

Comparative Distribution of Tocotrienols in Livers of Suckling and Adult Rats

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Abstract: This study was carried out to compare the patterns of hepatic distribution of palm vitamin E (palmvitee) in suckling and adult rats. Suckling and male adult Wistar rats were given palmvitee in a dose of 0, 30 or 60 mg/kg body weight intra peritoneally for 14 days. The palmvitee was administered in the neonates from day 1 of life. It contained α -tocopherol (α -TP, 21%), α -tocotrienol (α -T3, 17%), γ -tocopherol (γ -TP, 4%), γ -tocotrienol (γ -T3, 33%) and δ -tocotrienol (δ -T3, 24%). Twenty-four hours after the last injection of palmvitee, the rats were sacrificed and vitamin E concentrations in the liver of each rat were determined. All isomers of vitamin E were detected in groups given palmvitee. Administration of palmvitee increased total vitamin E and its isomers in suckling rats, and in adult rats that received 60 mg/kg palmvitee compared to the respective control groups. In adult rats given 30 mg/kg palmvitee, all tocotrienol isomers and total vitamin E but not α -TP and γ -TP were raised. The patterns of hepatic vitamin E distribution in both groups of palmvitee-treated neonates and adult rats treated with 60 mg/kg palmvitee corresponded well with the composition of palmvitee used, dissimilar to the adult rats given with 30 mg/kg palmvitee which had the highest proportion in α -TP concentration (64%). This preliminary study showed that tocotrienols were distributed differently in liver when given at 30 and 60 mg/kg body weight, postnatally or during adulthood.

Key words: Tocotrienol, suckling rats, neonates, adult rats, liver

Introduction

Vitamin E, a naturally occurring antioxidant is found in abundance in palm oil (Ong and Goh, 2002). It is an essential lipid soluble vitamin and is considered a generic name describing bioactivities of two of its derivatives, tocopherol and tocotrienol, which share a common general structure i.e. an aromatic chromanol head and a 16-carbon tail. Tocotrienol differs from tocopherol by the presence of an unsaturated tail. Each group comprises four different isomers i.e. α , β , γ and δ , which all have different biological activity (Azzi and Stocker, 2000).

Palmvitee is a vitamin E extract from palm oil, which contains both tocopherol (TP) (α and γ isomers) and tocotrienol (T3) (α , γ and δ isomers). Both types of vitamin E have been shown to possess high antioxidative activity (Soelaiman *et al.*, 2004; Asmadi *et al.*, 2005; Yoshida *et al.*, 2005). Besides its antioxidant property, tocotrienol is also claimed to have anticancer (Nesaretnam *et al.*, 2000; Iqbal *et al.*, 2003; Wada *et al.*, 2005) and anti-angiogenic (Inokuchi *et al.*, 2003; Miyazawa *et al.*, 2004) activities, as well as a potent inhibitory effect on β -hydroxy-3-methylglutaryl-coA (HMGCoA) reductase, a rate-limiting enzyme of cholesterol biosynthesis (Raederstoff *et al.*, 2002; Iqbal *et al.*, 2003).

Vitamin E used as a therapeutic agent may have a beneficial role in newborns. It has been reported to

improve conditions associated with oxidative stress in neonates such as retinopathy (Brion *et al.*, 2004), jaundice (Gross, 1979; Rudenko *et al.*, 1990) and bronchopulmonary dysplasia (Ehrenkranz *et al.*, 1979). Many pharmacokinetic studies on tocopherol in fetuses or neonates have been documented (Hidiroglou *et al.*, 2001; Hidiroglou *et al.*, 2003; Pressman *et al.*, 2003), but studies involving tocotrienol are lacking.

α -Tocopherol transfer protein, tocopherol-associated protein and tocopherol binding protein are the tocopherol-regulatory proteins that determine the tissue tocopherol concentrations (Blatt *et al.*, 2001). It is thought that α -tocopherol transfer protein plays an important role in the biodiscrimination of vitamin E isomers. Therefore in adult rats as well as humans, α -TP is preferentially retained in various organs as compared to tocotrienols (Ikeda *et al.*, 2003; Fairus *et al.*, 2004) and thus, a substantial increase in tocotrienol concentrations cannot be achieved even though the intake of tocotrienol-enriched diet is high (O'Byrne *et al.*, 2000). However, the tocotrienols could still be detected in various tissues and plasma, even though α -T3 and γ -T3 were reported to be accumulated in high concentrations in skin and adipose tissues in adult rats (Podda *et al.*, 1996; Ikeda *et al.*, 2000; Ikeda *et al.*, 2003).

