PREDICTION OF PHENYTOIN CLEARANCE IN-VIVO FROM IN-VITRO PHENYTOIN OXIDASE ACTIVITY

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Phenytoin is a widely used anticonvulsant with a well defined therapeutic plasma concentration window. In man, as well as in rodents, the low Km for the metabolism of phenytoin results in nonlinear pharmacokinetics within the therapeutic dose range. Hepatic cytochrome P450 oxidises phenytoin to produce phenols and dihydrodials via an arene oxide. We have investigated in rats the kinetics of the initial oxidation of phenytoin *in vivo* and *in vitro* using hepatic microsomes and isolated hepatocytes. The use of the *in vitro* data to predict *in vivo* clearance of phenytoin has been evaluated.

A radiometric procedure (Gerber *et al*, 1971) has been used to characterise the kinetics of phenytoin oxidation *in vitro* using hepatic microsomes and isolated hepatocytes prepared from untreated male Sprague-Dawley rats using standard methodology. Over a range of 0.1-100μM substrate concentrations the kinetics were consistent with two components of enzyme activity - a high affinity, low capacity site and a low affinity, high capacity site. The activity of the low affinity site (as assessed by Vmax/Km ratio) was approximately one order of magnitude greater than the activity of the high affinity site in both systems. The difference between the Km for the high affinity site (0.6 and 19μM for microsomes and cells respectively) was partially due to non-specific binding in cells (58%±1%).

Five separate groups of rats, unanaesthetised, with previously implanted cannulae in the jugular and femoral veins received both an intravenous bolus and an intravenous infusion of [^{14}C]-phenytoin over a three hour time period. Jugular blood samples were specifically assayed for phenytoin and demonstrated that by varying the bolus dose and infusion rate (1.5-10.7 μmoles/h) steady-state concentrations of 1.6-90μM were achieved. The increase in steady-state concentration with increase in infusion rate was disproportionate and could be analysed in terms of a modified Michaelis-Menten relationship with a Vmax of 93 nmoles/min/250g body weight and a Km of 11μM for the high affinity site. When expressed in terms of unbound concentration, the Km and intrinsic clearance of phenytoin was calculated to be 2μM and 47 ml/min/250g body weight, respectively. As with the *in vitro* systems a second low affinity site was evident with an unbound clearance of 7 ml/min/250g body weight.

In vitro data was scaled up to provide prediction of *in vivo* pharmacokinetic parameters by use of microsomal protein yield, hepatocyte yield, liver weight and degree of non-specific binding within the hepatocytes. Between the three systems, there was good agreement in the relative importance of the two classes of sites and reasonable agreement for the high affinity unbound Km. However the scaled Vmax was overestimated using cell data and underestimated using microsomal data. These discrepancies resulted in poor prediction of *in vivo* clearance from *in vitro* data.

Gerber, N. *et al* (1971) J. Pharmac. Exp. Ther. 178 : 567-579