Abnormal vitamin K metabolism in the presence of normal clotting factor activity in factory workers exposed to 4-hydroxycoumarins

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- 1 The case histories of two patients exposed to the novel anticoagulants brodifacoum and difenacoum are reported.
- 2 Abnormal vitamin K₁ metabolism, as indicated by elevated vitamin K₁ 2,3-epoxide plasma concentrations after i.v. administration of vitamin K₁, could be detected for more than 18 months after exposure to the anticoagulants.
- 3 There was a marked prolongation of prothrombin time (> 50 s) in both cases, at the time of exposure. However, subsequent haematological investigations (prothrombin time and vitamin K-dependent clotting factor activity) have been shown to be normal in both cases for at least 18 months.
- 4 These cases confirm the long-acting nature of brodifacoum and difenacoum and present an apparent dissociation between the effect of coumarin anticoagulants on vitamin K₁ metabolism and clotting factor activity.

Keywords vitamin K brodifacoum difenacoum clotting factor

Introduction

Vitamin K is an essential co-factor for the post-ribosomal synthesis of clotting factors II, VII, IX and X (Stenflo & Suttie, 1977). During clotting factor synthesis vitamin K₁ is converted into an inactive metabolite vitamin K₁ 2,3-epoxide, which is rapidly reduced back to the vitamin by a microsomal epoxide reductase (Willingham & Matschiner, 1974; Bell, 1978). The cyclic inter-conversion of vitamin and epoxide is referred to as the vitamin K-epoxide cycle. It has been proposed that 4-hydroxycoumarin anticoagulants, such as warfarin, block clotting factor synthesis by inhibiting the regeneration of vitamin K₁ from the inactive vitamin K₁ 2,3-epoxide in plasma (Bell & Matschiner, 1972; Whitton *et al.*, 1978). Consistent with this hypothesis, it has been shown that administration of coumarin

anticoagulants such as warfarin, difenacoum and brodifacoum cause an accumulation of vitamin K₁ 2, 3-epoxide in plasma, in man and experimental animals, after administration of either a physiological or pharmacological dose of the vitamin (Shearer *et al.*, 1977; Park *et al.*, 1979, 1984; Choonara *et al.*, 1985). Furthermore, the effect of warfarin on vitamin K₁ metabolism is dose-dependent (Shearer *et al.*, 1977).

We wish to report an apparent dissociation between inhibition of vitamin K₁ 2,3-epoxide reduction and anticoagulant effect of the long-acting coumarin anticoagulants difenacoum and brodifacoum in two patients accidentally exposed, and consider the implications of these findings with regard to the mechanism of coumarin anticoagulation.

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Methods

The pharmacokinetics of vitamin K₁ and vitamin K₁ 2,3-epoxide were determined in plasma after intravenous administration of vitamin K₁ (Konakion; 10 mg) in each patient on several occasions as previously described (Park *et al.*, 1984). The limit of sensitivity for detection of both compounds was 25 ng ml⁻¹. Plasma concentrations of coumarin anticoagulants were measured by high performance liquid chromatography (Breckenridge *et al.*, 1985). Prothrombin time and the activity of individual clotting factors were measured using established procedures (Hall & Malia, 1984). Factor II related antigen was determined by one-dimensional immunoelectrophoresis (Malia *et al.*, 1980).

Results

Case 1

The first patient was a 26 year old Caucasian male who commenced work in the manufacture of coumarin anticoagulants (brodifacoum, difenacoum and warfarin) in 1978. During 1980 his prothrombin time became intermittently abnormal on routine examination at work and in May 1981 he was given oral vitamin K₁. By May 1982 his prothrombin time was abnormal despite daily vitamin K₁, which was then, however, stopped. He developed haematuria, bleeding gums and easy bruising resulting in his admission to hospital in June 1982. Investigations at this stage revealed the presence of difenacoum (60 ng ml⁻¹) in his plasma, a prothrombin time of 53 s (control 12.5 s) a partial thromboplastin time of 88 s (control 25 s), normal platelet count, thrombin time and fibrinogen level. He was discharged from hospital after responding to fresh frozen plasma and oral vitamin K₁. On discharge, his prothrombin time was still 20 s. He remained on oral vitamin K₁ until January 1983 by which time the prothrombin time was normal (12 s). He then recommenced work with coumarins and his prothrombin time subsequently again became elevated (66 s) in July 1983. Plasma analysis revealed the presence of both difenacoum and brodifacoum measured on several occasions and in the range of 30–50 ng ml⁻¹. From July 1983 onwards, he had no further industrial contact with 4-hydroxycoumarins. In order to determine the biochemical effect of coumarin exposure, we measured vitamin K₁ and vitamin K₁ 2,3-epoxide in plasma after i.v. administration of 10 mg of the vitamin (Park *et al.*, 1984) on several

occasions. Concentrations of vitamin K₁ 2,3-epoxide (300–500 ng ml⁻¹) were measured from 2–24 h after vitamin K₁ administration. The intravenous vitamin K₁ administration reduced prothrombin time from 66 to 30 s within 12 h. This study was repeated on a number of occasions during the next 18 months. Vitamin K₁ 2,3-epoxide concentrations were found to remain elevated (Figure 1), but the prothrombin time was always within the normal range (13.0, 13.5, 13.5 and 13.5 s). A more detailed haematological investigation (September, 1984) showed normal activities of clotting factors II, VII, IX and X (Table 1). There was no consistent increase in vitamin K-dependent clotting factor activity

