Progress in CYP Enzymes Mechanisms of Induction and Its Applications

Zhanxu Meng
Tai 'an No. 1 Middle School, Tai 'an, Shandong Province, China

Keywords: Cytochrome P450 (CYP) Enzymes, Induction Mechanisms, Drug Metabolism, In vitro, In vivo.

Abstract: Cytochrome P450 (CYP) enzymes are pivotal in the metabolic processing of drugs, and their modulation via induction pathways plays an important role in the therapeutic efficacy, safety, and drug interaction profile. This comprehensive review delineates three focal points concerning CYP enzyme induction. The first domain scrutinizes the accuracy of in vitro methodologies—predominantly cryopreserved primary human hepatocytes (PHH) and Human HepaRG cells—in forecasting the in vivo dynamics of CYP enzymes. The second segment elucidates novel induction paradigms, highlighting the enduring induction of CYP1A enzymes via 3-methylcholanthrene (MC) in murine models and the atypical induction pathways that involve YAP/TEAD perturbation and consequent hepatocyte dedifferentiation. The terminal section evaluates the application of these findings in clinical settings, discussing the kinetic profiling of CYP3A modulation and the transposition of in vitro CYP repression to actual drug-drug interaction scenarios. The synthesis of these facets contributes to an enriched understanding of CYP induction mechanisms and their ramifications for drug discovery and tailored therapeutic approaches. Furthermore, the appraisal accentuates the reliability and pertinence of cryopreserved PHH and HepaRG cells as in vitro proxies for human CYP enzyme induction studies, potentially informing regulatory risk evaluation and elucidating drug metabolism and nuclear receptor-mediated regulatory anomalies in biochemical pathways.

1 INTRODUCTION

Cytochrome P450 (CYP) enzymes play a critical role in drug metabolism, facilitating the biotransformation of a wide range of endogenous and exogenous compounds within the body (Pelkonen et al. 2008). The regulation of CYP expression is essential for maintaining physiological balance and ensuring efficient drug metabolism (Li et al., 2019). Induction represents a primary mechanism through which CYP expression levels are modulated, whereby various xenobiotics and endogenous signalling molecules can upregulate these enzymes (Li et al., 2019). When the drug is cleared from the body more quickly, it leads to lower levels of the drug in the body, which can reduce its effectiveness. An example of this is when rifampin is taken alongside sulfonylureas, resulting in decreased levels of sulfonylureas in the blood and a diminished ability to lower blood glucose. This decrease in effectiveness may be caused by the induction of CYP2C9 enzyme by rifampin. Another factor contributing to the decreased drug concentrations and effectiveness could be the induction of P-glycoprotein by rifampin.

CYPenzymes play a critical role in drug metabolism, with induction and inhibition as key modulatory mechanisms. Induction of these enzymes is characterized by a time-dependent augmentation in enzyme levels, necessitating a period of adaptation to stabilize at a new homeostasis (Lin, 2006). Contrasting the immediate effect of CYP inhibition, induction involves intricate genetic regulation leading to an increase in enzyme synthesis. Activation of CYP enzymes within the families 1 to 3 is a complex process governed by three primary pathways, responding to exogenous compounds (Pelkonen et al. 2008). Under basal conditions, these receptors are sequestered in the cytoplasm. It is bound to heat shock protein 90 (Hsp90). Ligand binding instigates a conformational alteration, prompting dissociation from Hsp90, receptor activation, and nuclear translocation, thereby kick-starting gene transcription. Beyond these classical receptor-mediated mechanisms, CYP enzyme induction also encompasses pathways such as the direct and indirect

Meng, Z.
Progress in CYP Enzymes Mechanisms of Induction and Its Applications.
DOI: 10.5220/0013846300004914

In *Proceedings of the 2nd International Conference on Renewable Energy and Ecosystem (ICREE 2024)*, pages 76-86
ISBN: 978-989-758-776-4

glucocorticoid receptor-mediated induction, highlighting the nuanced regulatory landscape of CYP enzyme activity (Schuetz et al., 1996 & Pascussi et al., 2001). Moreover, the modulation of certain CYP enzymes transcends transcriptional control, involving post-transcriptional modifications that stabilize the mRNA and protein, further complexifying the regulation of CYP enzyme levels (Chen et al., 2017)

The process of CYP induction holds significant implications in pharmacology and toxicology, impacting drug efficacy, safety, and potential interactions. Understanding the molecular mechanisms underlying CYP induction is vital for optimizing drug therapy, predicting drug-drug interactions (DDIs), and comprehending the effects of environmental toxins on human health.

This review provides a detailed examination of CYP induction, highlighting its implications for drug metabolism and toxicity testing. Anchored by three primary perspectives, the analysis begins with an exploration of in vitro methodologies and their role in predicting in vivo CYP induction, a topic extensively addressed by Bernasconi et al. in 2019 (Bernasconi et al., 2019). The translation of in vitro findings to in vivo contexts is critical, particularly in understanding how induced CYP activity may affect metabolic processes. Recent advances have refined these in vitro techniques, capitalizing on human-based systems to simulate and study enzyme interactions.

