Maternal palmvitee administration reduced plasma total bilirubin and uridine diphosphate glucuronyltransferase activity in hyperbilirubinaemic rat neonates

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Abstract

Introduction: Hyperbilirubinaemia (jaundice) is common during the first days of postnatal life. α-Tocopherol was reported to decrease serum total bilirubin in jaundiced human newborns. This study was conducted to investigate the effect of maternal palmvitee administration on hyperbilirubinaemia induced by δ-aminolevulinic acid (ALA) in rat neonates.
Material and methods: Twenty-four successfully mated female Wistar rats were divided into three groups. They were given 50 or 200 mg palmvitee/kg body weight orally once daily from day 1 of pregnancy to delivery, while the other group was given olive oil (control). At postnatal day 14 (the optimal age to induce hyperbilirubinaemia as obtained earlier), the pups born to four dams of each group were induced with hyperbilirubinaemia, while the rest were given vehicle. Twenty-four hours after the induction, the neonates were sacrificed. Plasma total bilirubin, hepatic thiobarbituric acid reactive substance (TBARS), uridine diphosphate glucuronyltransferase (UGT) activity and vitamin E content were determined in the neonates.
Results: ALA administration increased plasma total bilirubin. In the palmvitee-treated groups, plasma total bilirubin was lower than in the controls (0.19 ± 0.01 and 0.10 ± 0.01 vs. 0.35 ± 0.18, $p<0.05$). ALA did not affect the hepatic UGT, but it was reduced in the palmvitee-treated groups (0.71 ± 0.10 and 0.55 ± 0.02 vs. 0.92 ± 0.07, $p<0.05$). Neither ALA nor palmvitee influenced hepatic TBARS level. Maternal pretreatment with 200 mg/kg palmvitee increased the neonatal hepatic vitamin E content.
Conclusions: The maternal administration of palmvitee showed a protective effect on hyperbilirubinaemia. However, this administration could lead to decreased hepatic glucuronidation activity in rat neonates.

Key words: tocotrienol, bilirubin, uridine diphosphate glucuronyltransferase.

Introduction

Jaundice or hyperbilirubinaemia is a common phenomenon occurring during the first days of postnatal life and affects nearly two thirds of human newborns. It causes increased hospitalization costs to both mothers and newborns, and also risk of neonatal death due to kernicterus [1].

Hyperbilirubinaemia in newborns results in part from exaggerated erythrocyte destruction due to a reduced erythrocyte lifespan, enhanced rates of tissue haem oxidation and immaturity of hepatic bilirubin conjugation [2, 3]. Imbalance between production and conjugation of bilirubin plays an important role in the mechanism of neonatal hyperbilirubinaemia [4]. Increased rate of erythrocyte destruction, which contributes about 75% of bilirubin metabolism [5] in neonates, will lead to increased production of bilirubin. Bilirubin is taken up by the liver and conjugated to glucuronides by the action of uridine diphosphate glucuronyltransferase (UGT) to form water-soluble and more easily excreted bilirubin [6]. Therefore, low activity of UGT will increase the level of unconjugated bilirubin, which is lipid soluble and neurotoxic [5]. A deficiency in UGT activity can cause Crigler-Najjar and Gilbert syndrome [7]. Rifampicin was shown to treat unconjugated hyperbilirubinaemia successfully via induction of UGT1A1, the isoenzyme of UGT that glucuronides bilirubin, in patients with Gilbert syndrome [8]. Phenobarbitone was also used for neonatal jaundice prophylaxis [9], which also acts via the induction of UGT1A1 [10].

α-Tocopherol, a type of vitamin E, has been demonstrated in a few studies to have a protective effect against hyperbilirubinaemia. Its administration caused decreases in serum total bilirubin and the duration of phototherapy in preterm [11] and full term infants [12]. In glucose-6-phosphate dehydrogenase deficient patients, it improved red blood cell life span, increased haemoglobin concentration and reduced reticulocytosis [13]. The protective effect of the α-tocopherol could be due to its antioxidative properties as well as its stabilizing effect on erythrocytes [14].

