Effects of Common Organic Solvents on the Activity of Recombinant Human Cytochrome P450s Expressed in *E. coli.*

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Abstract

It has been documented that human cytochrome P450s, both in human liver microsomes and when expressed in insect cells, are inhibited to varying degrees by solvents commonly used to dissolve new chemical entities, however, very little data is available relating to the inhibition of cytochrome P450s expressed in *E. coli* by organic solvents. As *E. coli* expressed recombinant human cytochrome P450s are widely used during drug discovery, we have investigated the effects of methanol, ethanol, acetonitrile, dimethylsulphoxide (DMSO) and polyethylene glycol 400 (PEG) on the activity of CYPs 3A4, 2D6, 1A2, 2C9 and 2C19 co-expressed in *E. coli* with human NADPH P450 reductase.

All of the solvents tested inhibited all of the CYPs at the highest concentration (10% v/v) tested but, at lower concentrations, some solvents had markedly different, enzyme- and solvent-specific effects on the activity of the CYPs. For example, 7-ethoxycoumarin turnover by CYP1A2 was significantly activated by acetonitrile up to a concentration of 5% v/v while CYP3A4 was inhibited at all concentrations of acetonitrile tested.

The varying effects of solvents on the activity of these five enzymes should be taken into account along with solubility considerations when conducting in vitro metabolism studies.

Introduction

New chemical entities (NCEs) are routinely screened *in vitro* for metabolism by, and inhibition of, cytochrome P450s, the principal enzyme family involved in the oxidation of xenobiotics. Because NCEs are often dissolved in organic solvents, it is important to know the effects these solvents may have when present in the assay mix. Although it has been shown that cytochrome P450s in human liver microsome preparations (Hickman, D. *et al* 1998, Chauret, N. *et al* 1998) and baculovirus expressed recombinant cytochrome P450s (Busby, W. F. *et al* 1999) are affected by the presence of organic solvents, there is little or no data relating to the effects of solvents on the activities of cytochrome P450s expressed in *E. coli*. As *E. coli* expressed enzymes are becoming widely used in routine screening programmes, we have investigated the effects of five solvents, acetonitrile, methanol, ethanol, dimethyl sulfoxide (DMSO) and polyethylene glycol (PEG) that are commonly used to dissolve NCEs on the activity of 5 human cytochrome P450s (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) co-expressed in *E. coli* with NADPH P450 reductase.

Materials & Methods

All chemicals were obtained from Sigma-Aldrich or VWR unless otherwise indicated. Recombinant human cytochrome P450s co-expressed in *E. coli* with human NADPH P450 reductase (Bactosomes) and 4' hydroxydiclofenac were supplied by Cypex Ltd..

Enzymes were assayed at 37°C for 5 minutes in 50 mM (CYP3A4 100 mM) potassium phosphate pH 7.4, 5 mM magnesium chloride total assay volume 1 ml. The reactions were started by the addition of 0.2 ml 5x NADPH generating system (5 mM NADPH, 25 mM glucose-6-phosphate, 5 U/ml glucose-6-phosphate dehydrogenase). Reactions were stopped by the addition of 0.1 ml 1 M HCl (3A4, 1A2 & 2D6), 0.5 ml acetonitrile (CYP2C19) or 1 ml methanol (2C9). Samples were centrifuged at 13,000 rpm in a microfuge and the supernatants analysed by HPLC.

Substrates were as follows:

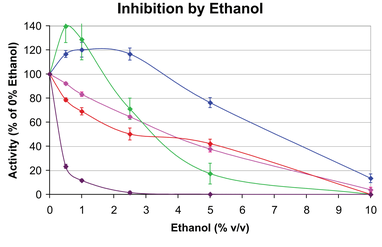
P450	Substrate	Concentration (at or close to Km)
CYP1A2	7-ethoxycoumarin	2 µM
CYP2C9	Diclofenac	2 µM
CYP2C19	Diazepam	20 µM
CYP2D6	Dextromethorphan	0.4 µM
CYP3A4	Testosterone	40 µM

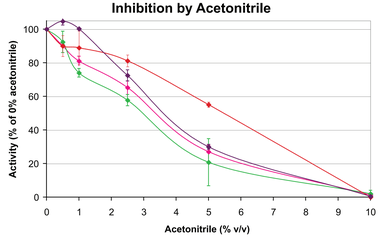
Results

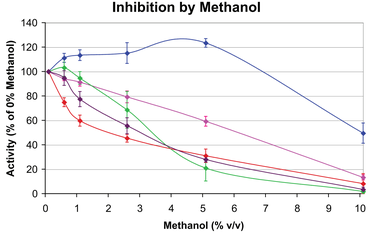
See graphs on right for results summary, values are mean of data from 3 different batches of P450 ± standard error.

