Comparative Distribution of Tocotrienols in Livers of Suckling and Adult Rats

Y. Kamisah1,*, M.Y. Norhayati1, B. Zakri2, M. Mazladiyana1, O. Faizah1, M.T. Gapor1 and A.Y. Asmadi1
Department of Pharmacology, 2Department of Surgery, 3Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia 4Agro Product Unit, Engineering and Processing Research Division, Malaysian Palm Oil Board, Kajang, Selangor, Malaysia

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 (αTP, 21%), α-tocotrienol (αT3, 17%), γ-tocopherol (γTP, 4%), γ-tocotrienol (γT3, 32%) and δ-tocotrienol (δT3, 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 αTP and γTP 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 αTP 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 antioxidantive 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 (HMG-CoA) reductase, a rate-limiting enzyme of cholesterol biosynthesis (Raederstorf 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 foetuses 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, αTP is preferentially retained in various organs as compared to tocotrienols (Ikeda et al., 2003; Fairs 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 αT3 and γT3 were reported to be accumulated in high concentrations in skin and adipose tissues in adult rats (Podda et al., 1990; 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 littersmates 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>
<thead>
<tr>
<th>Isomers</th>
<th>Percentage (%)</th>
<th>Amount in doses (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 mg/kg (PA30 and PN30)</td>
<td>60 mg/kg (PA60 and PN60)</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>21</td>
<td>6.3</td>
</tr>
<tr>
<td>α-Tocotrienol</td>
<td>17</td>
<td>5.1</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td>γ-Tocotrienol</td>
<td>33</td>
<td>0.9</td>
</tr>
<tr>
<td>δ-Tocotrienol</td>
<td>24</td>
<td>7.2</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>29.7</td>
</tr>
</tbody>
</table>

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**

Tocoids 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 8.5 mg/kg).

<table>
<thead>
<tr>
<th>Isomers</th>
<th>Levels (mg/kg).</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol acetate</td>
<td>10.20 ± 0.58</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>5.43 ± 0.16</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>0.87 ± 0.20</td>
</tr>
<tr>
<td>α-Tocotrienol</td>
<td>2.69 ± 0.09</td>
</tr>
<tr>
<td>γ-Tocotrienol</td>
<td>4.54 ± 0.23</td>
</tr>
<tr>
<td>δ-Tocotrienol</td>
<td>1.36 ± 0.07</td>
</tr>
<tr>
<td>Total</td>
<td>25.11</td>
</tr>
</tbody>
</table>

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 γT3 and the lowest γTP, in both groups (PN30 and PN60) (Fig. 1). There were however, only two isomers which were αTP and γTP 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 αTP
and γTP, in the group given 30 mg/kg palmvitee (PA30) in the adult rats (Fig. 2). The concentration of αTP was the highest in the PA30 group, whereas in the group given 60 mg/kg palmvitee (PA60), γT3 concentration was noted to be the highest and followed by αTP.

**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 γT3 followed by αTP, δT3, αT3 and γTP. The presence of αTP 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 αTP transfer protein which was purified from adult rats, had the highest affinity for natural αTP (RRR-αTP), followed by βTP, αT3 and γTP. As a consequence, αTP 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 αTP 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 αTP transfer protein in the neonatal rat liver. Kim et al. (1996) has reported that αTP 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 αT3 and αTP to the developing rat fetal brain. In their study, αT3 and αTP were both detected in the fetal brain after two weeks of maternal supplementation of the isomers mixture. Contrary to our findings, the concentration of αT3 was much lower than that of αTP in their study, although the amount of αT3 and αTP present in the mixture was comparable (119 mg αT3 and 110 mg αTP). The possible explanation for this discrepancy is that biodiscrimination of the tococols (TP and T3) by the αTP transfer protein occurs during gestation due to the presence of the protein which is localised at the implantation site of pregnant mouse uterus (Kaesmp-Ritzoll 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, αTP was detected in the highest amount, followed by αT3, δT3, γT3 and γTP 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 γT3, followed by αTP, αT3, δT3 and γTP. The traces amount of T3 found 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 αTP 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 αTP. 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 αTP transfer protein by the αTP at a relatively high concentration of vitamin E. Other than the activity of αTP 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
Acknowledgements

The authors wish to thank Professor Dr Nafeez Mohd. Ismail from Faculty of Medicine, MARA Technology University, for editorial assistance. This study was supported by a grant from Universiti Kebangsaan Malaysia (UKM F66/00).

References