Vitamin E extract from palm oil, known as palmvitee, contains both tocopherol and tocotrienol. Like tocopherol, tocotrienol has also been shown to possess high antioxidative activity [15-17]. Besides its antioxidant property, it is also claimed to have antitumour [18, 19] and anti-angiogenic [20, 21] activities, as well as a potent inhibitory effect on β-hydroxy-3-methylglutaryl-coA (HMGCoA) reductase, a rate-limiting enzyme of cholesterol biosynthesis [18, 22].

Since both tocopherol and tocotrienol can cross the placenta, as shown in many studies in both animals and humans [23, 24], we believe that maternal supplementation of the combination (palmvitee) could afford protection against hyperbilirubinaemia in neonates. Therefore, in this study, we investigated the effect of maternal administration of palmvitee on hyperbilirubinaemia induced in rat neonates.

Material and methods

### Animals, reagents and diet

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 × 28 × 20 cm. The mothers of the suckling rats were given free access to a commercial rat chow (Gold Coin Ltd., Malaysia) and water.

All chemicals and enzymes were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated. The palmvitee used in this study was a generous gift from Mr A Gapor Mat Top (Malaysian Palm Oil Board), comprising 21% α-tocopherol, 17% α-tocotrienol, 4% γ-tocopherol, 33% γ-tocotrienol and 24% δ-tocotrienol. It was dissolved in olive oil as the oil has the lowest vitamin E content compared to other edible oils [25]. The rat chow contained about 25.11 mg/kg total vitamin E and its composition is as follows (mg/kg food): α-tocopherol acetate 10.2, α-tocopherol 5.43, γ-tocopherol 0.87, α-tocotrienol 2.69, γ-tocotrienol 4.54 and δ-tocotrienol 1.38.

Experimental design

This study was divided into two phases. In the first phase, groups of Wistar rat neonates at the age of 6, 8, 10, 12, 14, 16 and 20 days were each divided into two groups designated control and δ-aminolevulinic acid (ALA)-treated, consisting of 10 neonates per group. ALA (500 μmol/kg body weight) was administered intraperitoneally in three separate doses, 24, 20 and 16 hours prior to sacrifice, following a procedure described by Drummond and Kappas [26]. Control animals were administered an equivalent volume of normal saline. The final volume of each injection was 0.1 ml. The rat neonates were housed together with their littermates and individual mothers in polyethylene cages during the course of the treatment. The neonates were killed 24 hours after the first dose of ALA. Blood samples were taken via cardiac puncture under diethyl ether anaesthesia, for plasma total bilirubin analysis.

In the second phase, twenty-four successfully mated female Wistar rats (180-220 g), were divided into three groups. Pregnancy at day 1 in the female rats was determined by the presence of spermatozoa in the vaginal smear using Shorr stain, after they were mated overnight with male rats of the same strain. These pregnant rats were given palmvitee once daily by oral gavage at a dose of 50 or 200 mg/kg body weight from day 1 of pregnancy to delivery, which was 21 to 22 days long, while another group was only given vehicle (control group). They were given free access to a commercial rat chow and water, and housed individually in cages sized 45 × 28 × 20 cm. Hyperbilirubinaemia was induced in the pups born
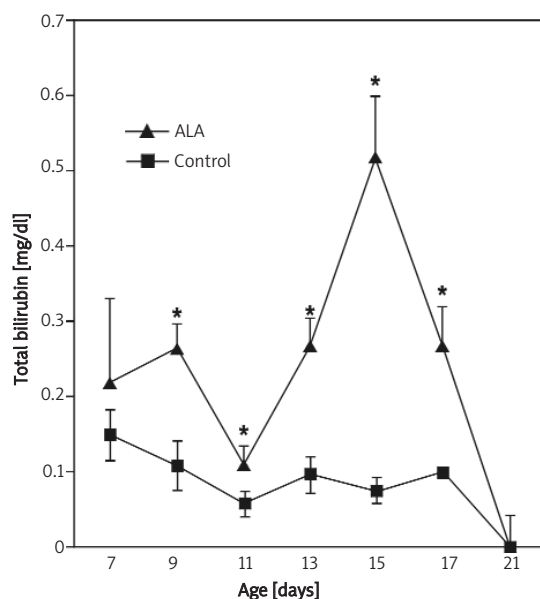
Figure 1. Plasma total bilirubin level after the administration of δ-aminolevulinic acid (ALA) in suckling rats. ALA (500 μmol/kg body weight) was administered intraperitoneally to neonates in three divided doses 24, 20 and 16 hours prior to sacrifice at the times indicated. Each timepoint represents mean ± standard error (n=10)

