Enzyme induction by phenobarbitone and vitamin K₁ disposition in man

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The plasma disposition of an intravenous pharmacological dose of vitamin K₁ was determined in seven healthy volunteers before and after chronic administration of phenobarbitone (100 mg *nocte*). Phenobarbitone produced a significant increase in antipyrine clearance and 6 β -hydroxycortisol excretion in all volunteers, indicating induction of hepatic microsomal mixed-function oxidase enzymes. However, phenobarbitone administration had no effect on the plasma disposition of vitamin K₁. We conclude therefore, that vitamin K₁ is not metabolized to any significant extent, by phenobarbitone-inducible mixed-function oxidase enzymes.

Keywords vitamin K₁ phenobarbitone enzyme induction epilepsy

Introduction

There are a number of reports which indicate that a haemorrhagic diathesis related to low prothrombin concentrations is a major complication of maternal anticonvulsant therapy (Mountain *et al.*, 1970; Blayer & Skinner, 1976; Deblay *et al.*, 1982). The coagulation defect is similar to that observed in vitamin K deficiency; there is a decrease in the activity of clotting factors II (prothrombin), VII, IX and X but clotting factors V, VIII and fibrinogen are normal (Mountain *et al.*, 1970). Nevertheless the coagulation factors measured in the mothers of neonates with a severe defect were normal.

It has been suggested that vitamin K₁ prophylaxis should be routine for epileptic mothers who are near term (Mountain *et al.*, 1970; Griffiths, 1981). Thus Deblay *et al.* (1982) found that 14 pregnant epileptic women who received an oral supplementation of vitamin K₁, gave birth to babies with no haemorrhage and normal prothrombin times. In the same study it was found that 20 out of 75 epileptics gave birth to children with abnormal prothrombin times.

In most of the cases where haemorrhagic diathesis was observed, the mother was taking a barbiturate or a drug such as primidone which is metabolized to a barbiturate. However in many

instances a second drug, such as phenytoin, was also given. Phenobarbitone is known to alter the disposition of a number of endogenous substances in man (Park & Breckenridge, 1981). In the rabbit, administration of phenobarbitone (40 mg kg⁻¹) significantly increased the plasma clearance of vitamin K₁ but did not alter the pharmacological response to the vitamin after coumarin anticoagulation (Wilson & Park, 1984).

We have therefore investigated the effect of short-term treatment with a therapeutic dose of phenobarbitone on vitamin K₁ disposition in volunteers. Hepatic microsomal enzyme induction was monitored by measuring antipyrine clearance and 6 β -hydroxycortisol excretion (Ohnhaus & Park, 1979).

Methods

Plan of study

The plasma pharmacokinetics of vitamin K₁ were determined in six male and one female healthy volunteers immediately before, and on the day after, administration of phenobarbitone

(100 mg *nocte*) for 14 days. Consent for the study was obtained from the local ethics committee (University of Essen). In addition, antipyrine clearance and urinary 6 β -hydroxycortisol excretion (as a ratio of 17-hydroxycorticosteroids) were measured before, and after, phenobarbitone pretreatment as described previously (Park, 1978; Sanghvi *et al.*, 1973; Ohnhaus & Park, 1979).

The plasma pharmacokinetics of vitamin K₁ were determined after a single 10 min intravenous infusion of Konaktion® (10 mg) in saline (50 ml). Blood samples were obtained at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 9, 12 and 24 h after administration of vitamin K₁, collected in heparinized tubes. Plasma was obtained by centrifugation (2000 g) and stored frozen (-56°) until assayed. Vitamin K₁ concentrations were determined using a normal phase high performance liquid chromatography method which has been developed for the estimation of pharmacological concentrations of vitamin K₁ in plasma (Wilson & Park, 1983). Using this assay *trans*-vitamin K₁ plasma concentrations could be measured up to 9 h after intravenous administration of a single 10 mg dose.

Statistical analysis

Pharmacokinetic analysis of the vitamin K₁ data was performed using least squares regression analysis according to the method of Nielsen-Kudsk (1980). Results are presented as the mean $\pm$ s.d. and were analysed using a paired Student's *t*-test.

Results

The following laboratory parameters were found to be normal, before and after the study, haemo-

globin, leucocytes, thrombocytes, serum transaminases.