CYP P450 enzymes, key players in xenobiotic metabolism, are underscored for their expansive presence and metabolic diversity. They are instrumental in detoxifying xenobiotics by metabolizing harmful substances into benign products or, conversely, converting non-toxic compounds into harmful metabolites. Beyond detoxification, these enzymes are essential for synthesizing various endogenous compounds. Therefore, the xenobiotic-induced alteration of CYP enzyme activity has far-reaching effects on metabolic stability and can precipitate adverse biological consequences. This review underscores two pivotal studies utilizing in vitro approaches to forecast drug interactions and refine clinical outcomes. The first study by Bernasconi et al. investigates the effects of tipranavir/ritonavir on enzymatic and transporter functions (Bernasconi et al., 2019). The second, by Dumond et al., presents cocktail phenotyping, a novel strategy for evaluating drug interaction potentials. Both studies furnish essential insights into the complex interplay of drug interactions, contributing to the enhancement of therapeutic protocols (Dumond et al., 2010). Consequently, the review will critically analyze and synthesize the findings, emphasizing their significant contributions to the field of in vitro prediction methods.

Furthermore, the review delves deeper into novel mechanisms of CYP enzyme induction, specifically highlighting the extended induction of CYP1A enzymes by MC in murine models, a phenomenon elucidated by Jiang et al. in 2009 (Jiang et al., 2009). The research has shed light on the persistent transcriptional activation of promoters associated with these enzymes. Considering the potent carcinogenicity of MC, a polycyclic aromatic hydrocarbon (PAH) prevalent in various environmental matrices, it's crucial to understand how its metabolism by CYP enzymes results in intermediates capable of DNA binding and potential carcinogenesis.

The induction of CYP1A1 & A2 enzymes by MC and the eventual decline post-exposure cessation present an enigmatic aspect of CYP regulation. This review aims to dissect the mechanisms behind the lasting transcriptional activation of these CYP enzymes, which have significant implications for environmental health. Moreover, the review addresses a comparative analysis of YAP/TEAD inhibitors within bidimensional and tridimensionality primary human hepatocyte cultures conducted by Oliva-Vilarnau et al. in 2023 (Oliva-Vilarnau et al., 2023). These inhibitors, initially developed as cancer therapeutics, have been observed to stimulate CYP enzymes in 2D hepatocyte cultures. Intriguingly, such induction is absent in 3D spheroid cultures, underscoring the importance of considering alternative induction pathways and adopting organotypic culture systems in drug development to predict CYP enzyme modulation.

Clinical trials form the apex of this review, particularly the meticulous quantification of CYP3A modulation dynamics through continuous midazolam (MDZ) infusion and the correlation of in vitro P450 downregulation with in vivo DDIs, especially regarding 13-cis-Retinoic Acid (13cisRA) (Li et al., 2019 & Stevison et al., 2019). The time-dependent modulation of CYP enzymes is critical in the design of DDI studies, which in turn influences the safe and efficacious application of drugs metabolized by CYP3A.

This compendium of studies coalesces into a comprehensive understanding of CYP enzyme induction, from experimental exploration to clinical relevance, imparting vital insights that have the potential to refine drug development and pave the way for tailored therapeutic approaches in personalized medicine.

2 USING IN VITRO METHODS FOR PREDICTING IN VIVO BEHAVIOUR

In vitro methodologies represent a cornerstone in the preclinical assessment of CYP enzyme expression upon exposure to pharmaceuticals. These methodologies utilize various hepatocyte-based models as proxies to the in vivo environment, offering a simplified yet controlled setting to circumvent the complexities and ethical considerations of animal testing.

According to the Food and Drug Administration (FDA) in U.S., a spectrum of in vitro hepatic models has been established. This suite includes fresh and cryopreserved primary hepatocytes, hepatocytes with stable or transient transfections, hepatic cell lines, and assays utilizing reporter genes. These platforms enable pharmaceutical entities to gauge the induction potential of new compounds, aligning their evaluation processes with the regulatory framework set forth by the FDA. A compound is flagged for further clinical drug-drug interaction studies and in vivo scrutiny if it elicits a CYP enzyme induction surpassing 40% relative to a positive control, as per FDA guidelines. Molecules demonstrating significant induction propensities might be withdrawn from the development pipeline to pre-empt adverse drug interactions (Ghosh et al., 2023).

An initial high-throughput screen can detect enhanced activation of nuclear receptors leading to upregulated CYP enzyme synthesis. One approach involves coalescing hepatoma cells with a CYP3A4 promoter region and a luciferase reporter or using a human pregnane X receptor (PXR) coupled with the CYP3A4-luciferase construct. Given CYP3A4's susceptibility to PXR-mediated induction, regulatory protocols advocate for its in vitro examination during early drug development. A negative outcome for CYP3A4 can generally rule out the induction potential for CYP2C, as PXR also governs this enzyme. Hepatic and immortalized cell lines that maintain hepatocyte characteristics and deliver reproducible findings are integral to the pharmaceutical industry. Among these, the HepaRG and Fa2N-4 cell lines are frequently used, with mRNA measurement serving as an accepted endpoint for induction in immortalized cells. Hepatoma lines, like HepG2, HepaRG, and BC2, see extensive application within industry settings (Ghosh et al., 2023 & Ingelman-Sundberg, 2004).