*significantly different from the control (p<0.05)
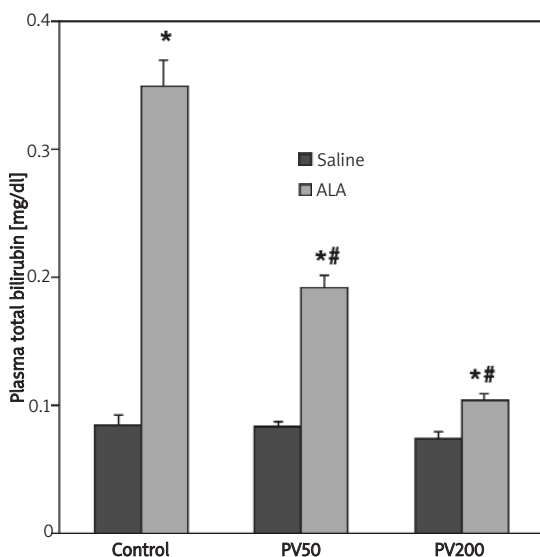
Figure 2. Plasma total bilirubin in neonatal rats which were maternally pretreated with oral palmvitee (PV) at 50 or 200 mg/kg daily from day 1 pregnancy through delivery, after intraperitoneal administration of δ-aminolevulinic acid (ALA 500 μmol/kg body weight) on day 14 postnatally, in three divided doses 24, 20 and 16 hours prior to sacrifice (10-15 neonates per group). Each bar represents mean ± standard error of mean.

*significantly different from saline groups (p<0.05)
#significantly different from other ALA-treated groups (p<0.05)

to four dams from each group on postnatal day 14 (the optimal age was obtained from phase 1 study), following the same procedure as in the first phase, while another half were given normal saline (10-15 neonates per subgroup). Pups with lysed blood and those that came from a litter size of less than six or more than ten were excluded because they tended to have lysed blood when withdrawn, which would affect the bilirubin measurement. Neonatal blood and livers were taken 24 hours after the induction for biochemical measurements.

The experimental procedure and animal handling were approved by the Institutional Animal Care and Use and Medical Research Ethics Committee (Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia), which conforms to Malaysian Ministry of Science, Technology and Innovations guidelines.

Biochemical parameter measurements

Plasma total bilirubin was determined using a commercial kit with an automated analyzer Cobas Mira S (Roche Diagnostic, Switzerland) according to the method of Wahlefeld et al. [27]. The hepatic microsomes were prepared following a standard procedure [28] prior to the measurement of uridine diphosphate glucuronyltransferase (UGT) activity [29]. Hepatic malondialdehyde measured as thiobarbituric acid reactive substance (TBARS) content was determined in the livers of the neonates following the method as described by Ledwozyw et al. [30]. Protein concentration was evaluated by the method of Lowry et al. [31] using bovine serum albumin as standard. Hepatic vitamin E was extracted as described [32] with some modifications [33] and analysed using analytical high performance liquid chromatography (HPLC; Waters Corp., Milford MA, USA).

Statistical analysis

Results are expressed as mean ± standard error of mean. Data were statistically compared using one way analysis of variance (ANOVA), followed by Tukey's multiple comparison test using GraphPad Prism 2.1 software (1997; GraphPad Software Inc., San Diego, CA, USA). A p value <0.05 was considered to indicate a significant difference between the groups.

Results

Effect of ALA on plasma total bilirubin

δ-Aminolevulinic acid (ALA) caused a significant elevation in neonatal plasma total bilirubin on day 9 to day 17 after birth, which peaked on day 15 and returned to normal value by day 21 (Figure 1).

