Differential effect of the enantiomers of phenprocoumon and warfarin on the vitamin K_1-epoxide/vitamin K_1 ratio in rat plasma

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Evidence has accumulated that the hypoprothrombinaemic effect of oral anticoagulant drugs is closely related to their ability to inhibit an enzyme which reduces the biologically inactive vitamin K_1-epoxide to the native vitamin (vitamin K epoxide reductase) thereby interrupting the physiologically important vitamin K_1-vitamin K_1-epoxide interconversion (Bell & Matschiner 1972; Matschiner et al 1974; Willingham & Matschiner 1974). As a consequence, vitamin K_1-epoxide accumulates in the livers of anticoagulant-treated rats (Matschiner et al 1970; Sadowski & Suttie 1974). It has also been shown that vitamin K_1-epoxide accumulates in the plasma of men treated with tritium labelled vitamin K_1 and warfarin (Shearer et al 1973, 1977). If the inhibition of vitamin K_1-epoxide reductase by oral anticoagulant drugs is linked to their anticoagulant activities, and if plasma serves as a simple tool to follow the metabolism of vitamin K_1, differences would be expected in the accumulation of vitamin K_1-epoxide in plasma when animals are treated with the individual enantiomers of phenprocoumon and warfarin, because these have been shown to differ in their intrinsic anticoagulant activities in that in man and rat the *S*(−)-enantiomer is about 2 to 4 times more potent than the *R*(+)-enantiomer (Breckenridge & Orme 1972; Breckenridge et al 1974; Levy et al 1974; O'Reilly 1974; Yacobi & Levy 1974; Jähnchen et al 1976; Schmidt & Jähnchen 1977).

Male inbred Lewis rats (Zentralinstitut für Versuchstiere, Hannover, Germany), 280–330 g, were used because of the small intersubject variations with respect to the measured parameters (cf. Fig. 2). A polyethylene cannula was implanted into the right jugular vein under slight ether anaesthesia. Four days later these rats received a single injection of [^{3}H]vitamin K_1† (45 μCi kg^{-1}, dissolved in Tween 80 and diluted with 0·9% w/v NaCl). The specific activity of [^{3}H]vitamin K_1 was 180 μCi mg^{-1}. The enantiomers of phenprocoumon and warfarin were injected through the cannula 30 min before the injection of [^{3}H]vitamin K_1. Blood samples of about 0·4 ml were taken at 0·25, 1, 2, 3, 4, 6 and 8 h following the injection of [^{3}H]vitamin K_1. Plasma samples of 0·1ml were extracted twice with chloroform–methanol (Shearer et al 1970) and the combined chloroform phases were evaporated to dryness under nitrogen. [^{3}H]vitamin K_1 and [^{3}H]vitamin K_1-epoxide were assayed by reversed-phase thin layer chromatography (Shearer et al 1973). As a reference substance, unlabelled vitamin K_1-epoxide was synthesized by oxidation of vitamin K_1 with alkaline H_2O_2 solution in ethanol (Tishler et al 1940). Proton magnetic resonance spectra indicated that the epoxide was formed exclusively on the naphthoquinone olefinic double bond and not on the phytyl side chain double bond.

Fig. 1 shows a typical experiment in a control rat (treated only with [^{3}H]vitamin K_1) and in a rat which received in addition a dose of 0·3 mg kg^{-1} racemic phenprocoumon. Plasma [^{3}H]vitamin K_1 declined at least biexponentially with time. There was apparently no difference in the plasma concentration/time course of vitamin K_1 between controls and anticoagulant-treated rats. This finding accords with experiments in man (Shearer et al 1977). The mean half-life for the two exponential components was 0·58 (0·09) (mean and s.d.) and 2·52 (0·27) h for the fast and slow component, respectively.

