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CYTOCHROME P450 INDUCTION STUDIES IN HUMAN CRYOPRESERVED HEPATOCYTES

Background

Many drug-drug interactions are metabolism based and mediated primarily via the Cytochrome P450 (CYP) family of enzymes. Ten CYP isoforms are expressed in a typical human liver (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1, and CYP3A4). Some of these enzymes may be induced following drug exposure (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/19, CYP2E1, and CYP3A4). The induction of these enzymes may have important clinical consequences. For example, induction of a CYP isoenzyme(s) by a xenobiotic may alter the metabolism of a co-administered drug resulting in a decrease in efficacy due to sub-therapeutic concentrations of the drug and/or adverse drug effects due to an increase in the formation of toxic metabolites. As detailed in the FDA's Draft Guidance document for Drug-Drug Interactions (2006)[1], the FDA has placed emphasis on evaluating the induction potential of a new chemical entity (NCE) at an earlier stage in drug-development, to identify and avoid potential adverse drug interactions.

Early assessment of an NCE's CYP induction potential can be achieved using human cryopreserved hepatocytes. Cryopreserved human hepatocytes are an intact cell model system of the human liver containing physiological levels of Phase I and Phase II metabolizing enzymes and physiological levels of co-factors. The culture of human hepatocytes offers a reliable and valuable *in vitro* system to test a compound's ability to induce the expression and the activity of human cytochrome P450 enzymes.[2] As a result, the use of human cryopreserved hepatocytes is accepted as the 'gold standard' for predicting human CYP450 induction.

Assay Outline

In accordance with the FDA Draft Guidance for Drug-Drug Interactions CYP 1A2 and CYP3A4 induction studies are carried out as follows:

- Cryopreserved human hepatocyte cultures from three individual donors are used
- Cells are treated with three concentrations of test compound(s) in triplicate
- As a positive control, cells are treated with FDA accepted prototypical inducers for CYP1A2 and for CYP3A4
- As a negative control, cells are treated with vehicle
- Following 48 hours of treatment, CYP1A2 and CYP3A4 activity is assessed by measuring the conversion of FDA-recommended substrates to metabolites quantified using validated LC/MS-MS methods
- The degree of induction by the test compound(s) is determined by comparing the inductive effect of the test compound to the inductive effect of the known prototypical inducer for the corresponding CYP isoenzyme

CYP Subtype	Known Inducer	Probe Substrate	Metabolite
CYP 1A2	3-methylcholanthrene (3-MC)	Phenacetin	Acetaminophen
CYP 3A4	Rifampicin	Testosterone	6-β-OH-Testosterone

Experimental Results

As an example, cryopreserved human hepatocytes were treated with prototypical CYP1A2 and CYP3A4 inducers, 3-MC and rifampicin, respectively, for 48 hours. The fold induction in CYP1A2 and CYP3A4 activity was determined as a measure of specific metabolite formation (acetaminophen and 6-β-OH-testosterone, respectively) relative to vehicle control cells (un-induced). The data (mean ± S.D) are depicted graphically in the figure below.

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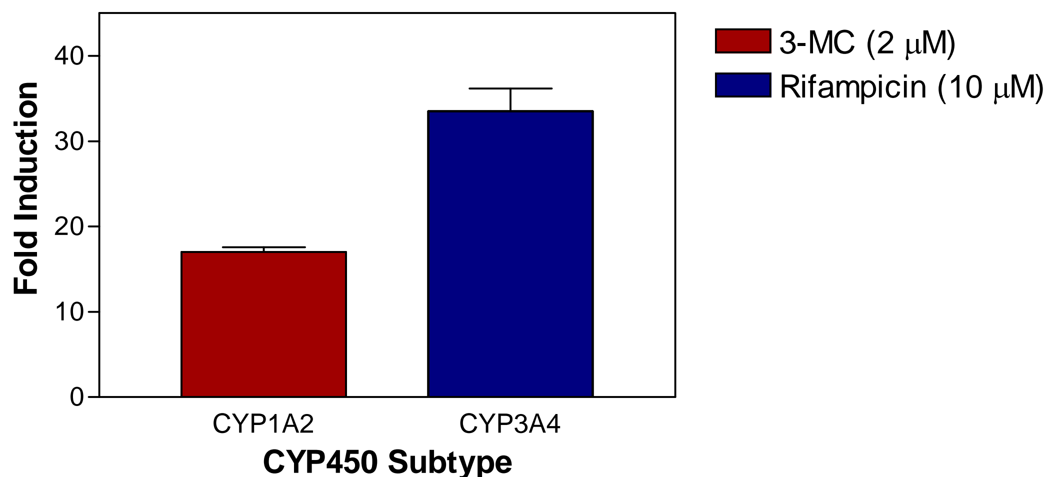
Assessment of the Induction of CYP1A2 and CYP3A4 Activity in Human Cryopreserved Hepatocytes following 48 hours of Treatment with 3-MC and Rifampicin



Treatment with 3-MC resulted in a 17.0 ± 1.30 (n=5) fold induction of CYP1A2 activity and treatment with rifampicin resulted in a 33.5 ± 5.35 (n=4) fold induction of CYP3A4 activity. These levels of CYP1A2 and CYP3A4 induction are within the range reported in the USFDA Draft Guidance Document for Drug-Drug Interactions[1]. The typical fold induction of CYP1A2 activity following treatment with 3-MC is reported to be in the range of 6 - 26, and the typical fold induction of CYP3A4 activity following treatment with rifampicin is reported to be in the range of 4 – 31.

NoAb's CYP450 induction assay allows the evaluation of the induction potential of a compound, which helps to identify and avoid potential adverse drug interactions. NoAb also offers a complementary activity based CYP450 inhibition assay, to evaluate the inhibitory potential of NCEs. In addition, NoAb's proprietary DTEx™ Gene Expression Analysis allows investigators to simultaneously survey drug related induction and suppression of ADME related genes (such as CYP450s, drug transporters and transcription factors) at the gene expression level, which is useful where activity based assays are unreliable or unavailable. All of these services are examples of NoAb's commitment to providing the best drug discovery tools for our clients, helping to shape drug discovery.

References:

1. USFDA (2006) Draft Guidance for Industry: Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling, U.S. Food and Drug Administration Publication
2. Roymans et al., Drug Metabolism and Disposition, Vol. 33 No.7 (2005)

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