Effect of palmvitee on plasma total bilirubin

Figure 2 shows the effect of maternal palmvitee supplementation on plasma total bilirubin induced

by the administration of ALA. As expected, ALA administration on postnatal day 14 caused an increase in plasma total bilirubin in rat neonates. Palmvitee pretreatment in the dams reduced the plasma total bilirubin in the neonates significantly and the reduction was dose-dependent.

Effect of palmvitee on hepatic UGT

ALA administration did not significantly influence the hepatic activity of uridine diphosphate glucuronyltransferase (UGT) in rat neonates. However, pretreatment with palmvitee reduced this enzyme activity in both saline and ALA-treated groups, compared to the control group (Figure 3). This reduction however, was not dose-dependent.

Effect of palmvitee on hepatic lipid peroxidation

Administration of neither ALA nor palmvitee pretreatment affected the TBARS content, a lipid peroxidation marker, measured in the livers of the rat neonates (Figure 4).

Effect of palmvitee administration on hepatic vitamin E content

Palmvitee administration in mothers at the dose of 200 mg/kg body weight increased the content of almost all vitamin E isomers in the livers of the neonates (Figure 5). The administration of ALA reduced the neonatal hepatic content of α-tocotrienol in the control and PV50 groups. It also reduced the hepatic γ-tocopherol and total vita-min E, but it increased the γ-tocotrienol content in the control group.

Discussion

In the first phase of our study, the administration of ALA had been proven to increase bilirubin production and it was age-dependent. The optimal age for hyperbilirubinaemia obtained in our study was at postnatal day 14, based on the highest increase in plasma total bilirubin seen at that age. The dose of ALA used (500 µmol/kg body weight) was one third of the one employed in Drummond and Kappas [26] study and the pattern of the increase was similar to that study. δ-Aminolevulinic acid (ALA) is a haem precursor. It increases haem synthesis and finally bilirubin production in animal models dose-dependently [26]. However, at this dose, increases in plasma total bilirubin still can be seen despite its smaller dose.