The plasma concentrations of vitamin K_1-epoxide, however, increased after treatment with phenprocoumon (Fig. 1). For the construction of log anticoagulant dose-response relationships the areas under the plasma concentration/time curves (from 0 to 8 h) of vitamin K_1 and vitamin K_1-epoxide were estimated by the trapezoid rule and the area ratios (vitamin K_1-epoxide/vitamin

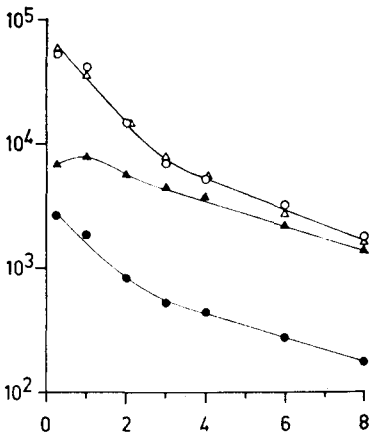

FIG. 1. Time course of [^{3}H]vitamin K_1 (open symbols) and vitamin K_1-epoxide (closed symbols) in the plasma of two rats. One rat received a single dose (45 μCi kg^{-1}) of [^{3}H]vitamin K_1 only (circles), the other rat received in addition a single dose (0·3 mg kg^{-1}) of racemic phenprocoumon 30 min before the injection of vitamin K_1 (triangles). Time zero indicates the time of injection of [^{3}H]vitamin K_1. Ordinate: dpm 100 μl^{-1}. Abscissa: time (hours).

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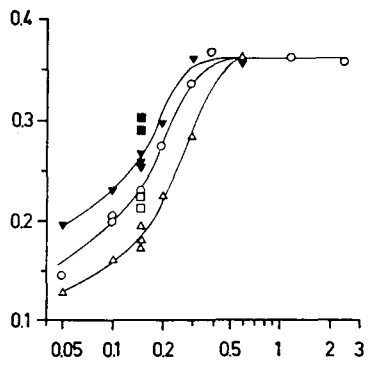

FIG. 2. Relationship between the ratios (area$_{\text{vitamin K}_1\text{-epoxide}}$/area$_{\text{vitamin K}_1}$) and the log of the dose of $R(+)$-phenprocoumon (△), racemic phenprocoumon (○), $S(-)$-phenprocoumon (▼), $R(+)$-warfarin (□), and $S(-)$-warfarin (■). Each symbol represents one rat. The mean ratio in 4 control rats (these rats received only vitamin K_1) was 0·054 (0·004) (mean with s.d.). Ordinate: area ratio. Abscissa: dose of anticoagulant (mg kg^{-1}).

K_1) were taken as a quantitative measure of epoxide formation. Fig. 2 shows the dose-response relationships obtained for the enantiomers of phenprocoumon and the racemate. For all three forms there is a gradual increase in the vitamin K_1-epoxide/vitamin K_1 area ratio when the dose of the three forms was increased from 0·05 to about 0·5 mg kg^{-1}. The maximal effect obtained seems to be similar for all forms (area ratio of about 0·36). However, the dose-response relationship for $S(-)$-phenprocoumon was shifted to the left when compared to $R(+)$-phenprocoumon. The dose-response relationship of racemic phenprocoumon (which consists of equal parts of $R(+)$- and $S(-)$-phenprocoumon) was between that of the enantiomers. In addition, when equal doses (0·15 mg kg^{-1}) of the warfarin enantiomers were compared the area ratio obtained following $S(-)$-warfarin was markedly greater than that of $R(+)$-warfarin (Fig. 2).

The dose-response relationship obtained for racemic phenoprocoumon in rats is similar to that obtained for racemic warfarin in man (Shearer et al 1977). These authors found no apparent differences in the accumulation of epoxide in the plasma following doses of 764 μg kg^{-1} $R(+)$- and $S(-)$-warfarin. These warfarin doses, however, were thought to be supramaximal (Shearer et al 1977).

Our results clearly demonstrate stereoselective differences in the accumulation of vitamin K_1-epoxide in the plasma in that the more anticoagulant active $S(-)$-enantiomers of phenprocoumon and warfarin also elicit a greater accumulation of the epoxide metabolite. This observation may be taken as further support for the hypothesis that the effect of anticoagulant drugs on the vitamin K_1-vitamin K_1-epoxide cycle is closely linked to the inhibition of prothrombin synthesis (Bell et al 1976; Ren et al 1977; Shearer et al 1977; Whitlon et al 1978).

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