Biphasic Michaelis-Menten kinetics for ethoxycoumarin and phenacetin metabolism by human and rat hepatic monooxygenases

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There is now considerable evidence from animal studies for the existence of multiple forms of cytochrome P-450, the terminal electron acceptor of the microsomal monooxygenase system involved in the metabolism of drugs and other foreign compounds. There is some, though less convincing, evidence for the existence of more than one form of cytochrome P-450 in man. Although different forms of cytochrome P-450 have different substrate specificities there is considerable overlap in these specificities. We now present data which are compatible with the existence of at least two different forms of cytochrome P-450 in human liver. Studies were performed with two substrates, 7-ethoxycoumarin and phenacetin.

Wedge biopsies of human liver were obtained as previously reported (Boobis, Brodie, Davies, Fletcher & Saunders, 1978) and microsomal fraction prepared by differential centrifugation. 7-Ethoxycoumarin 0-deethylase activity was assayed by a minor modification of the method of Greenlee & Poland (1978) using 18 substrate concentrations between 1 and 1000 μM. Incubations contained 25 μg microsomal protein. In studies with male Wistar rat liver microsomes, 10 μg protein were used. The product, 7-hydroxycoumarin, was extracted and measured fluorimetrically. Phenacetin 0-dealkylase activity was determined by measurement of the product, paracetamol by computerised gas chromatography-mass spectrometry (GC-MS). Incubations were performed with 15 concentrations of phenacetin between 1 and 5000 μM with 100 μg microsomal protein. Paracetamol was extracted differentially at alkaline pH and converted to the trimethylsilyl derivative before measurement by GC-MS.

Eadie-Hofstee plots were constructed from the data and these were biphasic for both substrates. Michaelis-Menten constants were calculated for both of the phases.

For 7-ethoxycoumarin 0-deethylase activity of human liver Km_1 was 1.9 ± 0.5 μM, V_{max_1} 88 ± 24 pmol/mg microsomal protein/min, Km_2 220 ± 28 μM and V_{max_2} 0.79 ± 0.21 nmol mg^{-1} min^{-1} (mean ± s.e. mean, $n = 7$) where Km is the apparent Michaelis-Menten constant and V_{max} the maximum velocity of the reaction. With rat liver, biphasic kinetics were also observed with constants Km_1 2.3 ± 0.4 μM, V_{max_1} 260 ± 31 pmol mg^{-1} min^{-1}, Km_2 240 ± 57 μM and V_{max_2} 3.5 ± 0.8 nmol mg^{-1} min^{-1} ($n = 4$).

With phenacetin as substrate the kinetic constants obtained using human liver were Km_1 6.3 ± 1.5 μM, V_{max_1} 440 ± 160 pmol mg^{-1} min^{-1}, Km_2 250 ± 75 μM and V_{max_2} 1.0 ± 0.25 nmol mg^{-1} min^{-1} ($n = 5$). Activities in rat liver were similar to those obtained with human liver.

Preliminary studies with selective inhibitors of monooxygenase activity such as α-naphthoflavone and metyrapone suggest that the two components of activity may be further distinguished.

It thus seems likely that two different forms of cytochrome P-450 can metabolise 7-ethoxycoumarin and phenacetin to specific products in man. Whether the same two forms of cytochrome P-450 are involved in the metabolism of both substrates remains to be established.

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References

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