Whether or not the tocotrienols are distributed in a similar pattern in neonates as it is in adults, still remains a question. Therefore, this preliminary study

was carried out in an attempt to obtain information on the extent of hepatic distribution of tocotrienols in suckling and adult rats given palmvitee.

Materials and Methods

Animals and housing: The Wistar rats used in this study were obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. The rat neonates were housed together with their littermates and individual mothers in polyethylene cages sized 45 cm x 28 cm x 20 cm, while the male adult Wistar rats were housed separately. The male adult and mothers of the suckling rats were given free access to a commercial rat chow (Gold Coin Ltd., Malaysia) and water.

Experimental procedure: The two groups of rats used in this study were the male adult rats (200-250 g) and suckling rats aged one day (5-6 g). Both groups were given palmvitee intra peritoneally at a dose of 30 (PN30 and PA30) or 60 (PN60 and PA60) mg/kg body weight for 14 days after which all the rats were killed, under ether anaesthesia and their livers isolated for vitamin E analysis. The control groups (PN0 and PA0) were only given vehicle (olive oil).

The palmvitee used was prepared by Malaysian Palm Oil Board (MPOB). Its composition of vitamin E is shown in Table 1. The experimental procedure and animal handling were approved by the Universiti Kebangsaan Malaysia Animal Care and Use Committee.

Table 1: Content of the vitamin E isomers in palmvitee

Isomers	Percentage (%)	Amount in doses (mg/kg)	
		30 mg/kg (PA30 and PN30)	60 mg/kg (PA60 and PN60)
" -Tocopherol	21	6.3	23.6
" -Tocotrienol	17	5.1	10.2
(-Tocopherol	4	1.2	2.4
(-Tocotrienol	33	9.9	19.8
* -Tocotrienol	24	7.2	14.4
Total	99	29.7	59.4

PA30, Adult rats given 30 mg palmvitee/kg body weight.

PN30, Suckling rats given 30 mg palmvitee/kg body weight.

PA60, Adult rats given 60 mg palmvitee/kg body weight.

PN60, Suckling rats given 60 mg palmvitee/kg body weight.

Vitamin E isomers analysis: The vitamin E in the liver was extracted as previously described (Podda *et al.*, 1996) with some modifications. Briefly, 100 mg of liver tissue was homogenized in a tube containing 50 µl ethanolic butylated hydroxytoluene (10 mg/ml) and 1 ml distilled water. One ml of sodium dodecyl sulfate (0.1 M) was then added to the homogenates. After addition of 1 ml ethanol, the homogenates were extracted with 3 ml hexane. An appropriate aliquot was dried up using vacuum concentrator (Heto Lab Equipment, Denmark)

and reconstituted in hexane.

The vitamin E in hexane lipid extract (20 µl sample) was analysed using an analytical high performance liquid chromatography (HPLC; Waters Corp., Milford, MA, USA). The chromatographic system consisted of an isocratic pump (Waters 1515) and a programmable fluorescence detector (Waters 474), set at 295 nm (excitation wavelength) and 330 nm (emission wavelength). The stationary phase was a 150 mm silica normal phase column (Spherisorb 55W, Waters) with an internal diameter 4.6 mm and particle size 5 µm, protected by a guard column (2 mm x 4.6 id mm). The mobile phase was hexane : isopropanol (99:1) at a flow rate of 1.2 ml/min.

The chow pellet was randomly selected for the determination of dietary vitamin E content using the same method (n=7).

Statistical analysis: The results were analysed by one way ANOVA followed by Tukey's Multiple Comparison Test as the data were normally distributed. Values of P<0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism 2.1[®] software (1997; GraphPad Software Incorporation, San Diego, CA, USA).

Results

Tocols levels in the diet: The chow contained about 25 mg vitamin E per kg food (Table 2). Tocopherol made up the major portion of the total vitamin E in the food that is approximately 16.5 mg/kg food, whilst the remaining were tocotrienols (about 8.6 mg/kg).

Table 2: The rat chow components of vitamin E.