From Table 1 it can be seen that phenobarbitone administration produced a significant increase in antipyrine clearance and a significant decrease in antipyrine half-life but did not alter the volume of distribution of the drug. Phenobarbitone also increased 6 β -hydroxycortisol excretion but had no effect on the excretion of 17-hydroxycorticosteroids. The changes in these indices of mixed-function oxidase enzyme induction are the same as reported previously (Ohnhaus & Park, 1979).

Vitamin K₁ concentrations in plasma declined biexponentially after intravenous administration of a single 10 mg dose (Figure 1). Phenobarbitone administration did not significantly alter plasma concentrations of the vitamin at any time-point and there was no difference between the control (1746 ± 591 ng ml⁻¹ h) and test (2171 ± 544 ng ml⁻¹ h) area under the plasma concentration curve which was measured between 0.25 h and 9 h using the trapezoidal rule. The control terminal half-life of vitamin K₁ (2.36 ± 0.8 h), which is similar to that reported previously for vitamin K₁ (Shearer *et al.*, 1972; Bjornsson *et al.*, 1979) was also not altered (2.02 ± 0.34 h) by phenobarbitone. Vitamin K₁ 2, 3-epoxide was not detected in plasma either before or after phenobarbitone treatment.

Discussion

Vitamin K₁ is a co-factor for the post-ribosomal synthesis of clotting factors II (prothrombin), VII, IX and X (Uotila & Suttie, 1982). During clotting factor synthesis, vitamin K₁ is converted into an inactive metabolite, vitamin K₁ 2, 3-epoxide, which is normally reduced back to the vitamin by an epoxide reductase. The intercon-

Table 1 The effect of phenobarbitone (100 mg daily for 14 days) on the plasma pharmacokinetic parameters of antipyrine and the urinary excretion of 6 β -hydroxycortisol (expressed as a ratio with 17-hydroxycorticosteroids). Values are mean $\pm$ s.d.

	Control	Phenobarbitone
Antipyrine half-life (h)	14.2 $\pm$ 4.4	10.4 $\pm$ 2.3*
Antipyrine clearance (ml min ⁻¹)	33.9 $\pm$ 5.8	54.9 $\pm$ 10.6**
Antipyrine volume of distribution (l)	41.4 $\pm$ 8.3	46.8 $\pm$ 7.1
Urinary 6 β -hydroxycortisol		
Urinary 17-hydroxycorticosteroids (ratio)	3.31 $\pm$ 0.61	7.13 $\pm$ 2.50*

* $P < 0.05$, ** $P < 0.001$; using paired Student's *t*-test, when compared to control.

Figure 1 Plasma concentrations of *trans*-vitamin K₁ in seven volunteers following intravenous administration of the vitamin (10 mg) before (●) and after (○) daily administration of phenobarbitone (100 mg) for 14 days.

version of the vitamin and its 2, 3-epoxide occurs primarily in the rough endoplasmic reticulum of hepatocytes and is referred to as the vitamin K₁-K₁ epoxide cycle (Willingham & Matschiner,

1974). However, the major route of vitamin K₁ metabolism is thought to involve ω -oxidation and β -oxidation in the mitochondria followed by glucuronidation in the endoplasmic reticulum (McBurney *et al.*, 1980). Metabolism via the 2, 3-epoxide only becomes apparent after administration of coumarin anticoagulants (Shearer *et al.*, 1977).

In the present study, we have found that short-term administration of a therapeutic dose of phenobarbitone does not appear to affect either route of vitamin K metabolism. There was no change in the pharmacokinetics of the vitamin, and vitamin K₁ 2,3-epoxide was not detected in plasma.

In all volunteers, there was a significant increase in hepatic mixed-function oxidase activity as measured by antipyrine clearance and 6 β -hydroxycortisol excretion. It is therefore unlikely that vitamin K₁ can be diverted from its normal metabolic pathways, and inactivated by cytochrome P-450 enzymes in the endoplasmic reticulum. Such a mechanism has been proposed for the 'wasting effect' on vitamin D by the enzyme inducing agents phenytoin, phenobarbitone and rifampicin (Hahn *et al.*, 1972; Brodie *et al.*, 1980). Therefore, the increased rate of metabolism of vitamin K₁ observed in rats and rabbits (Wilson & Park, 1984) given much larger daily doses (80 mg kg⁻¹ and 40 mg kg⁻¹ respectively) of phenobarbitone may be related to induction of other enzymes, increases in liver blood flow, or changes in the binding of the vitamin to plasma lipoproteins. We are now determining the disposition of vitamin K₁ in patients on long term anticonvulsant therapy, in order to investigate the effect of long term treatment on vitamin K₁ disposition.

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