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INFLUENCE OF PALM OIL OR ITS TOCOTRIENOL-RICH FRACTION ON THE LIPID PEROXIDATION POTENTIAL OF RAT LIVER MITOCHONDRIA AND MICROSOMES

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Summary: The effect of palm oil, a widely used vegetable oil, rich in tocotrienols, on peroxidation potential of rat liver was examined. Long-term feeding of rats with palm oil as one of the dietary components significantly reduced the peroxidation potential of hepatic mitochondria and microsomes. As compared to hepatic mitochondria isolated from rats fed control or corn oil-rich diet, those from palm oil-fed group showed significantly less susceptibility to peroxidation induced by ascorbate and NADPH. However, in microsomes, only NADPH-induced lipid peroxidation was significantly reduced in rats fed palm oil rich-diet. Though the accumulation of thiobarbituric acid reactive substances during ascorbate-induced lipid peroxidation in mitochondria from rats fed corn oil-rich diet supplemented with tocotrienol-rich fraction (TRF) of palm oil was similar to that of control rats, the initial rate of peroxidation was much slower than those from control or corn oil fed diets. Our *in vitro* studies as well as analyses of co-factors related to peroxidation potential indicated that the observed decrease in palm oil-fed rats may be due to increased amount of antioxidants in terms of tocotrienol as well as decrease in the availability of substrates for peroxidation.

Introduction

In recent years there has been considerable interest in the role of diet and nutrition related to human health. One of the areas which has attracted a great deal of attention is antioxidant nutrition in the control of degenerative diseases such as atherosclerosis and cancer (1,2). Peroxidation of the cellular membrane lipids is a basic reaction which results in the deterioration of unsaturated fatty acids in the membrane. This process has been implicated in several human diseases and in the toxicity of xenobiotics (3-5). If lipid peroxidation is

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triggered, it can inactivate cellular components and its products can have serious consequences on almost all the crucial molecules leading to diseased conditions (4,5). The peroxidation products can also cause the formation of 8-hydroxydeoxyguanosine whose presence in the genetic material can lead to mutagenesis and carcinogenesis (6,7).

The susceptibility of tissues to lipid peroxidation is influenced by the lipid and antioxidant composition of cellular membranes which in turn may be controlled by the dietary composition (8,9). Palm oil, an edible vegetable oil, forms an important component of the human diet in different parts of the world. It has 50% saturated, 40% monounsaturated and 10% polyunsaturated fatty acids besides an abundant amount (approximately 1000 ppm) of antioxidants in the form of vitamin E group of compounds consisting of tocotrienols and tocopherols (10,11). Unrefined palm oil is also rich in carotenoids, another group of dietary antioxidants (2,12). Recent studies have shown that palm oil and/or its antioxidant constituents can help in the control of diseases like atherosclerosis and some forms of cancer (2,13,14). Whereas, the exact mechanisms responsible for such beneficial effects of palm oil are not properly understood, antioxidants are believed to be implicated. The present study is an attempt in this direction and shows that long-term feeding of rats with palm oil or in some cases diet supplemented with its tocotrienol-rich fraction (TRF) can decrease the peroxidation potential of hepatic mitochondria and microsomes.

Materials and Methods

Female Sprague-Dawley rats were obtained from the Animal Breeding Unit, University of Malaya at 45 days of age. They were housed in temperature and light-dark controlled rooms with food and water ad libitum. Eighty rats were divided into four groups and were fed control diet (low fat-diet) consisting of casein (28.0%), dextrose (55.0%), fat (5%), cellulose (6.0%), vitamin mix (1.0%, AIN76), salt mix (4.5%, AIN76), methionine (0.3) and choline bitartarate (0.2%). The other three groups were fed with high fat-diet (having 20% fat) consisting of 28% casein, 40% dextrose, 6% cellulose, 1% vitamin mix, 4.5% salt mix, 0.3% methionine, 0.2% choline bitartarate and 20% fat. The latter can be either refined, bleached and deodorised palm oil (RBDPO), refined corn oil or corn oil supplemented with 1000 ppm TRF of palm oil. Chemicals used in our studies were obtained from either Sigma Chemical Co., St. Louis, U.S.A. or E.Merck, Germany.

Azobis(2-amidopropane)dihydrochloride (AAPH) was a gift from Prof. Lester Packer, University of California.