Primary hepatocytes are endorsed by industry and regulatory bodies due to their preservation of in vivo-like CYP metabolism post-isolation. For prolonged phenotypic stability, 3D cultures are preferred over 2D monolayers. With primary human hepatocyte cultures, CYP mRNA, protein levels, and microsomal activity can be accurately quantified. Furthermore, to address inter-individual metabolic variance, hepatocyte cultures sourced from multiple donors are utilized (Ghosh et al., 2023).

Despite their merits, the application of primary or cryopreserved hepatocytes is constrained by challenges such as limited availability, potential loss of enzymatic function due to cryopreservation, single-receptor pathway analysis limitations, and variability across different batches. As a result, immortalized and hepatic cell lines have surfaced as practical substitutes. These alternatives furnish manifold benefits: they facilitate the examination of multiple receptor-mediated mechanisms, offer an inexhaustible supply via cell propagation, and ensure uniform inducer responses. Current regulatory guidance also transitions the focus from enzyme activity to mRNA expression as the definitive endpoint for in vitro assays. This shift underscores mRNA levels as a more dependable metric for gauging CYP induction (Ghosh et al., 2023).

2.1 Vitro Methods: Human Cytochrome P450 Enzyme Induction

The 2019 research by Bernasconi et al. pursued the validation of two in vitro methodologies designed to assess chemical compounds' propensity to activate CYP enzymes—particularly CYP1A2, CYP2B6, and CYP3A4 (Bernasconi et al., 2019). These investigations employed two cellular models: cryopreserved PHH and HepaRG human cells.

The choice of cryopreserved PHH was predicated on their diverse array of native drug-processing enzymes and necessary cofactors, establishing them as a versatile tool for exploring toxicokinetic and toxicodynamic phenomena. PHH stands as a robust in vitro proxy for the human liver's metabolic processes. In parallel, HepaRG cells are recognized for their hepatic-like functionality, mirroring the metabolic processes of actual human hepatocytes. This includes the synthesis of key liver enzymes, the operation of nuclear receptors, and the facilitation of xenobiotic transporters. The criterion for validating these models' metabolic competence was the induction of CYP enzymes—a vital marker for evaluating the cellular expression machinery's integrity and functionality.

The methodology detailed by the authors leveraged differentiated HepaRG cells, preserved through cryopreservation, to monitor the stimulation of specific CYP enzymes. This process involved the application of chemical agents to the cells and the subsequent measurement of CYP1A2, CYP2B6, and CYP3A enzyme induction via metabolite quantification using advanced liquid chromatography/mass spectrometry techniques.

Results indicated that CYP enzyme induction is not merely a sensitive marker for protein synthesis but is also a critical parameter for determining hepatic metabolic capacity. Prior validations have confirmed the reliability of both PHH and HepaRG cells in gauging.the functional induction of the specified CYP enzymes. The robustness of these results is further corroborated by ring trial data demonstrating consistent reproducibility across different laboratories. These findings underscore the methods' translatability and applicability across research facilities equipped with cell culture and analytical chemistry capabilities. Moreover, these in vitro techniques have been corroborated for their predictive accuracy regarding in vivo CYP enzyme induction by various chemicals, evidenced by their correct identification of reference inducers and the successful prediction of in vivo human CYP induction for a majority of the chemicals tested. Ultimately, the decision to employ PHH or HepaRG cells hinges on the specific evaluative needs of the chemical assessment in question.

However, there have been discrepancies in prediction for certain chemicals in PHH. Carbamazepine, sulfinpyrazone, and rifampicin in PHH did not align with in vivo human CYP induction as expected (Table 2). This could be attributed to the absence of sufficient human data or variability in the hepatocyte batches used in the studies.

	HepaRG			PHH		
Test item	CYP1A2	CYP2B6	CYP3A4	CYP1A2	CYP2B6	CYP3A4
Omeprazole	N	N	N	N	N	N
Carbamazepine	Y	Y	Y	N	Y	Y
Phenytoin	Y	Y	Y	Y	Y	Y
Penicillin	N	N	N	N	N	N
Rifabutin	Not tested			N	Y	Y
Sulfinpyrazone	Y	Y	Y	N	Y	Y
Bosentan	Y	Y	Y	N	Y	Y
Artemisinin	N	Y	N	N	Y	N
Efavirenz	Not tested			N	Y	Y
Rifampicin	Y	Y	Y	N	Y	Y
Metoprolol	N	N	N	N	N	N
Sotalol	N	N	N	N	N	N

Figure 1: a comparative analysis of the predictive capabilities of HepaRG cells and PHHin evaluating the induction of CYP1A2, CYP2B6, and CYP3A4 (Bernasconi et al., 2019).

The current validation study supports the reliability and relevance of cryopreserved PHH and cryopreserved HepaRG cells as in vitro tools for assessing human CYP enzyme induction. These in vitro methodologies are proving valuable in regulatory risk assessment, offering insights into metabolic processes, thyroid disruption, and nuclear-receptor-mediated changes in biochemical pathways. Utilizing these approaches helps researchers understand the potential risks of xenobiotic exposure, guiding the development of more accurate and effective risk assessment frameworks.

2.2 CYP450 & P-Glycoprotein Interactions' Prediction

The Dumond et al. conducted a comprehensive study to evaluate the potential for DDIs in the combination therapy of tipranavir/ritonavir (TPV/r), used to treat HIV-1 infections. Tipranavir, a protease inhibitor with high efficacy against drug-resistant strains, is known to induce certain cytochrome P450 enzymes, particularly CYP3A (Dumond et al., 2010). To offset this, it is coadministered with ritonavir (RTV), a CYP3A inhibitor that helps sustain therapeutic levels of tipranavir. This combination, however, complicates the accurate prediction of drug interactions because of its varying effects on metabolic enzymes and transporters, a challenge that this study aimed to address.