Isomers	Levels (mg/kg).
" -Tocopherol acetate	10.20 ± 0.58
" -Tocopherol	5.43 ± 0.16
(-Tocopherol	0.87 ± 0.20
" -Tocotrienol	2.69 ± 0.09
(-Tocotrienol	4.54 ± 0.23
* -Tocotrienol	1.38 ± 0.07
Total	25.11

Values represent mean ± standard error (n = 7)

Hepatic vitamin E in the sucklings: In the sucklings, all the five isomers present in the palmvitee, were detected in the liver of palmvitee-treated groups, with the highest amount being (T3 and the lowest (TP, in both groups (PN30 and PN60) (Fig. 1). There were however, only two isomers which were " TP and (TP detected in the control group (PN0). The concentrations of the individual isomers and total vitamin E were increased dose-dependently.

Hepatic vitamin E in adult rats: The administration of palmvitee for 14 days, increased the hepatic vitamin isomer concentrations dose-dependently except for " TP

and (TP, in the group given 30 mg/kg palmvitee (PA30) in the adult rats (Fig. 2). The concentration of " TP was the highest in the PA30 group, whereas in the group given 60 mg/kg palmvitee (PA60), (T3 concentration was noted to be the highest and followed by " TP.

Discussion

In the suckling rats treated with palmvitee, there was an increase in both T3 and TP concentrations in the liver, indicating that both tocotrienol and tocopherol were taken up by the liver. Surprisingly, the patterns of hepatic vitamin E distribution in all palmvitee-treated groups were similar to the composition of palmvitee used i.e. the highest being the (T3 followed by " TP, *T3, " T3 and (TP. The presence of " TP in the control neonates might be from the mothers which were fed on the rat chow and transferred to the neonates through milk. The finding from the present study is contradictory to an *in vitro* work reported by Hosomi *et al.* (1997). They showed that " TP transfer protein which was purified from adult rats, had the highest affinity for natural " TP (RRR-" TP), followed by \$TP, " T3 and (TP. As a consequence, " TP would be preferably retained in the tissue as compared to other isomers. It is noteworthy to mention that our study was a preliminary study. We did not determine the " TP transfer protein activity and its expression in this study. However, our study suggests that lack of biodiscrimination of vitamin E hepatic uptake in the neonates could be due to a poorly expression of " TP transfer protein in the neonatal rat liver. Kim *et al.* (1996) has reported that " TP transfer protein expression was very low immediately after birth and only increased steadily during the two weeks of life before weaning. The bioavailability of the tocotrienol has not been extensively studied. Roy *et al.* (2002) studied the maternal transfer of " T3 and " TP to the developing rat fetal brain. In their study, " T3 and " TP were both detected in the fetal brain after two weeks of maternal supplementation of the isomers mixture. Contrary to our findings, the concentration of " T3 was much lower than that of " TP in their study, although the amount of " T3 and " TP present in the mixture was comparable (119 mg " T3 and 110 mg " TP). The possible explanation for this discrepancy is that biodiscrimination of the tocols (TP and T3) by the " TP transfer protein occurs during gestation due to the presence of the protein which is localised at the implantation site of pregnant mouse uterus (Kaempff-Rotzoll *et al.*, 2002).

In the adult rats, the pattern of hepatic distribution of vitamin E was dose-dependent. In the group treated with 30 mg/kg palmvitee, " TP was detected in the highest amount, followed by " T3, *T3, (T3 and (TP being the lowest. Dissimilar to the former group, the highest concentration of the vitamin detected in the adults treated with 60 mg/kg palmvitee was (T3, followed by " TP, " T3, *T3 and (TP. The traces amount of T3 found

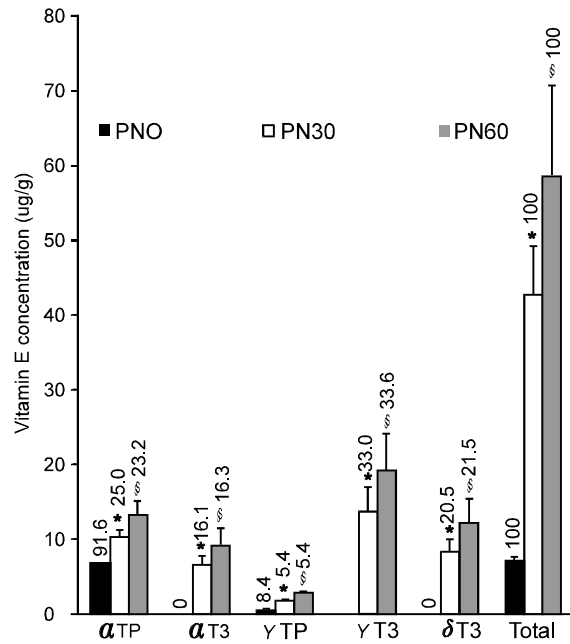


Fig. 1: The effect of palmvitee administration (0, 30 or 60 mg/kg bw) for 14 days on neonatal hepatic vitamin E distribution. Each bar represents mean ± standard error (n=10). Numbers in italic on each bar indicate fractions (%) of the individual isomer detected from each group of treatment. *Significantly different from PNO and PN60 (P<0.05). §Significantly different from PNO and PN30 (P<0.05).

