ANTICOAGULANT ACTIVITY OF THE ENANTIOMERS OF ACENOCOUMAROL

The mono-coumarin derivatives warfarin, phenprocoumon and acenocoumarol used clinically are racemic mixtures, consisting of equal parts of R(+) and S(−) enantiomers. For warfarin and phenprocoumon the S(−) enantiomers were found to be 2 to 5 times more potent than the R(+) enantiomers in both man (Hewick & McEwan, 1973; Breckenridge, Orme, Wesseling, Lewis & Gibbons, 1974; O'Reilly, 1974; Levy, O'Reilly & Wingard, 1974; Jähnchen, Meinertz, Gilfrich, Groth & Martini, 1976) and rats (Ebele, West & Link, 1966; Breckenridge & Orme, 1972; Hewick, 1972; Yacobi & Levy, 1974; West & Link, 1965; Schmidt & Jähnchen, 1977). From these findings it was concluded that the configuration greatly influences the pharmacologic effect of these drugs (Lewis, Trager, Chan, Breckenridge, Orme, Roland & Schary, 1974; Jähnchen *et al.*, 1976).

We were surprised by the observation that for the structurally related drug acenocoumarol the R(+) enantiomer is more potent as anticoagulant than the S(−) enantiomer. We briefly report on the anticoagulant activity of these enantiomers in man and rats.

Racemic acenocoumarol and the optical enantiomers of acenocoumarol were kindly supplied by Ciba-Geigy, Basel. The optical rotation of the S(−) acenocoumarol was -225° (1% in 0.5 N NaOH) and

of R(+) acenocoumarol + 223° (1% in 0.5 N NaOH), as performed by Ciba-Geigy, Basel. Racemic acenocoumarol was an equal mixture of the two enantiomers.

Three randomly selected groups each of five Sprague-Dawley rats (weighing from 250–350 g) received a single i.v. dose (1.5 mg/kg) of each form of acenocoumarol.

Collections of several blood samples were performed by puncture of the tail artery and clotting times were measured in plasma samples of 100 μ l using Thromborel (Behringwerke AG, Marburg). The samples had been kept frozen at -20°C until analysis.

Four healthy male volunteers received in a cross-over experiment 0.25 mg/kg of both enantiomers i.v. One subject received in addition 0.5 mg/kg S(−) acenocoumarol. An explorative determination of prothrombin times was performed in each freshly drawn plasma sample. Values presented were determined twice at the end of each study in plasma samples which had been kept frozen at -20°C and were thawed just before measurements. The measurements were always performed by the same investigator using the same batch of Thromborel throughout.

The time course of the anticoagulant effect of the three forms of acenocoumarol in rats is shown in Figure 1. For R(+) acenocoumarol the mean maximum prolongation of the prothrombin time was

Figure 1 Prothrombin times (mean $\pm$ s.e. mean, $n = 5$) in three groups of rats following a single dose (1.5 mg/kg i.v.) of R,S-(Δ), S-($\bullet$) and R-acenocoumarol ($\circ$). The range of prothrombin times in untreated animals is from 13–14.5 s (shaded area).

Figure 2 Prothrombin times obtained in cross-over experiments in four volunteers following a single dose (0.25 mg/kg i.v.) of R-($\circ$) and S-acenocoumarol ($\bullet$). Subject B received in addition 0.5 mg/kg S-acenocoumarol i.v. ($\bullet$). The anticoagulant response in subject C required treatment with vitamin K (40 mg orally). The normal range of prothrombin times is shown by the shaded area.

34.5 seconds and occurred after 18 h following a single i.v. dose of 1.5 mg/kg. For S(-) acenocoumarol the mean maximum prolongation was 19.3 s and occurred after 12 h. The mean activity of racemic acenocoumarol was always between that of the enantiomers. In additional experiments the anticoagulant activity per dose of 0.6 mg/kg R(+) acenocoumarol was compared with 4.5 mg/kg S(-) acenocoumarol by measuring the area under the prothrombin time vs. time curve. In these experiments the area of S(-) acenocoumarol was about 75% of that of R(+) acenocoumarol.

In the four healthy volunteers 0.25 mg/kg of R(+) acenocoumarol showed a distinct prolongation of the prothrombin time (Figure 2). There were however considerable intersubject differences in the anticoagulant response. Whereas in subject D only a slight effect was observed, the anticoagulant response in subject C required treatment with vitamin K (40 mg orally). The maximum effect occurred after 24–36 h. The administration of the same dose of S(-) acenocoumarol was ineffective in all but subject C. An additional experiment was performed in Subject B using a dose of 0.5 mg/kg S(-) acenocoumarol. Even this dose produced only a slight anticoagulant effect (Figure 2).

The results show that in man and rat (R+) acenocoumarol is several times more potent as anticoagulant than S(-) acenocoumarol. This is opposite to

results obtained with the enantiomers of warfarin and phenprocoumon. This is surprising, since acenocoumarol differs from warfarin only by a nitrogroup in para position of the phenyl ring. Whether the greater anticoagulant activity of R(+) over the S(-) acenocoumarol reflects stereoselective differences at the receptor site or whether stereoselective differences in the rate of elimination are responsible has to be determined. Stereoselective differences in the metabolism could also generate pharmacologic active metabolites predominantly from the R(+) form. Such metabolites with an even higher anticoagulant activity than the parent drug (when tested in mice) have recently been detected in human plasma and urine after administration of racemic [¹⁴C]-acenocoumarol (Dieterle, Faigle, Montigel, Sulc & Theobald, 1977). In either way, when acenocoumarol is used in therapy the R(+) form seems to be the pharmacologic active component. This just opposite to phenprocoumon and warfarin.

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