Hyperbilirubinaemia induction before the age of 7 days in neonates produces inconsistent increases in plasma total bilirubin due to inconsistencies in haem metabolism immediately after birth up to day 7 of age. Before the age of 21 days old, the hepatic glucuronidation system is not fully matured in rats.
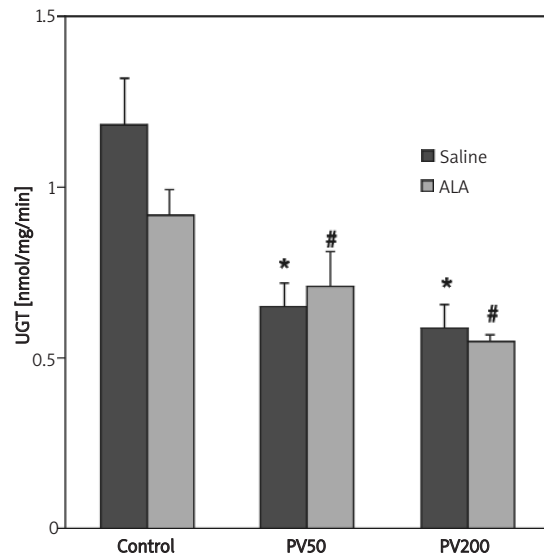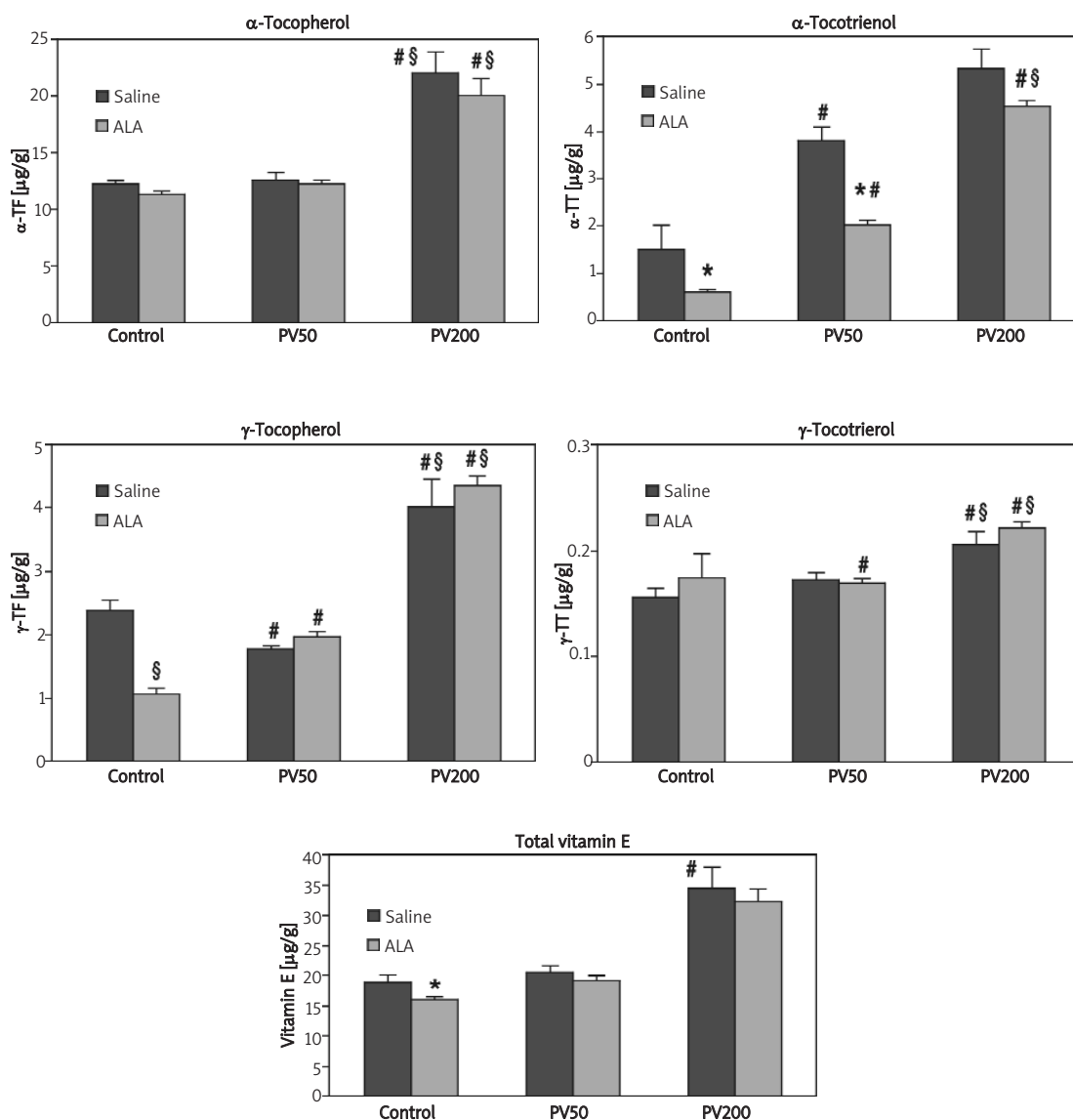

