PHARMACOKINETICS OF ETHOXYCOUMARIN AND HYDROXYCOUMARIN IN THE URETHANE-ANAESTHETISED RAT

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One of the most frequently employed enzyme assays for assessing drug metabolising capacity in vitro involves the O-deethylation of ethoxycoumarin (EC) to yield hydroxycoumarin (HC) (Prough et al 1978). This reaction has been extensively studied in microsomes and isolated cells from several animal species under a variety of perturbed states. However the complete lack of in vivo information on EC deethylation limits the interpretation of the in vitro data. The present investigations are concerned with delineating the pharmacokinetics of EC and HC using a newly developed analytical method.

Male Sprague-Dawley rats (250g) were anaesthetised with urethane (1.3g/kg) and the jugular vein and carotid artery cannulated. Either EC (130μmoles/kg) or HC (3μmoles/kg) were administered iv and arterial samples taken serially over 3h. Blood samples (250μl) were mixed with heparin and internal standard (propoxycoumarin) and extracted with chloroform. The concentrated extracts were analysed using a 25cm Hypersil SAS 5μm column and a mobile phase consisting of 25% acetonitrile in water containing 0.5% acetic acid (0.2M). Quantitation was achieved by means of UV and fluorimetric detectors in series; UV detection at 315nm for EC and fluorescence (380nm excitation, 470nm emission; following a post column eluent change to pH 11 with sodium hydroxide-glycine buffer) for HC. Replicate analysis (n = 5) of EC and HC at a concentration of 50 and 5μmoles/L, respectively, gave a coefficient of variation of 5% or less.

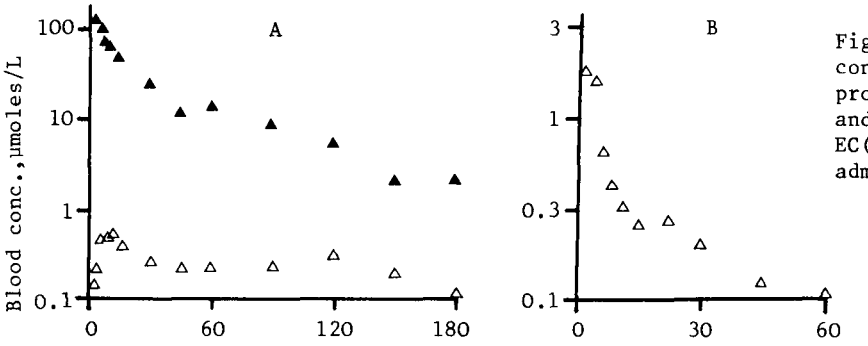

Fig. 1.Blood conc.-time profiles for EC(▲) and HC(△) after EC(A) or HC(B) administration

As illustrated in Fig. 1, following administration of EC (n = 7) blood concentrations of EC decrease in a biexponential fashion with initial and terminal half-lives of 4 ± 2 and 43 ± 12 min, respectively. After HC administration (n = 7) HC blood concentrations also decline in a biexponential fashion with on average shorter half-lives than observed with EC, 2 ± 0.4 and 35 ± 9 min. The volume of distribution is similar for both compounds 2.9 ± 1.2 and 7.4 ± 2.1 L/kg for EC and HC respectively. Both compounds are highly cleared; EC 48 ± 19 and HC 137 ± 36 mls/min/kg. HC blood concentrations increase rapidly after EC dosing and maximum concentrations of 0.62 ± 0.24μmoles/L are achieved by approximately 5 min. The concentration-time profiles for HC after EC dosing and HC after administration per se are similar at times after 5 min, despite the approximate 50 fold difference in dose. We believe the large differential in HC concentrations observed in the first 5 min is a consequence of the rapid sequential metabolism of HC to HC conjugates prior to leaving the site(s) of formation since urinary recovery studies indicate that conversion of EC to HC represents the predominant pathway for elimination of EC.

Prough, R.A., Burke, M.D., Mayer, R.T. (1978). Meth.Enz. 52 : 372-377.