IN-VITRO AND IN-VIVO ACTION OF MECHANISM-BASED INHIBITORS OF OXIDATIVE DRUG METABOLISM

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The quantitative and qualitative nature of the hepatic microsomal monooxygenase (cytochrome P450) system in an individual liver sample may be characterised *in vitro* using a battery of probe substrates and/or inhibitors (Burke & Wolf, 1987). The use of suicide or mechanism-based inhibitors offer a greater potential than most competitive inhibitors due to the irreversible nature of their effect. 1-aminobenzotriazole (ABT) is a suicide inhibitor of P450 acting via production of the highly reactive benzyne which covalently binds to P450 bridging two vicinal N atoms in the protoporphyrin ring system (Ortiz de Montellano, 1986). Chloramphenicol (CAP) inactivates P450 by virtue of the covalent modification of the apoprotein via a lysine residue (Halpert & Neal, 1980). We have compared the isozyme specificity of ABT and CAP in hepatic microsomes, from male Sprague-Dawley rats which had either received no pretreatment (C) or had been dosed with an enzyme inducer i.p. once daily for 3 days: phenobarbitone (PB), 80mg/kg and β-naphthoflavone (BNF), 100mg/kg which elevates cytochromes P450IIB and P450I by 40- and 70-fold respectively. In addition we have compared the *in vivo* time course of the mechanism-based inhibition following either ABT (50mg/kg) or CAP (120mg/kg) administration and sacrificing the rats after various times up to 36h.

Oxidative metabolising capacity was assessed by three assays which measured appearance of a fluorimetric metabolite-ethoxycoumarin O-deethylase (ECOD, a non-specific probe), methoxycourmarin O-demethylase (MCOD), a P450IIB specific probe) and ethoxyresorufin (EROD, a P450I specific probe). Induced microsomes were diluted to give comparable total P450 concentrations to C microsomes for these assays. Inhibitors were studied over a concentration range of 1μM-10mM. A 5 minute preincubation of microsomes with inhibitor and cofactor was carried out prior to substrate addition. In control microsomes both ABT and CAP were potent inhibitors. Concentrations as low as 1μM produced a measurable effect whereas a concentration of 30μM caused 50% inhibition of ECOD. PB and BNF microsomes were less sensitive to ABT requiring a concentration of approximately 100μM for 50% inhibition of ECOD. ABT inhibition of MCOD in C and PB microsomes showed marked similarities (50% inhibition at 70μM), as did inhibition of EROD in control and BNF microsomes (50% inhibition at 80μM). CAP inhibited ECOD and MCOD to similar degrees in C microsomes and these responses were similar to ABT. However there was a marked increase in potency in PB microsomes; the concentration of CAP producing 50% inhibition decreased by an order of magnitude.

In the *in vivo* studies ECOD, MCOD and EROD were maximally inhibited by ABT at 3 hours to 15-20% of control. EROD was fully restored at 12 hours, ECOD at 36 hours whilst MCOD was still depressed at 36 hours. CAP inhibition was also maximal at 3 hours although the effect was substantially less than ABT (30-50% of control). Recovery from CAP was uniformly fast and complete by 24 hours. Hence both ABT and CAP are effective mechanism-based inhibitors both *in vitro* and *in vivo* showing different patterns of selectivity towards P450 isozymes.

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