Figure 3. Uridine diphosphate glucuronyltransferase (UGT) activities in neonatal rats which were maternally pretreated with palmvitee (PV) at 50 or 200 mg/kg/day orally from day 1 pregnancy through delivery (10-15 neonates per group) after δ-aminolevulinic acid (ALA 500 µmol/kg body weight intraperitoneally) administration in three divided dose 24, 20 and 16 hours prior to sacrifice on day 14 postnatal. Each bar represents mean ± standard error of mean
*significantly different from control saline groups (p<0.05)
#significantly different from control ALA-treated groups (p<0.05)
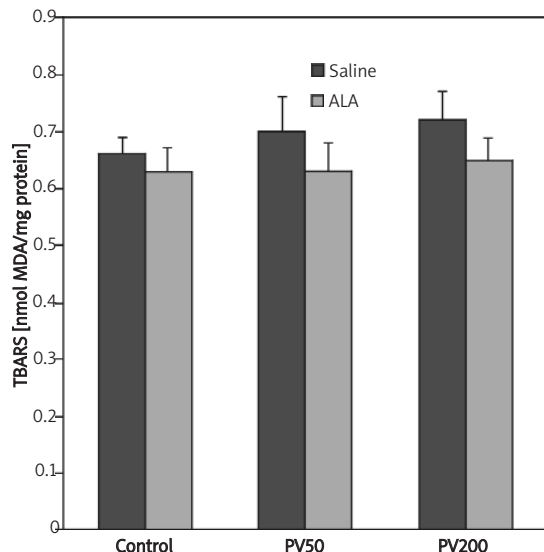
Figure 4. The hepatic thiobarbituric acid reactive substance (TBARS) in neonates which were maternally pretreated with palmvitee (50 or 200 mg/kg/day orally) during pregnancy (20 neonates per group) after peritoneal administration of δ-aminolevulinic acid (ALA 500 µmol/kg body weight) in three divided doses 24, 20 and 16 hours prior to sacrifice on day 14 postnatal. Each bar represents mean ± standard error of mean

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Figure 5. Hepatic tocopherol and tocotrienol isomer contents (μg/g liver) in rat neonates (15-20 neonates per group) which were maternal pretreated with palmvitee (50 and 200 mg/kg body weight) during gestational period after administration of δ-aminolevulinic acid (ALA). ALA (500 μmol/kg body weight intraperitoneally) was given in three divided doses 24, 20 and 16 hours prior to sacrifice on day 14 postnatal. Each bar represents mean ± standard error of mean

*significantly different from its saline group respectively ($p < 0.05$)

#significantly different from the control group ($p < 0.001$)

 significantly different from PV50 ($p < 0.001$)

Therefore, the hyperbilirubinaemia induction is normally done between postnatal days 7 and 21 because the effect of ALA will be easily seen during this period [26].

As previously obtained, the administration of ALA on day 14 (second phase of study) increased plasma total bilirubin significantly compared to the control group. Palmvitee pretreatment throughout pregnancy reduced this parameter dose-dependently. To the best of our knowledge, there are no other recent studies done regarding the

effect of palm vitamin E, especially the tocotrienols, on hyperbilirubinaemia. The administration of α-tocopherol in newborns was shown to reduce neonatal hyperbilirubinaemia in a few studies [11, 12]. Vitamin E deficiency has been reported to be one of the factors that causes hyperbilirubinaemia in neonates [34]. Low antioxidant levels in preterm babies may predispose them to increased oxidative stress and cause hyperbilirubinaemia [35].

In our study, ALA administration did not affect the activity of uridine diphosphate glucuronyltransferase

(UGT). However, unexpectedly, palmvitee at both doses inhibited the enzyme activity significantly, but no significant difference was seen between ALA- and saline-treated groups. We expected that the enzyme activity would be increased based on the reduced level of plasma total bilirubin. Therefore, it can be suggested that palmvitee reduced hyperbilirubinaemia possibly not through the increase in the activity of the bilirubin conjugation process. α-Tocopherol was shown to reduce haem oxygenase activity, the rate-limiting enzyme in haem synthesis [36, 37] which was found to be increased after ALA administration [26, 38].

Silymarin, which is also an antioxidant purified from milk thistle, and its main constituent, silybin, were demonstrated to inhibit UGT1A1 [39, 40], a UGT isoenzyme involved in bilirubin glucuronidation. It is hypothesized that the extract competes with bilirubin for the binding site in the enzyme or produces conformational changes that impede the binding of the substrate [40]. Chrungoo et al. [41] also showed that silymarin depleted hepatic UDP-glucuronic acid. It could be that the palmvitee, either the tocopherol and/or tocotrienol, acted the same way as silymarin. However, this needs further investigation. The activity of UGT1A1 is detectable from day 22 of gestation, gradually increases after birth and reaches the levels of adult life in rats [42].