The researchers utilized a modified cocktail phenotyping approach to evaluate potential drug interactions resulting from the coadministration of TPV and RTV. The study employed the caffeine, warfarin, omeprazole, dextromethorphan, intravenous MDZ, and vitamin K, to gauge the activity of key hepatic and intestinal proteins and explore the impact of TPV/RTV on them.

In vitro analyses had indicated that the TPV/RTV combination inhibits several CYP enzymes, such as CYP3A4, CYP1A2, CYP2C19, and CYP2D6. Yet, in clinical settings with HIV-1-infected patients on TPV/RTV and other CYP3A4 substrates, a decrease in exposure to coadministered protease inhibitors was observed, contrary to expectations. The study aimed to resolve this inconsistency by investigating how TPV/RTV influences various CYP enzymes and by exploring genotype-phenotype correlations and the genetic factors that contribute to drug interactions.

The recent investigation has furnished a multifaceted analysis of the drug-drug interaction potential attributed to tipranavir/ritonavir (TPV/RTV) via a comprehensive utilization of probe substrates and extensive genotyping. This

encompasses the genotypic characterization of critical CYP isoforms and P-glycoprotein (P-gp), endeavoring to articulate the interaction landscape at various pharmacokinetic junctures—baseline, subsequent to acute exposure, and upon achievement of a steady state. Study participants were administered a cocktail of probe substrates and digoxin, both via oral and intravenous routes, across three distinct stages to delineate the pharmacodynamic profile: baseline, after the triad of initial TPV/r dosages, and at the steady-state concentration.

Empirical results divulged that an inaugural dose of TPV/r exerts negligible modulation on CYP1A2 and CYP2C9 activities. Contrastingly, it mediates a mild inhibitory effect on CYP2C19 and P-gp, while imposing a pronounced inhibition on the activity of CYP2D6 and CYP3A enzymes. The investigative outcomes underscore a spectrum of induction and inhibition effects, thereby enriching the comprehension of drug interaction mechanisms inherent to TPV/RTV. Such insights are indispensable for the refinement of clinical deployment strategies for TPV/RTV.

The research emphasizes the efficaciousness of a phenotyping methodology in the prognostication of complex drug interactions and advocates for the application of biomarker probes in clinical pharmacokinetics. However, the translation of these findings to alternative therapeutic agents that display similar mixed inhibitory and inductive propensities should be approached with circumspection. The solicitation of further investigative efforts is necessary to unravel the intricate web of interactions that TPV/RTV may engage with a diverse array of pharmacological entities.

3 NOVEL MECHANISMS OF INDUCTION

3.1 Induction of CYP Enzymes in Mice

In the realm of toxicological research, the elucidation of mechanisms underlying the induction of CYP enzymes by xenobiotic substances remains a domain of significant scientific inquiry. Jiang et al. embarked on an incisive exploration of the molecular mechanisms driving the persistent induction of CYP1A1 and CYP1A2 enzymes after 3-methylcholanthrene (MC) exposure, a potent carcinogenic constituent among polycyclic aromatic hydrocarbons (PAHs) (Jiang et al., 2009). The investigators postulated a theory suggesting that MC catalyzes a long-standing transcriptional activation of the CYP1A1 and CYP1A2 gene promoters, thus inciting extended enzyme activity (Chen et al., 2017; Jiang et al., 2009 & Gibson et al., 2002).

To substantiate their hypothesis, the researchers designed a study utilizing both adult male wildtype (WT) mice and genetically modified counterparts, engineered to harbor human CYP1A1 or murine CYP1A2 promoter sequences. The investigative protocol entailed the administration of MC to these models, with subsequent assessments focused on gauging promoter-specific transcriptional activity.

The study treated mice with MC or a vehicle control (corn oil) daily for four consecutive days. The researchers utilized bioluminescent imaging to assess luciferase reporter gene expression, serving as a proxy for promoter activity, at intervals of 1, 8, 15, and 22 days after cessation of MC treatment.

The results indicated that MC treatment substantially increased luciferase expression driven by both CYP1A1 and CYP1A2 promoters in the transgenic mice. This elevated luciferase activity persisted for up to 22 days, with a more significant effect observed in the CYP1A1-luc mice. The MC-induced increases in CYP1A1 and CYP1A2 activity, as demonstrated by luciferase expression, supported the initial hypothesis regarding sustained transcriptional activation (Chen et al., 2017; Jiang et al., 2009 & Gibson et al., 2002).

Endogenous CYP1A1 and CYP1A2 expression was persistently induced in WT, CYP1A1-luc, and CYP1A2-luc mice, corroborating the sustained impact of MC exposure. Analysis of the findings indicated a 15-fold increase in CYP1A1-luc expression (Figure 2B). In contrast, the induction of CYP1A2-luc by MC was less pronounced (Figure 2C, D).

