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Kinetics and Inhibition by Fluvoxamine of Phenacetin O-Deethylation in V79 Cells Expressing Human CYP1A2

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Abstract: The kinetics of phenacetin O-deethylation and its inhibition by fluvoxamine was investigated in a V79 cell line (V79MZh1A2) transfected with human CYP1A2. In four sets of experiments the apparent Km values for phenacetin O-deethylation ranged from 35 to 95 μM and the Ki for fluvoxamine-mediated inhibition of the reaction ranged from 2.7 to 14.5 nM, i.e. comparable to values obtained in human liver microsomes. The kinetic performance of the V79MZh1A2 cell line demonstrates its usefulness as an analytical tool in a variety of toxicological and drug metabolism studies involving CYP1A2.

Cell lines expressing specific cytochromes P450 (CYP) are important tools in the study of foreign compound metabolism and toxicity. A series of V79 cell lines devoid of spontaneous CYP activity has been constructed for stable expression of c-DNA's encoding a number of rat and human CYP forms (Doehmer *et al.* 1992; Wölfel *et al.* 1992). CYP1A2 plays a role in the elimination of a number of drugs as well as the generation of toxic and carcinogenic metabolites from paracetamol and aromatic amines, respectively. A V79 cell line expressing rat CYP1A2 has been shown to correspond to freshly isolated hepatocytes with respect to catalytic activity towards phenacetin, a prototype substrate (Jensen *et al.* 1993a). Recently, a V79 cell line transfected with human CYP1A2 (V79MZh1A2) showed catalytic activity towards the substrates, caffeine and theophylline (Fuhr *et al.* 1992; Wölfel *et al.* 1992). However, the kinetic performance of cell lines expressing this enzyme has yet to be determined. We have investigated the kinetics of phenacetin O-deethylation in this cell line with and without co-incubation with fluvoxamine, a selective serotonin reuptake inhibitor, which has been shown to be a potent and specific inhibitor of CYP1A2 (Brøsen *et al.* 1993; Rasmussen *et al.* 1994).

Materials and Methods

The construction of the V79MZh1A2 (formerly named XEMhI-A2MZ) cell line has been described in details elsewhere (Wölfel *et al.* 1992). The cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Uxbridge, U.K.) supplemented with 7.5% heat-inactivated mycoplasma- and virus-screened foetal calf serum (Gibco), penicillin (100 U/ml), and streptomycin (100 μg/ml). Selection of plasmid-containing cells was maintained by addition of G418 400 μg/ml (Gibco). Cells were kept from reaching confluence at any time. All incubations were performed at 37° in 5% CO_2 in air. Cells (400,000) were seeded in plastic petri dishes (9.6 cm^2) and incubated for 24 hr giving asynchronously growing populations. After being rinsed twice with 2.5 ml Hanks balanced salt solution the cultures were incubated for 4 hr with phenacetin (Sigma St. Louis, MO, U.S.A.) in 6 concentrations from 5 to 200 μM in 2.5 ml full medium without G418 and with and without fluvoxamine (Duphar, B. V. Weesp, Holland). Four sets of experiments were performed, one with fluvoxamine in 5 concentrations from 10 to 200 μM and three with 5 concentrations from 10 to 100 nM. The supernatant was removed and frozen at −20° for later analysis of phenacetin and paracetamol (Nordisk Droge, Copenhagen, Denmark) by HPLC as previously described (Jensen *et al.* 1993a). The Michaëlis-Menten constants V_{max} and K_m for formation of paracetamol and the inhibition constant K_i for fluvoxamine were determined by weighted (the square of the reciprocal value) least square regression analysis using the standard equation for competitive inhibition:

$$V = \frac{V_{max} \times S}{K_m \times \left(1 + \frac{I}{K_i}\right) + S}$$

where V is the rate and S and I are the substrate and inhibitor concentrations, respectively.

Results

No paracetamol could be detected in native V79 cells incubated with phenacetin. In the V79MZh1A2 cells the formation of paracetamol was linear with time up to 4 hr and seeded cell number up to 600,000 (data not shown). Less than 2% of the substrate was consumed during incubation. In seperate experiments in the cell lines incubated with paracetamol 1 μM or 5 mM for 4 hr no further metabolism by disappearance of paracetamol or formation of sulfate and glucuronide conjugate could be detected, respectively. The relationship between the rate of O-deethylation and phenacetin concentration adhered to Michaëlis-Menten kinetics (table 1) whereas the effect of fluvoxamine was compatible with competitive inhibition in Dixon (fig. 1) as well as Cornish-Bowden plots (not shown).

