipid peroxidation, as suggested by a comparative study of the respective protective effects of these two compounds against necrosis in the rat skin flap model (Faramarz et al., 1994). Moreover, the administration of curcumin to rats in which diabetes had been induced by streptozotocin decreased the blood levels of LDL and VLDL cholesterol, triglycerides and phospholipids (Suresh Babu and Srinivasan, 1997), whereas an hydroalcoholic extract of the rhizome of *C. longa* administered to rabbits also showed hypocholesterolemic effects and decreased the susceptibility of plasma LDL to oxidation (Ramírez-Tortosa et al., 1999).

The above, in addition to our present apolipoprotein data and previous finding of a lowering effect of the curcuma extract on blood lipid peroxide, lipoprotein peroxide and fibrinogen (Ramírez-Boscá et al., 1995, 1997, 2000) justifies further work to investigate the effects of this extract on hyperlipidemic patients as a complement to the treatment of these patients with drugs and diet supplementation with physiological antioxidants. The curcuma lipid-lowering extract might be especially useful as an anti-atherogenic agent in those conditions accompanied by a marked increase in the level of blood lipid peroxidation such as miocardial infarction (Santos et al., 1989), diabetes (Yagi, 1989) and dislipemias in women, after menopause (Miquel et al., 1998).

References

- American Heart Association, 1995. Heart and Stroke Facts Statistical Supplement, 2–15.
- Beaudeau, J.L., Guillausseau, P.J., Peynet, J., Flourie, F., Assayag, M., Tielmans, D., Warnet, A., Rousselet, F., 1995. Enhanced susceptibility of low-density lipoprotein to in vitro oxidation in type 1 and type 2 diabetic patients. *Clin. Chim. Acta* 239, 131–141.
- Bjorkham, I., Henriksson-Freyschuss, A., Breuer, O., Diczfalusy, U., Berglund, L., Henrikson, P., 1991. The antioxidant butylated hydroxytoluene protects against atherosclerosis. *Arterioscler. Thromb.* 11, 15–22.

- Chaput, E., Maubrou-Sanchez, D., Bellamy, F.D., Edgar, A.D., 1999. Fenofibrate protects lipoproteins from lipid peroxidation: synergistic interaction with alpha-tocopherol. *Lipids* 34, 497–502.
- Faramarz, A., Suzuki, S., Hua-Zhou, J., Isshiki, N., Miyachi, Y., 1994. Involvement of lipid peroxidation in necrosis of skin flaps and its suppression by ellagic acid. *Plast. Reconstr. Surg.* 94, 1027–1034.
- Haberland, M.E., Fong, D., Cheng, I., 1988. Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science* 241, 215–218.
- Hashimoto, R., Narita, S., Yamada, Y., Tanaka, K., Kojo, S., 2000. Unusually high reactivity of apolipoprotein B-100 among proteins to radical reactions induced in human plasma. *Biochem. Biophys. Acta* 1483, 236–240.
- Johnson, E.J., 1993. Aging and carotene nutrition. *Age* 16, 59–66.
- Kakuyami et al., 1994.
- Keidar, S., Kaplan, M., Shapira, C., Brook, J.G., Aviram, M., 1994. Low density lipoprotein isolated from patients with essential hypertension exhibits increased propensity for oxidation and enhanced uptake by macrophages: A possible role for angiotensin II. *Atherosclerosis* 107, 71–84.
- Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N., Ooshima, A., Yoshida, H., Kawai, C., 1987. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA* 84, 5928–5931.
- Lavy, A., Brook, G.J., Dankner, G., Ben Amotz, A., Aviram, M., 1991. Enhanced in vitro oxidation of plasma lipoproteins derived from hypercholesterolemic patients. *Metabolism* 40, 794–799.
- Martens, J.S., Lougheed, M., Gomez-Muñoz, A., Steinbrecher, U.P., 1999. A modification of apolipoprotein B accounts for most of the induction of macrophage growth by oxidized low density lipoprotein. *J. Biol. Chem.* 274, 10903–10910.
- Masuda, T., Isobe, J., Jitoe, A., Nakatani, N., 1992. Antioxidative curcuminoids from rhizomes of *Curcuma xanthorrhiza*. *Phytochemistry* 31, 3645–3647.
- Miquel, J., Martínez, M., Díez, A., De Juan, E., Soler, A., Ramírez, A., Laborda, J., Carrión, M., 1995. Effects of turmeric on blood and liver lipoperoxide levels of mice: lack of toxicity. *Age* 18, 171–174.
- Miquel, J., Ramírez-Boscá, A., Soler, A., Díez, A., Carrión-Gutiérrez, A., Díez-Alperi, J., Quintanilla-Ripoll, E., Bernd, A., Quintanilla-Almagro, E., 1998. Increase with age of serum lipid peroxides. *Mech. Ageing Dev.* 100, 17–24.
- Palinski, W., Rosenfeld, M.E., Ylä-Herttuala, S., Gurtner, G.C., Socher, S.A., Butler, S., Parthasarathy, S., Carew, T.E., Steinberg, D., Witztum, J.L., 1989. Low density lipoprotein undergoes oxidative modification in vivo. *Proc. Natl. Acad. Sci. USA* 86, 1372–1376.
- Palinski, W., Ylä-Herttuala, S., Rosenfeld, M.E., Butler, S., Socher, S.A., Parthasarathy, S., Curtiss, L.K., Witztum, J.L., 1990. *Arteriosclerosis* 10, 325–335.
- Ramírez-Boscá, A., Soler, A., Carrión Gutiérrez, M.A., Laborda Alvarez, A., Quintanilla Almagro, E., 1995. Antioxidant curcuma extracts decrease the blood lipid peroxide levels of human subjects. *Age* 18, 167–169.
- Ramírez-Boscá, A., Carrión Gutiérrez, M.A., Soler, A., Puerta, C., Díez, A., Quintanilla, E., Bernd, A., Miquel, J., 1997. Effects of the antioxidant turmeric on lipoprotein peroxides: implications for the prevention of atherosclerosis. *Age* 20, 165–168.
- Ramírez-Boscá, A., Soler, A., Carrión-Gutiérrez, M.A., Pamies Mira, D., Pardo Zapata, J., Díaz-Alperi, J., Bernd, A., Quintanilla Almagro, E., Miquel, J., 2000. An hydroalcoholic extract of *Curcuma longa* lowers the abnormally high values of human-plasma fibrinogen. *Mech. Ageing Dev.* 114, 207–210.
- Ramírez-Tortosa, M.C., Mesa, M.D., Aguilera, M.C., Quijiles, L., Baro, L., Ramírez-Tortosa, C.L., Martínez-Victoria, C., Gil, A., 1999. Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. *Atherosclerosis* 147, 371–378.
- Rimm, E.B., Stampfer, M.J., Aschario, A., Giovannucci, G.A., Colditz, B., Willett, W., 1993. Vitamin E consumption and the risk of coronary heart disease in men. *N. Engl. J. Med.* 328, 1450–1456.
- Ross, R., 1995. Cell biology of atherosclerosis. *Ann. Rev. Physiol.* 57, 791–804.
- Santos, M.T., Vallés, J., Aznar, J., 1989. Plasma lipid peroxides in patients with vascular disease and in middle-aged normal subjects with a high risk of atherosclerosis. In: Miquel, J., Quintanilha, A., Weber, H. (Eds.), *CRC Handbook of Free Radicals and Antioxidants in Biomedicine*, vol. 1. CRC Press, Boca Raton, FL, pp. 237–254.