These data underscore the differential and prolonged effects of MC exposure on the induction of CYP1A1 & CYP1A2 enzymes. The utilization of transgenic mouse models expressing luciferase reporters driven by the CYP1A1 and CYP1A2 promoters represents a powerful tool for elucidating the molecular mechanisms underlying persistent enzyme induction, particularly in the context of PAH-induced carcinogenesis.

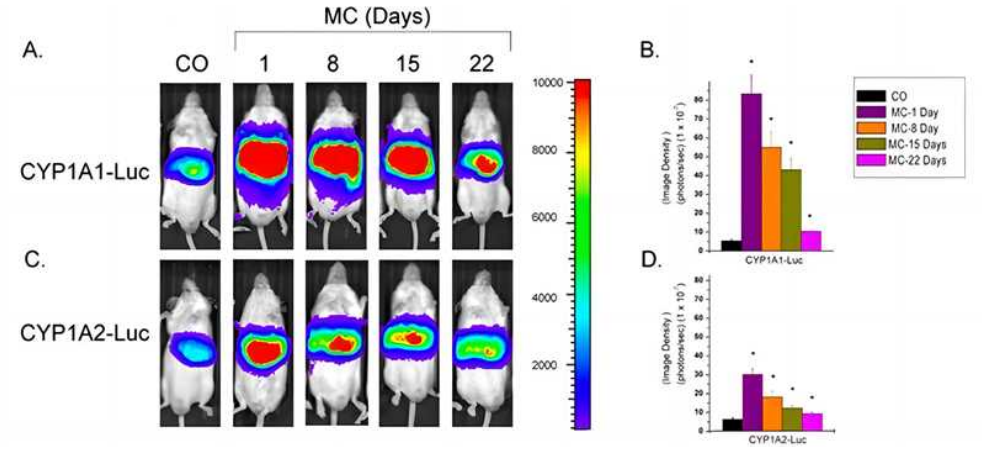

Figure 2: Bioluminescent imaging of CYP1A1-luc & CYP1A2-luc mice of MC treatment (Dumond et al., 2010).

3.2 Novel CYP Induction Mechanism by YAP/TEAD Inhibitors in Human Hepatocytes

DDIs which is mediated by the induction of CYPenzymes represent a significant challenge in drug development, necessitating comprehensive assessment during preclinical studies. A recent study focuses on the Hippo signalling pathway, which is known to govern cell fate, proliferation, and apoptosis, emphasizing its significance in the regulation of cellular processes (Zhang et al., 2024). At the molecular fulcrum of the Hippo signalling cascade lies the intricate intracellular trafficking of Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), which transits between the cytoplasm and nucleus. Ordinarily, in the quiescent state of the Hippo pathway, YAP/TAZ are permitted nuclear ingress, instigating the transcription of genes that promulgate cell proliferation and survival. This nuclear localization embodies the core of a cellular growth-promoting regime. In stark contrast, the activation of the Hippo pathway signals for a shift—YAP/TAZ are sequestered within the cytoplasm, an event that curtails their role as transcriptional regulators and, by extension, serves as a biochemical clamp on cellular proliferation. The dichotomy of YAP/TAZ localization thus constitutes a critical regulatory axis in cell growth control, embodying a cellular barometer that modulates gene expression in accordance with the proliferative or inhibitory cues of the Hippo pathway (Chen et al., 2017; Jiang et al., 2009 & Gibson et al., 2002).

The YAP/TEAD (TEA domain family member) signalling pathway has gained attention as a potential therapeutic target in oncology due to its role in cell growth regulation. Various inhibitors of YAP/TEAD are progressing through clinical development, each with distinct chemical structures and mechanisms of action. The pathway is also influenced by cell-cell contacts and biomechanical factors. YAP/TEAD activity is further modulated by cell geometry, which contributes to the complexity of the system.

Oliva-Vilarnau et al. conducted a pivotal study to evaluate the potential for CYP induction by assessing multiple YAP/TEAD inhibitors with varying selectivity profiles for TEAD isoforms (Oliva-Vilarnau et al., 2023). This study explored both traditional 2D cultures & 3D spheroids of PHH. The results indicated that YAP/TEAD inhibition caused extensive CYP enzyme induction in 2D monolayers but significantly reduced induction in 3D spheroids, suggesting a critical relationship between cell geometry and CYP regulation.

Further in-depth analysis through RNA sequencing revealed that YAP/TEAD signalling was more pronounced in 2D cultures compared to 3D, likely due to alterations in mechanoenzyme. The hyperactivation of YAP/TEAD in these cultures contributed to increased activity of other interacting transcription factors. This hyperactivation led to hepatocyte dedifferentiation, with a corresponding increase in hepatic function, including CYP enzyme induction. This induction was, therefore, an indirect consequence of YAP/TEAD inhibition.

These findings underscore the relevance of the Hippo pathway in drug development and its broader implications in pharmacokinetics. It highlights the necessity for organotypic 3D cultures in preclinical studies to better simulate clinical conditions and accurately assess pharmacokinetic profiles. Consequently, the results advocate for advanced testing models to refine the drug development process and enhance safety evaluations.