Dills and Klaassen [43] reported that diethyl ether anaesthesia reduced bilirubin glucuronidation by inhibiting endogenous hepatic UDP-glucuronic acid, which could directly affect the UGT activity. In our study, we had done some preliminary work to assess the effect of diethyl ether anaesthesia on plasma total bilirubin and the activity of UGT, and found no significant difference between the exposed and non-exposed groups (data not shown). The difference between their study and ours could be due to the length of diethyl ether exposure. In our study, the blood and liver taking procedures took less than one minute, while in their study the duration of exposure to diethyl ether was 30 min up to 4 hours. Some recent studies involving experimental jaundice and hepatic glucuronidation have also used diethyl ether for anaesthesia [42, 44].

There was no significant increase in hepatic thiobarbituric acid reactive substances (TBARS) following ALA administration. Palmvitee pretreatment also did not produce any significant changes in this parameter, indicating that the plasma total bilirubin reduction by palmvitee was not via its antioxidative mechanism. However, a study by Noriega et al. [45] had shown that intraperitoneal administration of ALA at 40 mg/kg body weight for 10 days increased hepatic lipid peroxidation significantly. That a similar result was not seen in our study could be due to the difference in duration of exposure to ALA. Even though TBARS was claimed not to be a sensitive marker for lipid peroxidation, it has been shown that palmvitee, especially tocotrienol, pretreatment reduced TBARS level in our other studies [16, 46] and it is still being used in many recent studies involving oxidative stress [47-49].

Yigit et al. [50] showed there was an association between serum malondialdehyde and bilirubin in neonates with hyperbilirubinaemia. The malondialdehyde level was found to positively correlate with the bilirubin level but a positive correlation was only obtained in neonates with haemolytic hyperbilirubinaemia. This discrepancy of the finding with our study could be due to a difference in the source of the bilirubin. In our study, the bilirubin was from the increased haem metabolism, while in their study it was from the lysed erythrocytes.

Our findings showed that palmvitee administration at high dose (200 mg/kg body weight) to pregnant rats throughout pregnancy increased both hepatic tocopherol and tocotrienol contents. Generally, ALA administration did not affect the vitamin content very much except that it reduced the α-tocotrienol in the control and PV50 groups, and γ-tocopherol in the control group, but increased the γ-tocotrienol in the control group. The reductions in certain vitamin E isomers after ALA administration could be due to the consumption of the vitamin E to overcome the detrimental effect of ALA. However, a slight increase in γ-tocotrienol after ALA administration in the control group was not understandable. The presence of vitamin E isomers in the control neonates could be from the dams which were fed on the rat chow that also contained vitamin E. The hepatic vitamin E contents in the neonates from maternally palmvitee-pretreated groups might be obtained from their mothers through placental transfer as well as during lactation.

It is noteworthy that more than 70% of the hepatic total vitamin E detected was in the form of α-tocopherol, and the second highest amount was α-tocotrienol, whilst γ-tocotrienol was the lowest despite its highest (γ-tocotrienol) concentration in the palmvitee preparation. δ-Tocotrienol was not detectable at all. This finding was in agreement with other findings showing that the body has a clear preference for α-tocopherol compared to other vitamin E isomers due to the biodiscriminational uptake of α-tocopherol into tissues by α-tocopherol transfer protein [51, 52], which was reported to be mainly expressed in the mouse uteri [53]. We believe this protein is also expressed in the rat uteri. As a consequence, α-tocopherol would be preferably retained in the tissue compared to other isomers. Another possible explanation for the lack of bioavailability of tocotrienol in vivo may be poor absorption or rapid clearance from plasma [54].

In conclusion the results obtained in this study demonstrate promising therapeutic effects of

palmvitee in neonatal hyperbilirubinaemia. However, the exact mechanisms of how palmvitee reduced plasma total bilirubin should be pursued further, it is probably not through an increase of the glucuronyl conjugation process. The outcome of this UGT inhibition by palmvitee on the management of drugs which are coadministered with vitamin E and undergo glucuronidation needs further studies. Palmvitee may inhibit the metabolism of these drugs when given together and this may increase the toxicity level of the latter drugs.

Acknowledgments

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