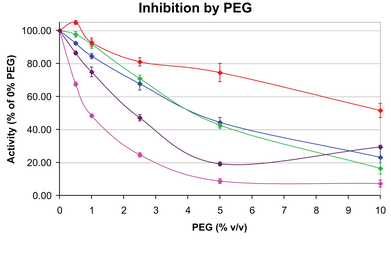
At the highest solvent concentration tested (10% v/v), all of the cytochrome P450s were inhibited by all of the solvents, however, at lower concentrations the effects of including solvent in the assay mix varied. The activity of most of the P450s was reduced by at least 20% by solvent concentrations in the assay mix above 2.5% v/v. The exception was CYP1A2 which demonstrated a six fold increase in 7-ethoxycoumarin-O-deethylase activity by the inclusion of acetonitrile at 2.5% v/v and was activated, albeit by a lesser amount, by low concentrations (2.5% v/v or less) of ethanol, methanol and DMSO. The dextromethorphan-O-demethylase activity of CYP2D6 was also increased in the presence of 1% v/v or less ethanol.

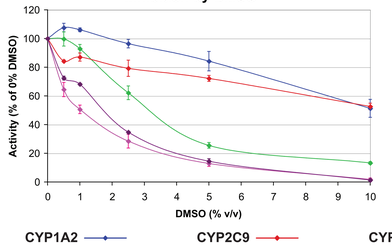
There was no solvent tested that stood out as a universal strong inhibitor for all of the P450s, indeed, the P450s varied considerably in their sensitivity to the different solvents. For example, CYP3A4 was strongly inhibited by PEG and DMSO (50% inhibition at 1% v/v) while the diazepam-N-demethylase activity of CYP2C19 was most sensitive to ethanol (nearly 80% reduction in activity at 0.5% v/v) and was only reduced by 25% by 1% v/v PEG.

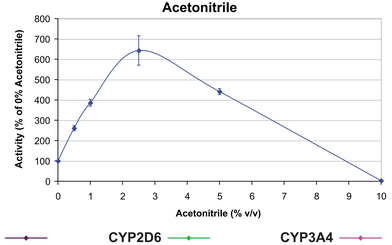












CYP1A2 — CYP2C9 — CYP2C19 — CYP2D6 — CYP3A4

Conclusions

As with other sources of cytochrome P450 used in *in vitro* assay systems, recombinant enzymes produced in *E. coli* are affected by the presence of solvents commonly used for dissolving new chemical entities. The effects are solvent and P450 specific and range from strong inhibition (e.g. ethanol and CYP2C19) to activation (e.g. acetonitrile and CYP1A2).

In general, the effects of the solvents on *E. coli* expressed cytochrome P450s mirrored those seen with baculovirus expressed P450s and human liver microsomes (see refs below). Where a difference was seen, e.g. CYP2C19 Bactosomes inhibited by 32% by 1% DMSO compared to 62% in HLM (Chauret *et al* (1998)), it could be attributed to the use of a different substrate to perform the assay. The substrate we used was diazepam whereas Chauret *et al* used (*S*)-mephenytoin. This is supported by the fact that variations are seen between separate studies using human liver microsomes: Chauret *et al* (1998) found the phenacetin-O-deethylase activity of CYP1A2 to be unaffected by 1% methanol while Hickman *et al* (1998) found the caffeine-N3-demethylation activity of CYP1A2 to be strongly inhibited (> 70% inhibition) by 1% methanol.

We have demonstrated that *E. coli* expressed CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 respond in a similar way to the same P450s in human liver microsomes and those produced using the baculovirus expression system. The same care needs to be taken, therefore, when using *E. coli* expressed cytochrome P450, to include the appropriate controls to account for solvent effects, as when using cytochrome P450s derived from other sources.

References

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