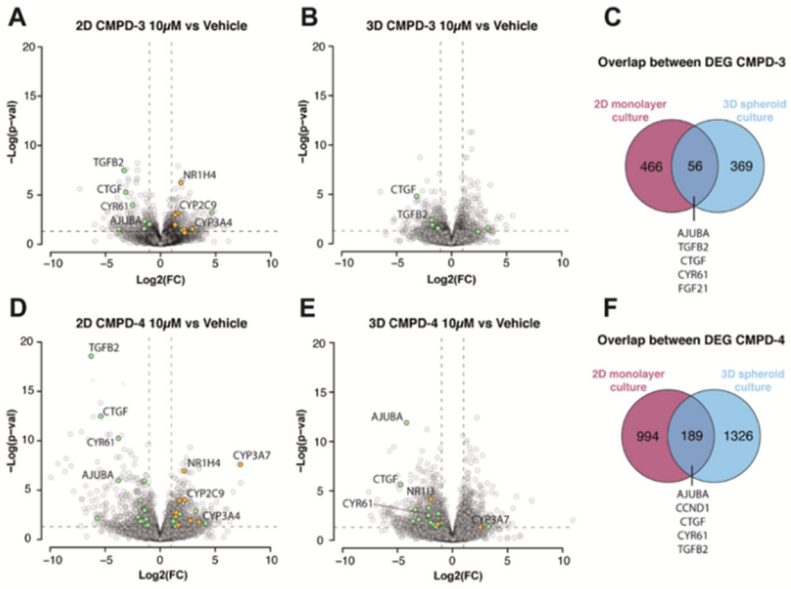

Figure 3: Comparative Gene Expression Response to YAP/TEAD Inhibition in 2D vs. 3D Primary Human Hepatocyte Cultures (Oliva-Vilarnau et al., 2023)

A-B: The figure presents volcano plots illustrating the gene expression impact of Compound 3 (CMPD-3) on PHHcultured in two dimensions (2D) (A) and three dimensions (3D) (B). Differentially expressed genes (DEGs) are highlighted.

C: A Venn diagram details the intersection of DEGs between 2D and 3D PHH cultures under CMPD-3 influence, underscoring the distinctive gene expression profiles elicited by YAP/TEAD inhibition in differing culture formats.

D-E: Volcano plots convey the gene expression ramifications of Compound 4 (CMPD-4) on PHH in 2D and 3D contexts. As with CMPD-3, DEGs connected to ADME are denoted in yellow, and those linked to Hippo signalling are in green.

F: Another Venn diagram showcases the commonalities and discrepancies of DEGs across 2D and 3D PHH cultures when subjected to CMPD-4, highlighting the dependency of cellular responses to YAP/TEAD inhibition on the cultural environment.

4 CLINICAL TRIALS

In the domain of drug metabolism research, in vitro assays are a cornerstone; however, their predictive validity for in vivo CYP induction is inherently limited. Hence, in vivo assays emerge as the gold standard, offering a more faithful reflection of CYP induction within a living system. The direct measurement of enzyme quantity and activity in vivo presents practical challenges, especially in humans. Consequently, an indirect methodology prevails, primarily involving comparative analysis of a drug's AUC before and after the introduction of a novel drug entity or potential inductive agent.

Preclinical evaluation often recruits animal models—ranging from mice and rats to monkeys and dogs—to ascertain CYP induction as a precursor to human testing. It is crucial to recognize the distinct discrepancies in enzyme systems and receptor affinities across species, which often result in divergent metabolic responses. For example, omeprazole's induction effect on CYP1A2 is exclusive to humans and does not extend to murine or rat models. To mitigate such species-specific limitations, research has pivoted towards the development of humanized mice through genetic engineering or the engraftment of human hepatocytes into immunocompromised mice, circumventing the confounding influence of murine hepatic enzymes.

Animal models, notwithstanding the interspecies variation in CYP induction, serve to generate initial pharmacokinetic profiles. Nevertheless, human subjects remain the epitome for assessing CYP induction. In human studies, the characterization of enzyme induction is conducted using selected CYP probe substrates, adhering to rigorous criteria such as enzyme specificity, minimal cross-enzyme inhibition, and optimal pharmacokinetic characteristics, like minimal rapid metabolism or shorter half-lives.

An alternative in vivo measurement strategy involves evaluating pharmacological parameters like the EC50 and Emax, mindful of the interindividual variances that arise due to CYP polymorphisms, which can influence the response to induction probes. Per FDA directives, data from in vitro assays and preliminary clinical assessments should inform the decision to advance to comprehensive human in vivo or clinical evaluations. A drug is earmarked for in vivo investigation only if it elicits an induction exceeding 40%. Furthermore, the FDA's regulatory framework allows for the exclusion of certain enzymes from in vivo scrutiny if in vitro results are conclusively negative (Ghosh et al., 2023).

4.1 Time Course Quantification of CYP3A Modulation with Micro Dosed MDZ

The enzymatic activity of CYP3A is important in the biotransformation and clearance of various pharmaceuticals, predominantly in hepatic and enteric regions. Perpetrator drugs that modulate the activity of CYP3A enzymes can profoundly influence the pharmacokinetic profile of CYP3A substrates, potentially culminating in clinically relevant DDIs. Traditional studies on DDIs tend to prioritize static exposure levels as endpoints, thus overlooking the dynamic nature of these enzymatic interactions over time. A comprehensive understanding of the temporal modulation of CYP3A could shed light on critical periods of altered enzymatic activity, thereby enhancing the strategic planning and oversight of DDIs in clinical settings.