After feeding the rats for 20 weeks with respective diets, they were killed by cervical dislocation, livers excised, pooled and stored at -70°C until use. These tissue samples were homogenised in 0.25 M sucrose containing 0.1 mM EDTA. The homogenate was spun at 650 x g for 10 min to remove nuclei and cell debris. The resultant supernatant was centrifuged at 10,000 x g for 30 min to sediment mitochondria. Post mitochondrial supernatant was centrifuged at 105,000 x g for 60 min to sediment microsomes. Both mitochondria and microsomes were washed free of sucrose in 0.15 M Tris-HCl buffer, pH 7.4. Protein from these fractions was estimated and samples were suspended in the above buffer at a concentration of 5 mg protein/ml, distributed into small aliquots, frozen in liquid nitrogen and stored at -70°C. These fractions were never stored for more than a week.

The lipid peroxidation potential, as studied by ascorbate- and NADPH- induced systems and measured by the formation of thiobarbituric acid reactive substances (TBARS), was assayed as described previously (15,16). Lipid peroxidation induced by the peroxyl radical initiator AAPH, was carried out by incubating 10 mM of this compound with tissue fractions in 0.15 M Tris-HCl buffer, pH 7.4, at 37°C and estimating the TBARS formed. Phospholipid and glutathione contents were estimated by standard methods as quoted in an earlier reference (15). Results were analyzed statistically using Student's **t** test.

Results and Discussion

Data related to the influence of different diets on the peroxidation potential of rat liver mitochondria and microsomes are given in Fig.1. Basal lipid peroxidation, as measured by the TBARS formed by incubation without the addition of exogenous cofactors, is a reflection of conditions *in vivo* which determine the peroxidizability of cellular membranes. In both hepatic microsomes and mitochondria corn oil- as well as corn oil+TRF -rich diets significantly increased basal lipid peroxidation whereas palm oil-rich diet as well as low-fat control diet had no effect. Ascorbate- and NADPH-induced systems are two of the dominant systems which induce lipid peroxidation in tissues by non-enzymatic and enzymatic means respectively (17,18). The susceptibilities of hepatic mitochondria from control rats to these two systems of peroxidation were similar. However, liver microsomes were more susceptible to the enzymatic NADPH-induced lipid peroxidation than that induced by ascorbate. Palm oil feeding significantly decreased the peroxidation potential of mitochondria with both ascorbate- and NADPH-induced systems

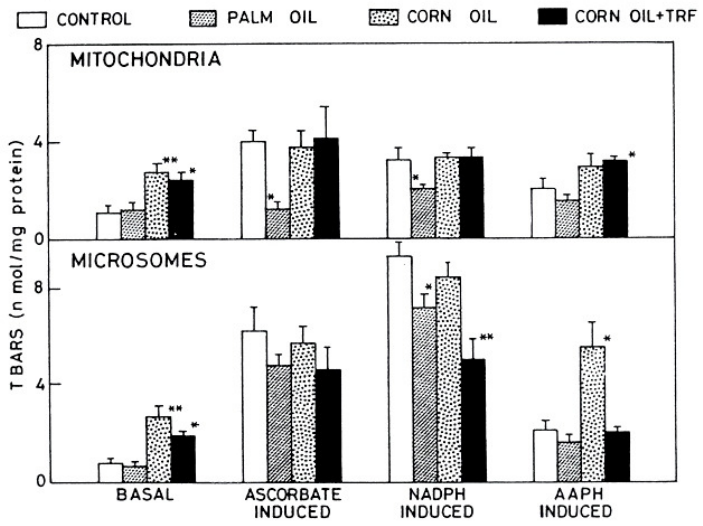

Fig.1. Effect of feeding palm oil, corn oil or corn oil + tocotrienol rich fraction (TRF) on the peroxidation potential of rat hepatic microsomes and mitochondria. Thiobarbituric acid reactive substances (TBARS) were estimated as malonaldehyde equivalents. Basal lipid peroxidation was carried out by incubating the tissue fractions in Tris-HCl buffer, pH 7.4 for 60 min. Incubations, as mentioned in the "Materials and Methods", were carried out at 37°C in a shaking water bath. Values represented are mean ± S.E. from 4 batches of 4 rats each. * $P < 0.05$, ** $P < 0.01$, as compared to respective controls.

whereas corn oil or corn oil+TRF diets did not show any significant effect. In microsomes, however, palm oil-rich diet decreased only the NADPH-induced lipid peroxidation. Though corn oil-rich diet did not have any significant influence on ascorbate- or NADPH-induced lipid peroxidation in microsomes and mitochondria, it significantly increased AAPH-induced lipid peroxidation in microsomes which involves the initiation of peroxidation by peroxyl radicals (19). Hence in both mitochondria and microsomes the basal lipid peroxidation as well as peroxidation induced by three different systems were either significantly decreased or remained unaffected by palm oil-rich diet.

