Metabolically Active Nuclear Receptor Reporter Assays: Enhancing Detection of Prodrug Activity with Hepatocyte Co-Cultures

Samar Maalouf[1], Nathan Connolly[1], Andrew Woodman[1], John P. Vanden Heuvel[1,2]

[1]INDIGO Biosciences, Inc., 3006 Research Drive, State College, PA, USA, and [2]Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA, USA

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Introduction

Cell-based reporter assay systems are valuable high-throughput tools widely used to efficiently screen large numbers of candidate drugs, chemicals, or environmental samples for biological activity such as toxicity and drug-drug interactions. However, when evaluating prodrugs which require metabolic activation, additional assays, such as S9 liver microsomes or primary hepatocytes, are often used to simulate the metabolic processes in the body.

S9 liver microsomes, while commonly used, have inconsistent metabolic enzyme expression, limiting their ability to replicate full in vivo metabolism. Their batch-to-batch variability, potential toxicity, and the need for careful optimization further complicate drug testing.

Primary hepatocytes offer a more physiologically relevant model but are limited in supply from any one donor. In addition, the lack of propagation capability poses challenges to their routine use for iterative, comparative drug screening.

To address these limitations, we evaluated metabolically active nuclear receptor reporter cell systems that offer better alignment with *in vivo* drug metabolism and activity. upcyte®hepatocytes are donor-derived hepatocytes engineered for limited proliferation while retaining their native xenobiotic-metabolizing enzyme activities, bridging the gap between primary hepatocytes and immortalized cell lines.

Our results highlight the utility of upcyte®hepatocytes in evaluating metabolic activity in the *in vitro* drug screening process. Their co-culture with reporter cell models enables direct evidence of metabolism without the challenges associated with S9 liver microsomes, or the availability of primary hepatocytes, offering a more efficient and cost-effective alternative.

Materials and Methods

Cell-based Reporter Assays for Xenobiotic-Sensing and/or Nuclear Receptors. INDIGO Biosciences' reporter cells express either full-length receptors (AhR) or Gal4-DBD hybrid receptors (PPARα & PXR). For each assay, the reporter gene is firefly luciferase functionally linked to the appropriate upstream regulatory element. Changes in luciferase activity provide a direct correlation to the receptor's transcriptional activity. Relative Light Units (RLU) are quantified using a plate-reading luminometer.

Human upcyte® hepatocytes, developed by upcyte® technologies GmbH, are derived from donor 10-03, an adult Caucasian female.

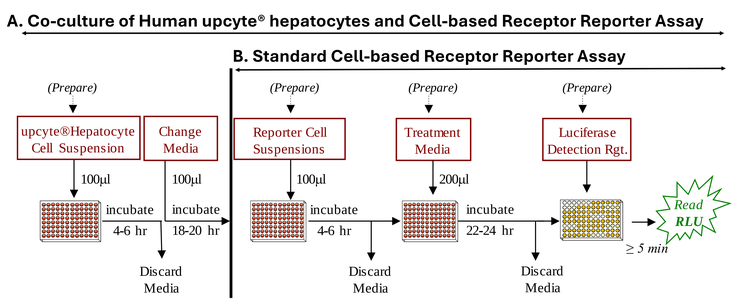

Figure 1: (**A**) Co-culture of human upcyte® hepatocytes and receptor reporter assay workflow. (**B**) Standard cell-based reporter assay workflow, in the absence of hepatocytes.

Results

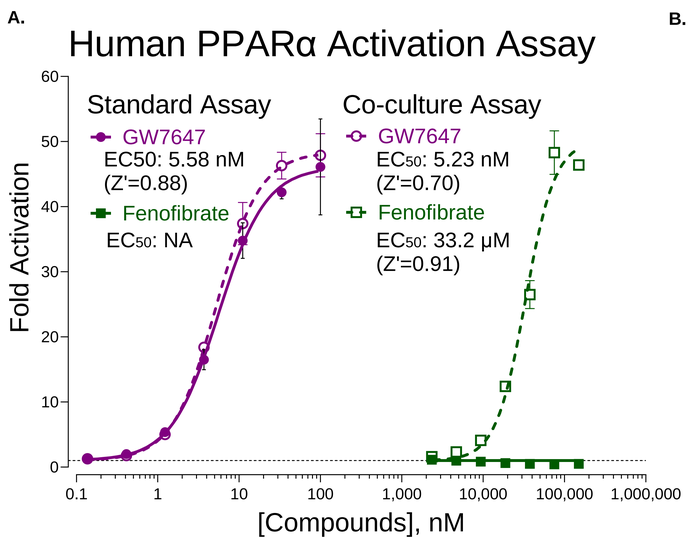

Figure 2: Human PPARα Activation Assay: (A) Activation Assay was performed using GW7647 and Fenofibrate (Cayman Chemical). Human PPARα reporter cells were seeded either alone (solid line, filled symbols) or co-cultured with Human upcyte® hepatocytes (donor 10-03; dashed line, open symbols). Cells were treated as described in Materials and Methods. Data show the mean fold activation and percent coefficient of variation (% CV) for dose-response curves (n=3, representative experiment performed at least twice). Curves were generated using non-linear regression analysis. NA=no activity. (B) Representative cell morphology of PPARα reporter cells and adult human upcyte® hepatocytes (donor 10-03) alone or in co-culture.