To bridge this knowledge gap, Stevison, F et al. incorporated the use of surrogate probe substrates, such as MDZ, in their investigation of drug interactions (Stevison et al., 2019). Given that MDZ is extensively metabolized by CYP3A, its pharmacokinetic profile serves as a reliable reflection of CYP3A activity. The linearity of MDZ pharmacokinetics across a broad dosage spectrum facilitates the assessment of CYP3A activity without eliciting notable pharmacodynamic responses.

This clinical investigation aimed to delineate the temporal patterns and degree of in vivo modulation of hepatic CYP3A activity by various perpetrator drugs. The researchers implemented a continuous micro dosing regimen involving intravenous MDZ, which allowed for precise quantification of metabolic DDIs in a healthy cohort. The study also endeavored to characterize different modulatory mechanisms.

The study's protocol included 24 healthy participants who received an initial bolus of intravenous MDZ. Subjects were stratified into four cohorts, each receiving a distinct CYP3A perpetrator drug: voriconazole, rifampicin, or efavirenz, with two placebo-controlled individuals per group. Following the MDZ infusion, perpetrator drugs were introduced after a 2-hour interval. Regular blood sampling facilitated the measurement of MDZ and its primary metabolite, 1'-hydroxyMDZ. The study's foremost aim was to quantify the temporal modulation of CYP3A activity by comparing MDZ clearance among the treatment and placebo cohorts.

The findings demonstrated unique temporal signatures and intensities of CYP3A modulation by each perpetrator drug. Notably, efavirenz, recognized as a CYP3A enhancer, displayed a swift onset of modulation, attaining peak impact within 2 to 3 hours. Conversely, rifampicin, a CYP3A inductor, manifested a protracted onset, with maximal impact noted after 28 to 30 hours, and subsequently a rapid return to baseline within 1 to 2 hours. Voriconazole, in both oral and intravenous forms, displayed a sustained inhibitory effect on CYP3A, maintaining suppression over the duration of the sampling interval, which extended to 8 hours post-administration. The study charted the differential peak clearance alterations induced by efavirenz, rifampicin, and both administrations of voriconazole, as depicted in Figure 4.

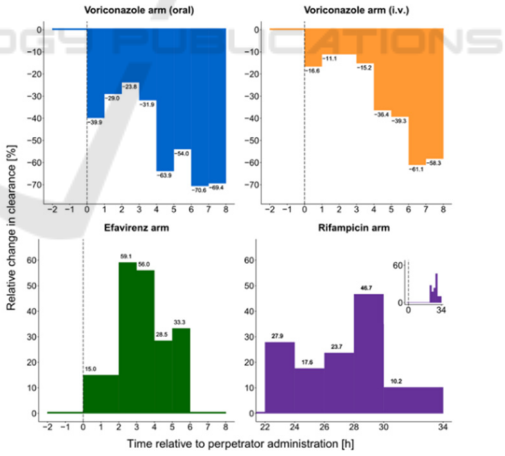

Figure 4: The percentage change in MDZ clearance. over time in comparison to the placebo group, following the administration of perpetrator drugs (Stevison et al., 2019).

The perpetrator drugs are categorized into different arms. The vertical dashed line indicates the time when the perpetrator drug was administered.

4.2 Translating Cytochrome P450 Downregulation from In Vitro to In Vivo

Surrogate probe substrates such as MDZ are instrumental in drug interaction research, providing a scientifically robust method to evaluate CYP enzyme activity. MDZ, primarily metabolized by the CYP3A isozyme, serves as an accurate barometer for CYP3A functionality due to its linear pharmacokinetics across extensive dosing ranges, thus enabling the assessment of CYP3A without eliciting marked pharmacological outcomes (Ingelman, 2004).

A 2019 investigation by Li et al. delved into the impact of all-trans-retinoic acid (atRA), the metabolite of vitamin A on CYP2D6 expression in diverse experimental systems (Li et al., 2019). Prior studies identified that atRA diminishes CYP2D6 expression in cellular models and murine systems by activating the transcriptional corepressor small heterodimer partner (SHP). Yet, whether this suppressive action translates to human physiology remained ambiguous. Notably, atRA does not interfere with the enzymatic function of CYP2D6, offering a unique vantage point to study DDIs that arise exclusively from transcriptional downregulation (Chen et al., 2017; Jiang et al., 2009 & Gibson et al., 2002).

The atRA isomer 13cisRA, is particularly suited for scrutinizing the translational aspects of CYP downregulation. Following administration, 13cisRA is isomerized to atRA and subsequently metabolized into 4-oxo-13cisRA in humans. Consequently, any DDIs observed post-13cisRA administration could be ascribed to 13cisRA itself or a collective effect of these metabolites.

The primary objective of Li et al.'s research was to elucidate the impact of three compounds, namely 13cisRA, atRA, and 4-oxo-13cisRA, on the expression of CYP2D6 within hepatic cells in humans (Li et al., 2019). Additionally, the study aimed to determine whether the findings from in vitro experiments could serve as predictors of potential DDIs in clinical settings. The research findings suggested a theoretical reduction of approximately 50% in CYP2D6 activity following the administration of 13cisRA, as observed in in vitro assays. However, analysis of clinical data, specifically the area under the plasma concentration-time curve for dextromethorphan, a substrate of CYP2D6, revealed only a minor increase in the drug's metabolic clearance after 13cisRA therapy. Similarly, in murine models, the administration of 4-oxo-13cisRA resulted in increased mRNA expression of several Cyp2d isoforms; however, this effect did not strongly correlate with in vivo modulation of CYP2D6 activity.