Lipid peroxidation in cellular membranes is controlled by the amount of substrate availability in the form of unsaturated fatty acids present in phospholipids, inducers in the form of free radicals and excited states which are capable of abstracting an allelic hydrogen atom adjacent to the unsaturated double bond,

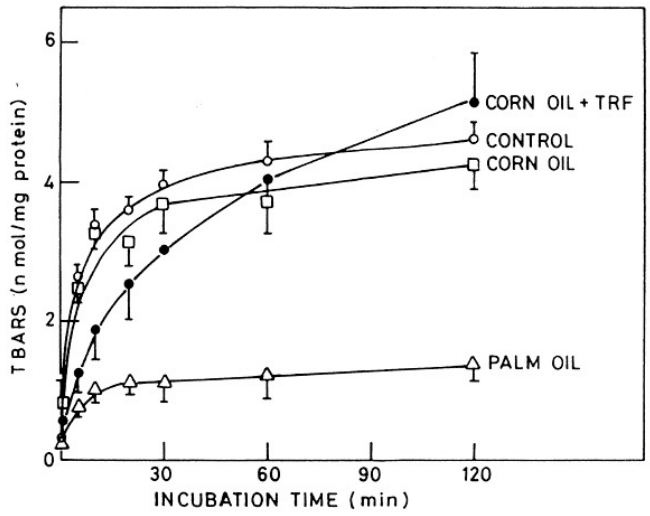

Fig.2. Ascorbate-induced lipid peroxidation in rat liver mitochondria as a function of incubation time in rats fed control ○ , palm oil △ , corn oil □ or corn oil + tocotrienol rich fraction (TRF) ● . Details as per legend to Fig. 1.

besides the enzymatic and non enzymatic antioxidants (4,20,21). It is likely that corn oil and corn oil+TRF diets adversely alter one or more of these factors thereby causing increase in the basal lipid peroxidation. Earlier studies have shown that the vitamin E content of tissue fractions from rats fed palm oil-rich diets was significantly increased (2). Corn oil contains less of vitamin E (800 ppm) than palm oil (1000 ppm). Besides, most of the vitamin E in the former is in the form of tocopherol whereas palm oil contains equal quantities of both tocopherols and tocotrienols (10). These unsaturated derivatives of tocopherols i.e. tocotrienols, possess more potent free radical scavenging activity in vitro (22). Feeding of rats or hamsters with palm oil-rich diet or TRF-rich diet has been shown to increase the amount of tocotrienols in liver (23,24).

Fig.2 shows the time course of ascorbate-induced lipid peroxidation in mitochondria isolated from rats fed different diets. Compared to other groups, mitochondria from palm oil-fed rats showed significantly lower rate of peroxidation as well as lesser accumulation of TBARS. The mitochondria from corn oil+TRF fed rats showed an intermediate rate of peroxidation in the

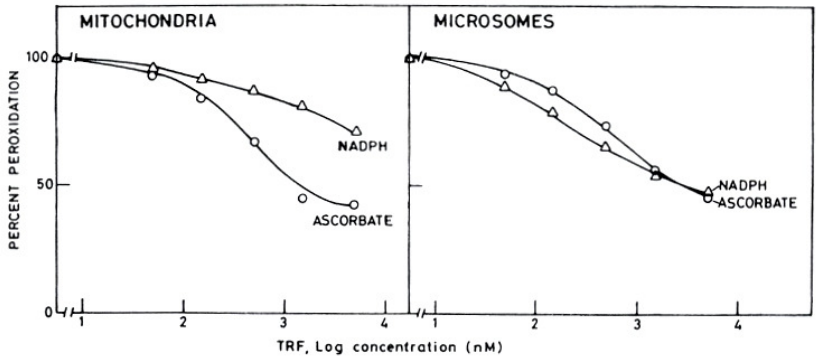

Fig.3. Effect of tocotrienol rich fraction (TRF) in vitro on the peroxidation potential of rat hepatic mitochondria and microsomes. Cell fractions from control rats were incubated with cofactors necessary for the NADPH- △ and ascorbate-induced- ○ systems in the presence of varying amounts of TRF at 37°C for 60 min. Each value represents mean of 4 experiments. The respective values obtained without the addition of TRF were taken as 100 percent.

initial stages but the accumulation of TBARS is similar to that of mitochondria from control or corn oil fed rats where highest rates were observed. The increase in the level of antioxidants probably in the form of tocotrienol in the mitochondria from corn oil+TRF fed rats may be responsible for the observed initial lower rates of peroxidation. Earlier studies have shown that dietary TRF enhances tocotrienol content of liver in rats (23). The subcellular distribution of this antioxidant, however, is not clear.