Figure 1 (Case 1) Plasma concentration-time profiles for (a) vitamin K₁ and (b) vitamin K₁ 2,3-epoxide after i.v. administration of vitamin K₁ (10 mg). Vitamin K₁ was given on various occasions, May 1983 (○), November 1983 (□), January 1984 (△), May 1984 (●), January 1985 (■), after the last exposure to coumarin anticoagulants in July 1983.

Table 1 The effect of vitamin K₁ administration (10 mg; i.v.) on the activity of the individual vitamin K₁-dependent clotting factors in two patients exposed to 4-hydroxycoumarin anticoagulants

	II	VII	IX	X
<i>Case 1*</i>				
Pre	100	115	76	98
0.5 h	100	98	74	100
1.0	100	110	69	102
2.0	92	145	76	105
4.0	100	150	72	102
8.0	96	135	79	102
<i>Case 2*</i>				
Pre	90	135	69	115
0.5 h	106	145	67	120
1.0	130	135	84	125
2.0	130	160	83	120
4.0	105	160	117	120
8.0	106	145	90	115

* Data obtained after vitamin K₁ administration to Case 1 (September 1984) and Case 2 (November 1984). The activities of the individual clotting factors are expressed as a percentage of the normal value obtained using pooled serum. The normal range for the factors are as follows; II (60–167%), VII (58–162%), IX (56–175%), X (58–124%).

following the administration of vitamin K₁. Although there was a transient rise in factor VII (2–4 h), the activity remained within the normal range. Further immunochemical analysis showed factor II related antigen (Malia *et al.*, 1980) was in the normal range and not influenced by the administration of vitamin K₁. Thus, there is no evidence for vitamin K₁-induced clotting factor synthesis (Choonara *et al.*, 1985). To investigate the possibility of a genetic deficiency in this individual, the metabolism of vitamin K₁ (10 mg) was studied in both parents and two female siblings; no vitamin K₁ 2,3-epoxide (< 25 ng ml⁻¹) could be detected during 12 h after vitamin K₁ administration.

Case 2

The patient was a 28-year-old Caucasian male who began work with coumarin anticoagulants in 1977. In July 1981 he was admitted to hospital with spontaneous bruising and haematuria, he had a prothrombin time of 100 s and partial thromboplastin time of 80 s. Platelet count, thrombin time and fibrinogen levels were all normal. He responded appropriately to administration of vitamin K₁ and fresh frozen plasma, was discharged from hospital and has remained asymptomatic since and has not been re-exposed to coumarin anticoagulants. Vitamin K₁ meta-

bolism was studied twice in this individual after his prothrombin time had been normal for 3 years (Figure 2). On both occasions vitamin K₁ 2,3-epoxide was detected in plasma (100–200 ng ml⁻¹) after intravenous administration of vitamin K₁ (10 mg). Vitamin K₁-dependent clotting factor activities were in the normal range (Table 1), and vitamin K₁ administration did not produce a consistent increase in activity in any of the individual factors or in factor II related antigen.

Discussion

In this study we have found unexpectedly high concentrations of vitamin K₁ 2,3-epoxide in two

Figure 2 (Case 2) Plasma concentration-time profiles for (a) vitamin K₁ and (b) vitamin K₁ 2,3-epoxide after i.v. administration of vitamin K₁ (10 mg). Vitamin K₁ was given on two occasions, July 1984 (○) and November 1984 (●), after the last exposure to coumarin anticoagulants in July 1981.

individuals who, although previously exposed to coumarin anticoagulants, had normal clotting factor activities and antigen levels. In normal volunteers given vitamin K₁ intravenously, vitamin K₁ 2,3-epoxide levels remain below the limit of detection of the assay used in this and previous studies (Park *et al.*, 1984). Plasma concentrations of the epoxide observed in case 1 are within the range observed in patients given oral anticoagulants such as warfarin for therapeutic purposes (prothrombin time 22–37 s) (Choonara *et al.*, 1985). A genetically determined disorder was unlikely since vitamin K₁ 2,3-epoxide could not be detected in the plasma of this individual's parents, or two sisters, after vitamin K₁ administration. Vitamin K₁ 2,3-epoxide was measured in plasma in Case 2, on two occasions, despite the fact that 3 years had elapsed since exposure to coumarin anticoagulants.