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Table 1.

Kinetics of phenacetin O-deethylation and inhibition by fluvoxamine in a V79 cell line (V79MZh1A2) expressing human CYP1A2.

Experiment no.	V_{max} (pmol/min./10^6 cells)	K_m (μM)	K_i (nM)
1	27±13	47±49	2.7±3.5
2	48±6	39±12	11.7±4.4
3	42±3	35±7	10.0±2.7
4	61±10	95±31	14.5±4.8

Values are estimates with S.D. obtained from weighted non-linear regression. fluvoxamine concentrations of 10–200 μM and 1–100 nM were used in experiment 1 and 2–4, respectively.

Discussion

The apparent K_m values for phenacetin O-deethylation (table 1) were completely in agreement with the corresponding values of 14–56 μM for the high affinity site estimated in human liver microsomes (Brøsen *et al.* 1993). The apparent V_{max} values in the present study were comparable to the values obtained in isolated rat hepatocytes and in V79 cells expressing rat CYP1A2 (Jensen *et al.* 1993a). The K_i values for the inhibition of paracetamol formation by fluvoxamine in the present V79 cells were approximately 10 times lower than the corresponding values of 120–240 nM obtained in human liver microsomes (Brosen *et al.* 1993; Rasmussen *et al.* 1994). This apparently more potent inhibition in the cell cultures may hypothetically be related to some extent of accumulation of fluvoxamine within the cells from the relatively large incubation volume. Tissue accumulation of fluvoxamine has been indicated by a volume of distribution in excess of 5 l/kg in animal studies (Dollery 1991). Regarding phenacetin and paracetamol the concentrations are identical within cells and in medium (Jensen *et al.* 1993a).

In the different series of incubation performed at different times, the kinetics of phenacetin O-deethylation and inhibition by fluvoxamine were reproducible (table 1). The simple approach of adding substrate to the medium and sampling from it for analysis of substrate disappearance and metabolite production resemble *in vivo* metabolism studies but with the advantage that only one CYP form is present. In human liver microsomes the contribution of other CYP forms to a catalytic activity, e.g. the low affinity phenacetin O-deethylation, frequently causes problems in interpretation of the role and/or inhibition of a high affinity site attributed to a specific CYP form. A number of toxicological end-points, including cytotoxicity and a whole battery of genotoxicity tests are applicable in CYP transfected V79 cells (Ellard *et al.* 1991; Doehmer *et al.* 1992; Jensen *et al.* 1993b; Kulka *et al.* 1993). Accordingly, the kinetic performance of the V79MZh1A2 cell line demonstrates its usefulness in toxicological and drug metabolism studies on the role and importance of human CYP1A2. Moreover, fluvoxamine as a potent inhibitor provides a valuable tool for comparative and mechanistic studies.

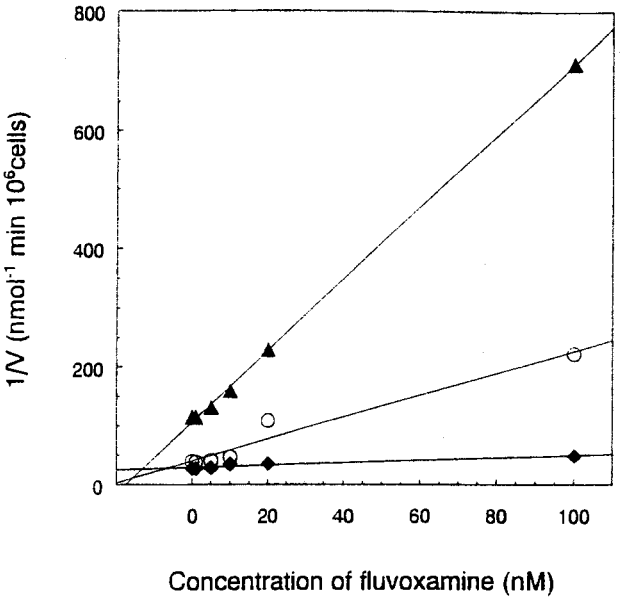

Fig. 1. Representative Dixon plot of the effect of fluvoxamine on the O-deethylation of phenacetin in a V79 cell line expressing human CYP1A2. Phenacetin concentrations: (▲) 10 μM, (○) 50 μM, (◆) 200 μM.

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