Results on the in vitro effect of TRF on peroxidation potential with ascorbate- and NADPH-induced systems are given in Fig.3. TRF inhibited lipid peroxidation in both mitochondria and microsomes in a dose-dependent manner. However, the responses differ in the two subcellular fractions. In mitochondria, TRF was more effective against ascorbate-induced lipid peroxidation than that induced by NADPH, whereas in microsomes both systems responded similarly to inhibition by this antioxidant. This may be because of the difference in the rates of peroxidation induced at the time point (60 min) used for comparison. TRF gave significant protection even at relatively low concentrations, [nM], in both the subcellular fractions.

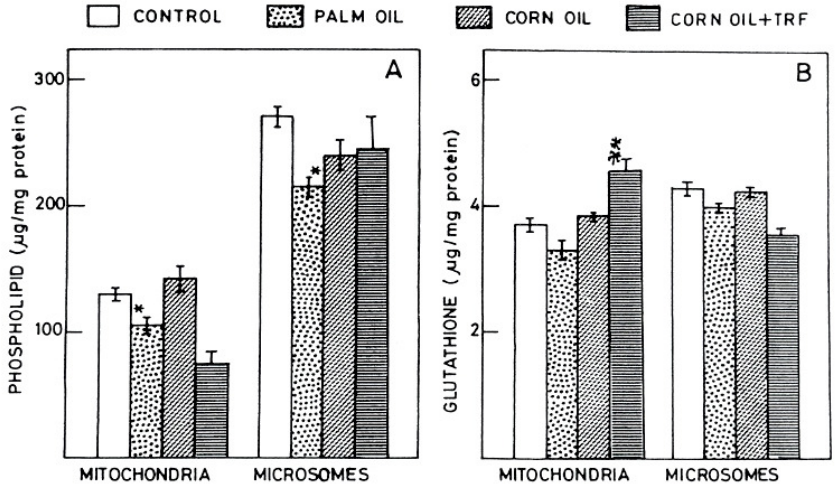

Fig.4. Effect of feeding palm oil or corn oil on the content of phospholipid and glutathione content of rat liver mitochondria and microsomes. Values given are mean ± S.E. from 4 batches of 4 rats each. *P < 0.05, **P < 0.01, as compared to respective controls.

Fig.4 shows that in both mitochondria and microsomes palm oil-rich diet significantly decreased the amount of phospholipids, the major substrates for peroxidation (3-5,18). Corn oil-rich diet did not significantly alter the amount of phospholipid in both fractions. However, corn oil+TRF rich-diet induces entirely different changes in mitochondria as compared to microsomes. This diet significantly reduces phospholipid in mitochondria but not in microsomes. Qureshi *et al*. (25) have shown that tocotrienol, a component of palm oil, inhibits HMG CoA reductase, a key enzyme in cholesterol biosynthesis. Though the mechanism by which dietary palm oil reduces tissue phospholipid remains unclear at present, a similar inhibition of a key enzyme is a possibility. Neither palm oil- nor corn oil-rich diet altered the content of glutathione, one of the major antioxidants and which can regenerate other antioxidant defense systems (26,20).

Compared to corn oil (54 %), palm oil contains less polyunsaturated fatty acids (10 %). The latter contains more monounsaturated fatty acids (40%) than corn oil (30%) (27). Recent studies by Bonanome *et al* (28) have shown that diets rich in monounsaturated fatty acids increase the resistance of plasma LDL

to oxidative modification, independent of their antioxidants content (28). Our present study shows that the same concept can be applied to the peroxidation potential of hepatic membranes. Hammer and Wills (8) have reported earlier that lipid components in the diet of rats regulated the fatty acid composition of hepatic microsomes as well as the lipid peroxidation in this subcellular fraction. More recent studies by Imaizumi et al. (29) indicated that dietary palm oil decreases the proportion of docosahexaenoic acid, a highly unsaturated fatty acid, in the rat liver. Our results also indicate that besides an increase in vitamin E concentration, reduction in the amount of substrates for peroxidation may also be essential for lowering peroxidation potential in mitochondria and to a lesser extent in microsomes. Hence, supplementation of corn oil-rich diet with 1000 ppm TRF, at the same concentration as present in palm oil, only affords a partial protection to subcellular membranes.

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