The plasma concentration–time profile for vitamin K₁ remained constant throughout the study in each patient (Figures 1 and 2). Coumarin anticoagulants, however, do not alter plasma disposition of either physiological or pharmacological doses of vitamin K₁ (Shearer *et al.*, 1977; Choonara *et al.*, 1985).

Both patients had been exposed to the rodenticides brodifacoum and difenacoum at work, and it is assumed that these agents are responsible for the abnormal vitamin K₁ metabolism observed. Indeed, both anticoagulants were detected in plasma in Case 1. The anticoagulants were not detected in the second patient (3 years after last exposure) but this does not preclude the presence of either brodifacoum or difenacoum in the liver, where they are known to accumulate (Breckenridge *et al.*, 1985). The source of exposure was not definitively determined. It was however probably due to either inhalation or percutaneous absorption of the 4-hydroxycoumarins during the manufacturing process. The exposure was of a chronic nature.

Previous studies have indicated that brodifacoum and difenacoum have a similar mode of action to warfarin (Park *et al.*, 1979), that is, inhibition of vitamin K epoxide reductase, but have a much longer duration of action in the rabbit (Park & Leck, 1982) and in man (Barlow *et al.*, 1982). The plasma half-life of brodifacoum in man determined in a single patient poisoned with the compound was 487 h (Breckenridge *et al.*, 1985). This is approximately tenfold greater than the plasma half-life for racemic warfarin in man (O'Reilly *et al.*, 1963). We have observed a gradual, but consistent, fall in the 24 h vitamin K₁ 2,3-epoxide plasma concentration (Figure 1) in case 1 over the period of investigation.

These cases raise two important questions about the mechanisms of action of vitamin K and 4-hydroxycoumarin anticoagulants. Firstly, the unexpectedly high concentrations of vitamin K₁ 2,3-epoxide observed in the presence of normal clotting factor activities and antigen levels, indicates an apparent dissociation between the inhibition of clotting factor synthesis and the inhibition of the enzyme vitamin K₁ 2,3-epoxide reductase. It is possible that partial inhibition of vitamin K₁ 2,3-epoxide reductase is not sufficient to interfere with clotting factor synthesis; we are exploring this possibility by examining the effects of low-dose warfarin on the relationship between vitamin K₁ metabolism and clotting factor synthesis. An alternative explanation, based on *in vitro* studies (Hildebrandt & Suttie, 1982; Preusch & Suttie, 1984), is that coumarin anticoagulants act by inhibiting other enzymes (e.g. vitamin K quinone reductase) in the physiologically important vitamin K-epoxide cycle.

Secondly, vitamin K epoxidation is thought to be chemically linked to the carboxylation event in clotting factor synthesis (Suttie *et al.*, 1978). Thus a direct relationship has been observed between these processes in man (Choonara *et al.*, 1985) and in animals (Bell 1978; Friedman & Smith, 1979). However, these two chemical reactions may be dissociated *in vitro*, either by addition of potassium cyanide (Larson *et al.*, 1981) or by changing the procedure for the preparation of hepatic microsomes (Canfield *et al.*, 1980). The high levels of vitamin K₁ 2,3-epoxide measured in the present study (Figures 1 and 2), without any concomitant change in clotting factor synthesis, suggest that such a dissociation may also occur *in vivo*.

Finally, we must consider the question of surveillance for industrial exposure to 4-hydroxycoumarin anticoagulants. Measurement of prothrombin time is a satisfactory method for monitoring patients on anticoagulant therapy, although it has been suggested recently that determination of native prothrombin antigen is superior (Furie *et al.*, 1984). However, if prothrombin time was the only criteria used for the two individuals in this report, both would have been considered suitable for working with 4-hydroxycoumarin anticoagulants again for the past 18 and 36 months, respectively, despite the fact that the vitamin K data strongly suggest the presence of coumarin anticoagulants in liver.

We suggest, therefore, that investigation of vitamin K metabolism might provide a more sensitive assessment of industrial coumarin exposure (accumulation), than monitoring prothrombin time and we are currently examining

the relationship between changes in vitamin K metabolism and changes in clotting factor synthesis induced by coumarin anticoagulants.

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Note added in proof

Determination of plasma epoxide concentrations in October 1985 have shown a marked fall in C_p max from 350–470 ng ml⁻¹ to 120 ng ml⁻¹ in case 1, and from 161–165 ng ml⁻¹ to 82 ng ml⁻¹ in case 2. There has been a steady fall in the epoxide concentration at 24 h over the last 2 years in case 1, with an apparent elimination half-life of 6.1

months. There is similar fall in the 5 h plasma epoxide concentration (the last measurable time point in October 1985) for case 2, apparent elimination half-life 6.9 months. The fall in epoxide concentrations provide further evidence that the abnormal vitamin K₁ metabolism described is a result of exposure to brodifacoum and difenacoum.