Introduction

Induction of cytochrome P450 (CYP) enzymes is one of the principal mechanisms of drug-drug interactions inasmuch as regulators recommend that new drug candidates be evaluated for their ability to induce CYP enzymes. The FDA (US), EMA (Europe) and PMDA (Japan) recently revised the guidelines for evaluating drug-drug interactions (FDA, 2012). According to this new guidance, a new chemical entity (NCE) should be evaluated at very high concentrations in vitro (e.g., (0.1-1.0) x 250 mM) or 50% the total Vmax (e.g., (0.1-1.0) x 100 μM). Achieving these concentrations is often possible if the test article is soluble in preferred solvents (e.g., 0.1% v/v DMSO) and/or cell culture medium. Consequently, test articles are evaluated for solubility in other solvents; however, potential cytotoxic effects and effects on CYP induction of these chemicals are often unknown.

In the present study, the effects of various solvents on CYP induction and cytotoxicity were evaluated in three preparations of sandwich-cultured cryopreserved human hepatocytes. These solvents include DMF, acetic acid, acetic acid, acetone, dimethylacetamide, ethyl alcohol, and methanol. CYP enzyme induction potential was evaluated by in situ marker substrate incubations and mRNA expression.

Materials & Methods

1X TE Buffer (Ambion); gene expression assay, high capacity cDNA reverse transcription kit, TaqMan fast advanced master mix (Applied Biosystems); hydroxybupropion, 1'-hydroxymidazolam (Cerilliant); acetic acid, acetone, ethyl alcohol, dimethylacetamide, ethyl acetate, methanol, benzyl alcohol, and methanol, RNeasy mini kit (Qiagen); acetaminophen-d4, hydroxybupropion-d6, 1X TE Buffer (Ambion); gene expression assay, high capacity cDNA reverse transcription kit, TaqMan fast advanced master mix (Applied Biosystems). The FDA (US), EMA (Europe) and PMDA (Japan) recently revised the guidelines for evaluating drug-drug interactions (FDA, 2012). According to this new guidance, a new chemical entity (NCE) should be evaluated at very high concentrations in vitro (e.g., (0.1-1.0) x 250 mM) or 50% the total Vmax (e.g., (0.1-1.0) x 100 μM). Achieving these concentrations is often possible if the test article is soluble in preferred solvents (e.g., 0.1% v/v DMSO) and/or cell culture medium. Consequently, test articles are evaluated for solubility in other solvents; however, potential cytotoxic effects and effects on CYP induction of these chemicals are often unknown.

In the present study, the effects of various solvents on CYP induction and cytotoxicity were evaluated in three preparations of sandwich-cultured cryopreserved human hepatocytes. These solvents include DMF, acetic acid, acetic acid, acetone, dimethylacetamide, ethyl alcohol, and methanol. CYP enzyme induction potential was evaluated by in situ marker substrate incubations and mRNA expression.

Results

Cytotoxicity was noted for several of the solvents tested (see Figure 1). Acetic acid caused up to a 22% increase in LDH release at 0.1% (the lowest concentration tested) following 24 hours of treatment, and complete cell death at all concentrations higher than 24 hours and all concentrations at 48 and 72 hours, and CYP activity and mRNA levels could not be determined. Hepatocytes treated with ethyl acetate or dimethylacetamide exhibited morphological changes (examined by light microscopy) including loss of membrane definition and increased cellular debris. Dimethylacetamide caused concentration-dependent increases in LDH release up to 58.5% following 48 hours of treatment, and complete cell death following 72 hours at 2% concentration. Treatment with up to 2% ethyl acetate caused concentration-dependent increases in LDH release up to 30.5% following 72 hours of treatment.

Unexpectedly, similar trends were not observed when CYP activity was evaluated. All concentrations of DMF, acetic acid, acetone, and methanol had little or no effect (± 2-fold change) on CYP activity with a few exceptions (i.e., CYP1A2; 2.0% acetic acid; CYP2B6; 0.5% DMF; CYP3A4: 2.0% DMF and 0.1% dimethylacetamide). However, dimethylacetamide and ethyl acetate caused greater than 50% decrease in CYP1A2, CYP2B6 and CYP3A4 activity, which may be a result of the cytotoxic effects of these solvents (see Figure 2, Figure 3 and Figure 4).

Conclusions

- Acetic acid (at 0.1% v/v), acetone (up to 2% v/v), and methanol (up to 2% v/v) are acceptable substitutions to 0.1% DMSO as they caused little to no effect on cytotoxicity, CYP enzyme activity and CYP enzyme mRNA expression levels.
- Acetic acid, dimethylacetamide, DMF (at ≥ 0.5% v/v) and acetic acid (at 0.2 to 2% v/v) were found to be unacceptable substitutions to 0.1% DMSO as they caused cytotoxic effects (morphological changes or increase LDH release or a concentration-dependent increase or decrease in CYP enzyme activity and CYP enzyme mRNA expression levels).
- Acetic acid (at 2.5% v/v) and ethyl acetate (up to 2% v/v) should be investigated further before accepting them as substitutions to 0.1% DMSO.
- The cytotoxic and enzyme inducing effects of the solvent should be considered and predetermined when an alternate solvent is used to evaluate CYP induction in cultured hepatocytes.

References


Figure 1. Cytotoxic evaluation: The effect of treating three lots of cultured human hepatocytes with various solvents on lactate dehydrogenase release (LDH). (A) Fold change = (ABCVM of High Control – ABCVM of Low Control) x 100 / ABCVM of Low Control. Calculations are as follows:

- $\Delta C_{\text{OCT}}$ = $C_{\text{OCT}}$ (target) – $C_{\text{OCT}}$ (endogenous control)
- $\Delta C_{\text{ACT}}$ = $C_{\text{ACT}}$ (treated sample) – $C_{\text{ACT}}$ (untreated control)
- Fold change in expression = $2^{-\Delta C}$

Figure 2. CYP1A2 expression: The effect of treating three lots of cultured human hepatocytes with various solvents on the expression of CYP1A2 activity and mRNA levels