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 (DDIs). According to this new guidance document, a new chemical entity (NCE) should be evaluated at very high concentrations in vitro (e.g., (0.1-1.0)%/250 mL or 500x the total Vmax [6]). Achieving these concentrations in vitro can be problematic if the test article is insoluble 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 DMSO, ascorbic acid, acetic acid, acetonitrile, dimethylacetamide, ethyl acetate, and methanol. Cytotoxicity and enzyme induction potential was evaluated by in situ marker substance incubations and mRNA expression.

Materials & Methods

1X TE Buffer (Ambion); gene expression assay, high capacity cDNA reverse transcription kit, TAOMan fast advanced master mix (Applied Biosystems); hydroxybupropion, 1’-hydroxymidazolam, 1’-hydroxy-midazolam-ol (Cerilliant); acetic acid, acetonitrile, DMSO, ethanol, ethyl acetate, β-mercaptoethanol, midazolam, phenacetin, triton X-100 (Sigma-Aldrich); acetaminophen, ascorbic acid, bupropion HCl, dexamethasone, dimethylacetamide, DMSO, ethanol, ethyl acetate, methanol, octanol, pH 7.4, phenobarbital, phenol, propofol, triton-X-100 (Sigma-Aldrich), LDH cytotoxicity detection kit (Roche Diagnostics; Supplemented MCM; XenoTech).

Hepatocyte cultures and LDH assay

Cryopreserved human hepatocytes were seeded and cultured at concentrations ranging from 0.26 – 0.33 x 10⁶ cell/well in a collagen-Matrigel sandwich configuration in 48-well tissue culture plates in a humidified culture chamber (37 ± 1°C at 95% relative humidity and 95/5% air/CO₂) for 24 hours. Cultures were then treated once daily for 3 consecutive days with MCM medium, one of four concentrations of DMSO, acetic acid (2 mM/L), acetonitrile, ascorbic acid, dimethylacetamide, ethyl acetate or methanol (0.1, 0.5, 1.0 or 2.0% for each solvent). Media samples from five treatment groups were collected immediately prior to dosing and at cell harvest. Three wells from the DMSO treatment group were treated with 1% Triton X-100 solution each day of treatment and incubated for 55 to 111 minutes. LDH activity for each sample was determined on a microplate reader (λ = 490 nm) with a Synergy HT Multi-Detection Microplate Reader (Bio-Tek Instruments, Inc.) warmed for 10 minutes at 2 to 8°C. An equal volume of substrate (0.1% v/v DMSO).

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 higher concentrations in 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.

Results

Cytotoxicity: The effect of treating three lots of cultured human hepatocytes with various solvents on lactate dehydrogenase release

Figure 1: Cytotoxicity: The effect of treating three lots of cultured human hepatocytes with various solvents on lactate dehydrogenase release

Conclusions

Acetic acid (at 0.1% v/v), acetaminophen (up to 0.5% v/v) and ascorbic acid (at 2% v/v) were found to be unacceptable substitutions to 0.1% DMSO as they caused little or no effect on cytotoxicity, CYP enzyme activity and CYP enzyme mRNA expression levels.

Unexpectedly, similar trends were not observed when CYP activity was evaluated. All concentrations of DMSO, acetic acid, ascorbic acid 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% DMSO; CYP3A4: 2.0% DMSO 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).

CYP3A4 expression: The effect of treating three lots of cultured human hepatocytes with various solvents on the expression of CYP3A4 activity and mRNA levels

Figure 4: CYP3A4 expression: The effect of treating three lots of cultured human hepatocytes with various solvents on the expression of CYP3A4 activity and mRNA levels

References