

P450 Induction Studies in HepaRG[®] Cells

Purpose

Primary hepatocytes derived from human liver tissue are considered as the gold standard for drug-drug interaction studies. However, the availability of freshly isolated human hepatocytes is limited, their isolation is an elaborate procedure and they have a limited survival time (Bjornsson et al., 2003). The commercially available hepatoma cell line HepaRG[®], derived from a human hepatocellular carcinoma, was shown to maintain hepatic functions and expression of liver-specific genes, such as P450s, Phase II enzymes, hepatic drug transporters and nuclear receptors (Aninat et al., 2006; Le Vee et al., 2006; Kanebratt & Anderson, 2008).

We propose HepaRG[®] cells as an alternative to human primary hepatocytes for investigations on P450 induction in order to overcome unpredictable tissue availability and the variations in functional activities. In terms of reproducibility and inter-donor variation, each batch of HepaRG[®] cells can be considered as hepatocytes derived from the one and the same donor. For evaluation of applicability of HepaRG[®] in pre-clinical drug testing, the response to classical P450 inducers was tested based on the recommendations of the FDA Draft Guidance for Industry (Drug-Drug Interactions, September 2006).

Assay Protocol

HepaRG[®] were cultured in the media recommended by the manufacturer (Biopredic International, Rennes) in 96-well-plates. For the determination of P450 induction, enzymatic activities were tested after exposure to prototypical chemical inducers for 48 hours. Subsequently, functional P450 enzyme activities (CYP1A2, CYP2B6, CYP2C9 and CYP3A4) were tested in a cocktail approach (Kanebratt & Anderson, 2008).

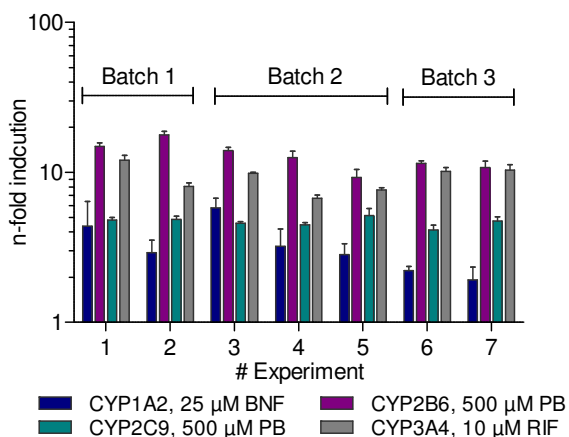


Figure 1: Reproducibility of P450 induction in HepaRG[®] (positive control inducer)

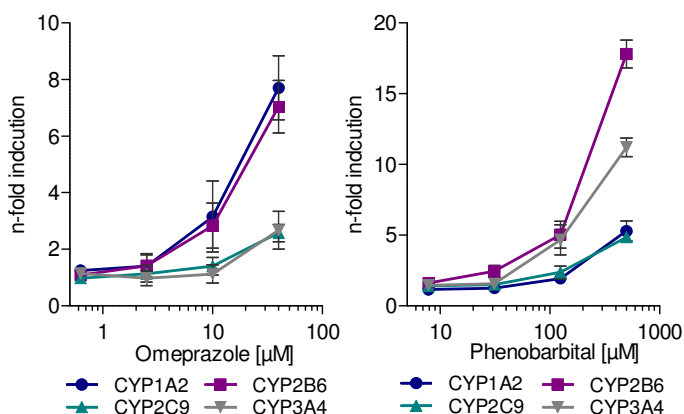


Figure 2: Concentration-response curves for omeprazole and phenobarbital

Results

β -Naphthoflavone, rifampicin or phenobarbital were tested as positive control for CYP1A2, CYP3A4 or CYP2B6 and CYP2C9 induction, respectively, in seven experiments, using three different HepaRG[®] batches. In all experiments, the acceptance criteria of the FDA Draft Guidance, i.e. > 2fold induction at a test concentration < 500 μ M, were met for each P450 isoform in consecutive experiments.

Both omeprazole and phenobarbital, prototypic P450 inducers, showed clear dose-response curves.

Conclusion

The HepaRG[®] model represents a suitable tool for induction studies. It benefits from the fact that cells of equal quality and responsiveness to P450 induction can be used for iterative studies, independent on tissue availability and inter-donor variation.

Please don't hesitate to contact us for a customized quotation

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