in the control group might be from the fed commercial rat chow that contained 8.61 mg T3/kg food. Our findings suggest that biodiscrimination for the " TP to be retained has occurred in the group given a lower dose of palmvitee (30 mg/kg body weight), but not at a relatively higher dose of palmvitee. In the current study, the findings observed with the lower dose of palmvitee are in agreement to those reported by other investigators (O'Byrne *et al.*, 2000; Ikeda *et al.*, 2001; Okabe *et al.*, 2002; Ikeda *et al.*, 2003), which emphasized that tissue uptake of tocotrienol did not increase with an increase in dose or if the major vitamin E isomer present was " TP. In the adults given 60 mg/kg palmvitee, the pattern of hepatic vitamin E distribution differed compared to the adults treated with 30 mg/kg body weight, suggesting the lack of biodiscrimination between the vitamin E isomers observed at a higher vitamin E administration dose. This may be due to the saturation of the " TP transfer protein by the " TP at a relatively high concentration of vitamin E. Other than the activity of " TP transfer protein, vitamin E is also transferred during chylomicron metabolism via lipoprotein lipase-mediated mechanism (Traber *et al.*, 1985). The conversion of the chylomicron to remnant particles results in the

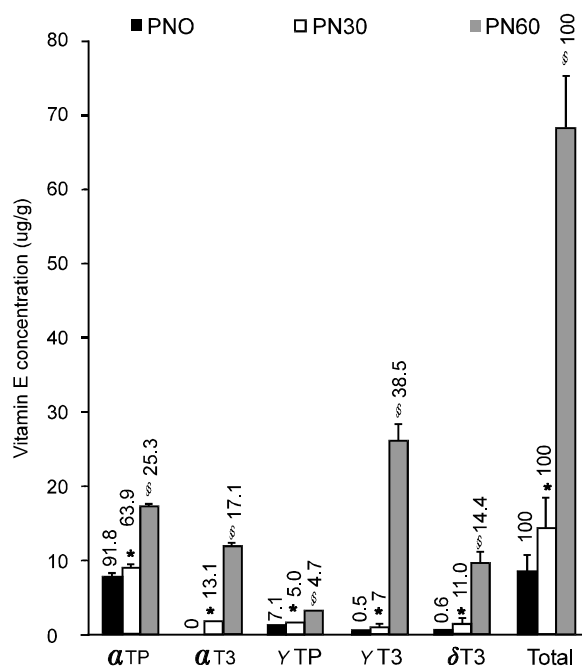


Fig. 2: The contents of tocopherols (TP) and tocotrienols (T3) (mg/g) in adult rat livers after palmvitee administration (0, 30 or 60 mg/kg bw) for 14 days. Each bar represents mean \pm standard error (n=10). Numbers in italic on each bar indicate fractions (%) of the individual isomer detected from each group of treatment. *Significantly different from PA0 and PA60 ($P < 0.05$). §Significantly different from PA0 and PA30 ($P < 0.05$).

distribution of newly absorbed vitamin E to all circulating lipoproteins and ultimately to tissues, without biodiscrimination (Traber and Sies, 1996). It is likely via this enrichment, tocotrienols are delivered to tissues. Therefore, we postulate that when α TP transfer protein gets saturated by the α TP, more of the vitamin E is being transferred to tissues by the activity of lipoprotein lipase. Thus, biodiscrimination cannot be detected. A similar finding by Behrens and Madere (1991) also demonstrated that the biodiscrimination between natural (RRR) α TP and all-rac- α TP was not observed at a relatively high concentration of administered α TP. In conclusion, this preliminary study showed that the patterns of distribution of tocotrienol in the rat liver were different when given in a relatively lower dose, postnatally or during adulthood. The mechanisms of uptake and transport of tocotrienols in organs and tissues are poorly understood in adults and more so in neonatal tissues. Therefore, further study is needed to obtain more information regarding the vitamin E distribution in neonates.

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