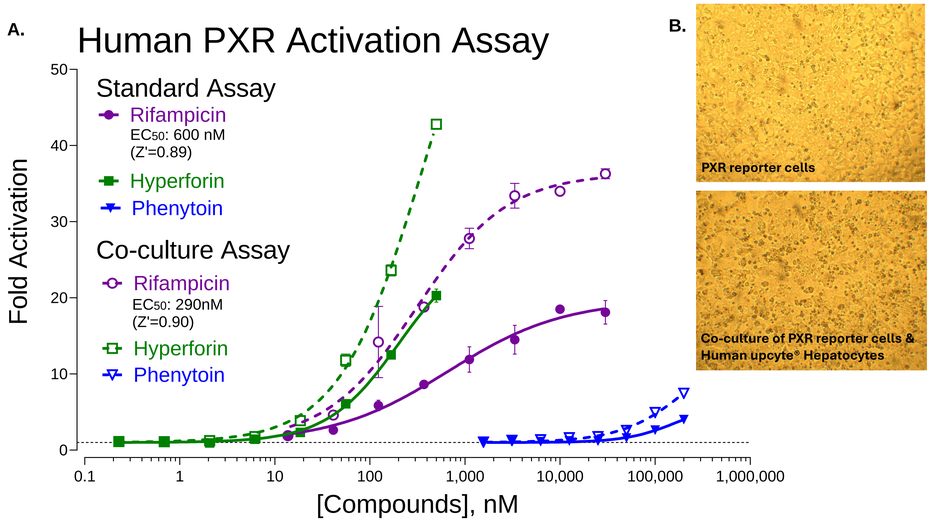

Figure 3: Human PXR Activation Assay: (A) Activation Assay was performed using Rifampicin, Hyperforin (Cayman Chemical), and Phenytoin (Selleckchem). Human PXR reporter cells were seeded either alone (solid line, filled symbols) or co-cultured with Human upcyte® hepatocytes (donor 10-03; dashed line, open symbols). Cells were treated as described in Materials and Methods. Data show the mean fold activation and % CV for dose -response curves (n=3, representative experiment performed at least twice). Curves were generated using non-linear regression analysis. (B) Representative cell morphology of PXR reporter cells alone or in co-culture with adult human upcyte® hepatocytes.

Results *(continued)*

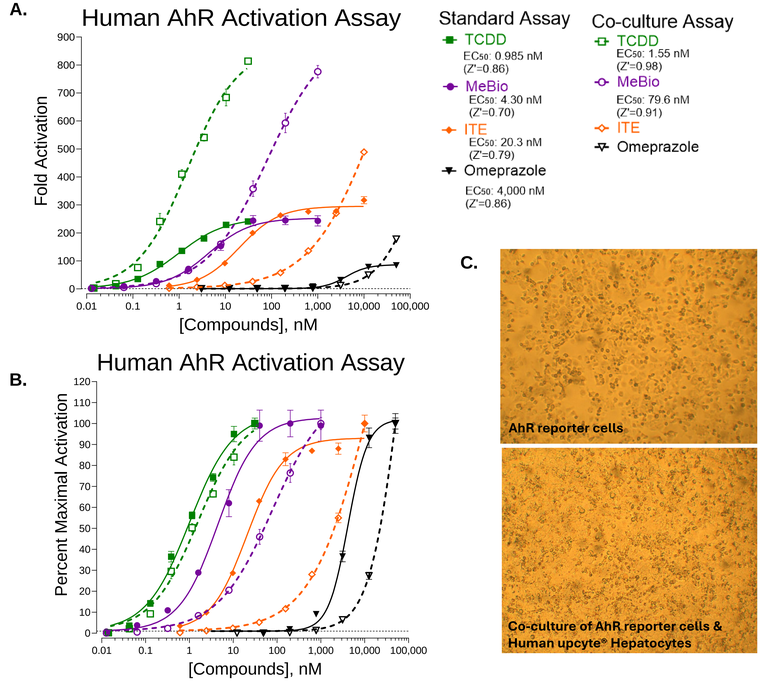

Figure 4: Human AhR Activation Assay: Activation Assay was performed using MeBio, ITE (2-(1H-indole-3-ylcarbonyl)-4-thiazolecarboxylic methyl ester), Omeprazole (Cayman Chemical), or TCDD (Cambridge Isotope). Human AhR reporter cells were seeded either alone (solid line, filled symbols) or co-cultured with Human upcyte® hepatocytes (donor 10-03; dashed line, open symbols). Cells were treated as described in Materials and Methods. Shown are **(A)** Fold Activation or **(B)** Percent of maximal activation and %CV for dose-response curves (n=3, representative experiment performed at least twice). **(C)** Representative cell morphology of AhR reporter cells alone or in co-culture with human upcyte® hepatocytes.

Conclusions

Metabolic Activation of PPARα by Fenofibrate - Fenofibrate required metabolic activation by human upcyte® hepatocytes to induce PPARα activity, while GW7647 activated PPARα independently of metabolism.

Enhanced Sensitivity of PXR Assay - PXR ligands activated the receptor in both standard and co-culture assays, but the presence of upcyte® hepatocytes increased assay sensitivity, as evidenced by higher luminescence and lower EC50 values for Rifampicin.

Differential Metabolic Effects on AhR Activation – All tested AhR ligands activated AhR, independent of upcyte® hepatocytes. However, in the co-culture system, the fold activation increased for all compounds, while the assay sensitivity decreased for all but TCDD (a forever compound), suggesting metabolic modification of these compounds.

Importantly, The increase in fold activation observed in PXR and AhR reporter assays was due to increase in luminescence in response to the treatments without significant changes in background between the standard and co-cultured systems.

These results demonstrate that upcyte® hepatocytes provide metabolically relevant conditions for nuclear receptor assays, offering a cost-effective and scalable alternative to S9 liver microsomes and primary hepatocytes. This approach enhances the ability to assess prodrugs and metabolites during early-stage drug discovery.