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In vitro **ADME & PK**

Cytochrome P450 Induction

Background Information

'Cultured hepatocytes (cryopreserved or fresh) are the preferred *in vitro* system for induction (and down-regulation) *in vitro* studies.'

[2]EMA (2012) Guideline on the investigation of drug interactions

- Induction of cytochrome P450 enzymes is associated with an increased prevalence of clinical drug-drug interactions.
- Cyprotex's Cytochrome P450 induction assay identifies the potential of test compounds to induce CYP1A2, CYP2B6 or CYP3A4 in cultured human hepatocytes by evaluating mRNA levels and/or catalytic activity. Assays are designed to meet FDA[1] and EMA[2] guidelines.
- Test drug concentrations should be based on the expected human plasma drug concentrations and dose. Solubility, cytotoxicity and plasma protein binding should also be taken into consideration.
- Cyprotex's Cytochrome P450 induction assay delivers fold-induction data normalised to vehicle control which can be compared to positive control responses. If appropriate, data is fit using non-linear regression analysis to four-parameter sigmoidal equation to produce E_{max} and EC_{50} values.
- The clinical consequences of induction may be therapeutic failure caused by a decreased systemic exposure of the drug itself or a co-administered therapy, or toxicity as a result of increased bioactivation.

Supplementary Assays

Preliminary aqueous solubility assessment

Cytotoxicity assessment in primary human hepatocytes or HepaRG (e.g. MTT)

Assessment of non-specific binding

Measurement of parent drug on final day of dosing

Protocol

Test System
Cryopreserved or fresh human hepatocytes (3 donors recommended)
HepaRG cells are available on request

Test Article Concentration
1, 3 or 6 concentrations (dependent upon unbound C_{max}, dose, solubility and cytotoxicity) plus vehicle control, in triplicate

CYP Isoforms
CYP1A2, CYP2B6 and CYP3A4
For CYP2C, UGT or transporter studies, please contact directly for information

Controls
Omeprazole (CYP1A2 positive control)
Phenobarbital (CYP2B6 positive control)
Rifampicin (CYP3A4 positive control)
Negative control (non-inducer)

Test Article Requirements
Dependent on top concentration (recommend 0.1% DMSO in incubation)

Exposure Period
72 hr (media changed every 24 hours)

Probe Substrates for Catalytic Activity
Phenacetin (CYP1A2)
Bupropion (CYP2B6)
Midazolam (CYP3A4)

Analysis Method
LC-MS/MS quantification of acetaminophen (CYP1A2), hydroxybupropion (CYP2B6) and 1-hydroxymidazolam (CYP3A4)
qRT-PCR for relative mRNA expression levels (CYP1A2, CYP2B6 and CYP3A4)

Data Delivery
Report detailing methodology, donor demographics, mRNA levels, fold induction relative to vehicle control, concentration of metabolite of probe substrate, E_{max}, EC_{50} and F2 (concentration which leads to a 2-fold increase above E_{min}) if appropriate.

To find out more contact **enquiries@cyprotex.com**

'The evaluation of CYP enzyme induction should begin with studies of CYP1A2, CYP2B6, CYP3A4 *in vitro*. If the *in vitro* induction results are positive according to predefined thresholds using basic models, the investigational drug is considered an enzyme inducer and further *in vivo* evaluation may be warranted.[1]'

Figure 1

Induction of CYP1A2 mRNA levels by omeprazole in cryopreserved human hepatocytes.

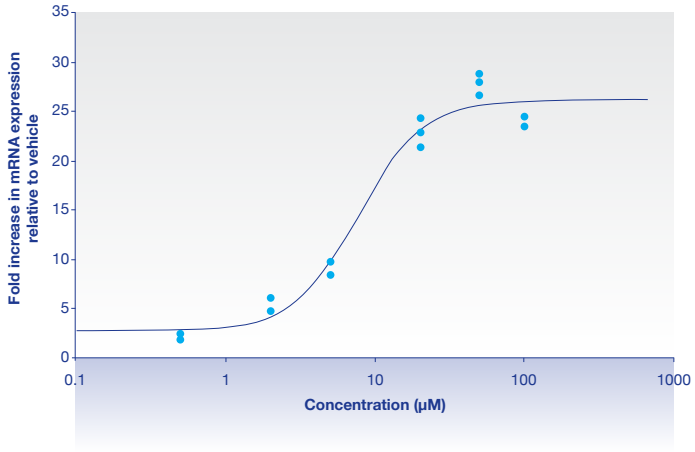


Figure 2

Induction of CYP2B6 mRNA levels by phenobarbital in cryopreserved human hepatocytes.

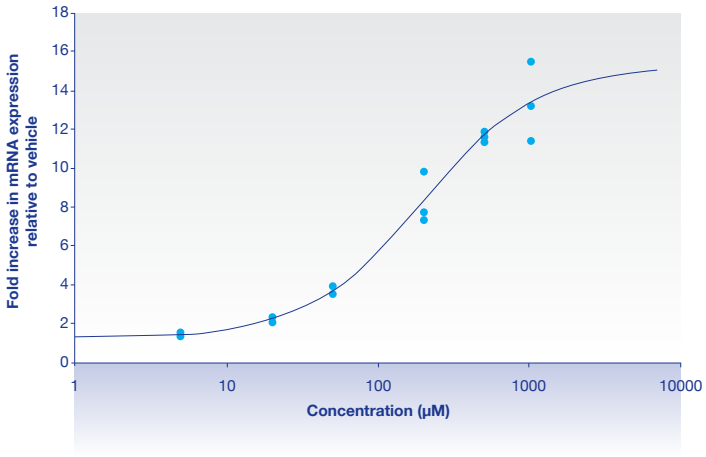
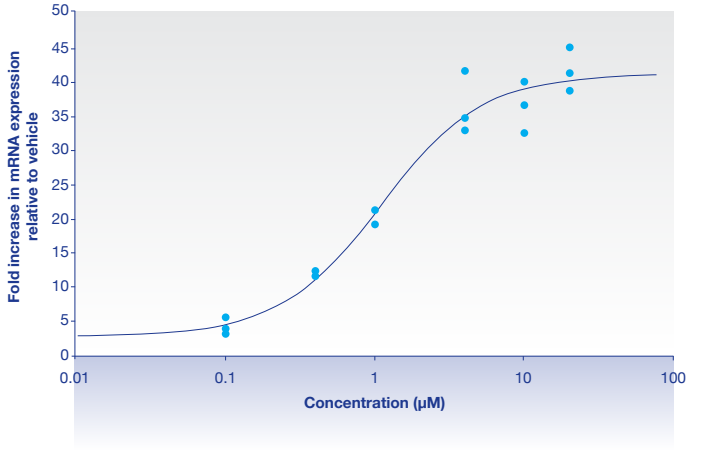


Figure 3

Induction of CYP3A4 mRNA levels by rifampicin in cryopreserved human hepatocytes.



References

[1] FDA (2012) Guidance for industry: drug interaction studies-study design, data analysis, implications for dosing, and labeling recommendations.

[2] EMA (2012) Guideline on the investigation of drug interactions.