- Sparrow, C.P., Doebber, T.W., Olszewski, J., Wu, M.S., Ventre, J., Stevens, K.A., Chao, Y.S., 1992. Low density lipoprotein is protected from oxidation and the progression of atherosclerosis is slowed in cholesterol-fed rabbits by the antioxidant *N,N'*-diphenyl-phenylenediamine. *J. Clin. Invest.* 89, 1885–1891.
- Stephens, N.G., Parson, A., Schofield, P.M., Kelly, F., Cheeseman, K., Mitchinson, M.J., 1996. Randomized controlled trial of vitamin E in patients with coronary disease: Cambridge heart antioxidant study (CHAOS). *Lancet* 347, 781–786.
- Suresh Babu, P., Srinivasan, K., 1997. Hypolipidemic action of curcumin, the active principle of turmeric (*Curcuma longa*) in streptozotocin induced diabetic rats. *Mol. Cell. Biochem.* 166, 169–175.
- Sutherland, W.H., Walker, R.J., Ball, M.J., Stapley, S.A., Robertson, M.C., 1995. Oxidation of low density lipoproteins from patients with renal failure or renal transplants. *Kidney Int.* 48, 227–236.
- Tangirala, R.K., Casanada, F., Miller, E., Witztum, J.L., Steinberg, D., Palinski, W., 1995. Effect of the antioxidant *N,N'*-diphenyl 1,4-phenylene diamine (DPPD) on atherosclerosis in apoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 15, 1625–1630.
- Witting, P.K., Pettersson, K., Östlund-Lindqvist, A.M., 1999. Inhibition by a coantioxidant of aortic lipoprotein lipid peroxidation and atherosclerosis in apolipoprotein E and low density lipoprotein receptor gene double knockout mice. *FASEB J.* 13, 667–675.
- Witztum, J.L., 1994. The oxidation hypothesis of atherosclerosis. *Lancet* 344, 793–795.
- Yagi, K., 1989. Serum lipid peroxides in subjects having certain diseases and ageing. In: Miquel, J., Quintanilha, A., Weber, H. (Eds.), *CRC Handbook of Free Radicals and Antioxidants in Biomedicine*, vol. 1. CRC Press, Boca Raton, FL, pp. 223–230.
- Yamamoto, A., Horibe, H., Mabuchi, H., Kita, T., Matsuzawa, Y., Saito, Y., Nayaka, N., Fujioka, T., Tenba, H., Kawaguchi, A., Nakamura, H., Goto, Y., 1999. Analysis of serum lipid levels in Japanese men and women according to body mass index. Increase in risk of atherosclerosis in postmenopausal women. Research Group on Serum Lipid Survey 1990 in Japan. *Atherosclerosis* 143, 55–73.
- Ylä-Herttuala, S., Palinski, W., Rosenfeld, M.E., Parthasarathy, S., Carew, T.E., Butler, S., Witztum, J.L., Steinberg, D., 1989. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J. Clin. Invest.* 84, 1086–1095.
- Zhang, S.H., Reddick, R.L., Piedrahita, J.A., Maeda, N., 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258, 468–471.