Moreover, a modest in vitro induction of CYP3A4 in PHH was associated with a correspondingly minor induction in vivo, thereby presenting a disparity between in vitro observations of CYP downregulation and the manifestation of clinical DDIs. These observations accentuate the need for an enriched comprehension of the mechanisms governing CYP downregulation to refine the prediction and management of DDIs in a clinical context.

5 CONCLUSION

The scientific quest to understand the induction of CYP enzymes has garnered considerable focus within the realms of drug development and toxicology (Carroccio, 1994). The goal is to foster a deeper, more precise scientific understanding. Although there has been considerable progress in decoding the mechanisms that underlie CYP induction, the intricacies of these molecular processes demand further elucidation. This need is heightened by the ongoing introduction of novel pharmaceuticals and environmental agents, each necessitating comprehensive evaluations to assess their potential for CYP induction and the resultant risk of undesirable side effects.

The field has seen substantial advancements with the introduction of innovative models for the study of CYP induction. These models operate both within controlled laboratory environments (in vitro) and living organisms (in vivo), significantly bolstering our capacity to examine and anticipate the behaviour of CYP induction. They have markedly enriched our knowledge of the signalling pathways and the diverse array of factors that govern CYP induction, including genetic variability and environmental influencers. Nevertheless, the predictive accuracy for CYP induction in relation to newly synthesized compounds is still encumbered by the multifaceted nature of these biological pathways.

Currently, the drug development industry is equipped with well-established experimental protocols for investigating the induction of CYP enzymes. The data derived from these laboratory analyses provide a foundational guide for subsequent biological investigations and are integral to the creation of predictive models that mirror the pharmacokinetic processes observed in actual physiological conditions. Despite these advanced in silico tools, empirical studies in human populations

are indispensable for a conclusive portrayal of the effects of CYP induction and inhibition, a requirement that is particularly salient for the attainment of regulatory approval. It is fortuitous that ongoing innovations in research methodologies have sharpened the ability to detect potential drug interactions that are mediated by CYP enzymes early in the drug development cycle, thereby mitigating unforeseen adverse effects in clinical settings. Early recognition of such interactions is crucial, steering the course of drug development away from entities that exhibit strong inhibitory or inductive effects on CYP enzymes. Nonetheless, we must acknowledge the presence of yet unidentified agents, possibly present in our diet, herbal treatments, and environmental exposures.

The profound enhancement of our understanding of CYP-mediated interactions has refined the drug development paradigm and the implementation of computational tools and databases to support medication prescribing practices has greatly facilitated their clinical deployment. These advancements are particularly vital considering the vast repository of data on DDIs, which presents a formidable challenge for clinicians to master single-handedly. It is pertinent to note that while the design of drugs has traditionally cantered on improving metabolic stability to diminish CYP-related interactions, it is also essential to consider the potential for interactions mediated by biological transport mechanisms.

Although the capability to predict CYP inhibition and induction is generally reliable, exceptional cases continue to emerge that defy expectations. For instance, the synergistic interaction between non-activating compounds and the PXR, resulting in receptor activation, exemplifies the complexity of predicting drug interactions. These synergies may manifest among pharmaceutical agents or in scenarios of exposure to complex environmental mixtures, relevant in toxicology. Hence, despite the substantial body of knowledge regarding CYP inhibition and induction accrued over the years, ongoing research is imperative. The landscape of drug interaction remains dynamic, with the ever-present prospect of unearthing new insights.

REFERENCES

Pelkonen, O.; Turpeinen, M.; Hakkola, J.; Honkakoski, P.; Hukkanen, J.; Raunio, H. (2008). Inhibition and induction of human cytochrome P450 enzymes: Current status. *Arch. Toxicol*. 82, 667–715.

Li Y, Meng Q, Yang M, Liu D, Hou X, Tang L, Wang X, Lyu Y, Chen X, Liu K, Yu AM. (2019). Current trends in drug metabolism and pharmacokinetics. *Acta Pharmaceutica Sinica B*. Nov 1;9(6):1113-44.

Lin, J. H. (2006). CYP induction mediated drug interactions: Invitro assessment and clinical implications. *Pharm. Res*. 23,1089–1116.

Schuetz, J.D.; Schuetz, E.G.; Thottassery, J.V.; Guzelian, P.S.; Strom, S.; Sun, D. (1996). Identification of a novel dexamethasone responsive enhancer in the human CYP3A5 gene and its activation in human and rat liver cells. *Mol. Pharmacol*. 49, 63–72.

Pascussi,J.M.;Drocourt,L.;Gerbal Chaloin, S. ; Fabre, J. M. ; Maurel, P. ; Vilarem, M. J. Dual effect of dexamethasoneon CYP3A4 gene expression in human hepatocytes. (2001). Sequential role of glucocorticoid receptor and pregnane X receptor. *Eur. J. Biochem*. 268, 6346–6358.

Chen, Y.; Zeng, L.; Wang, Y.; Tolleson, W.H.; Knox, B.; Chen, S.; Ren, Z.; Guo, L.; Mei, N.; Qian, F.; etal. (2017). The expression, induction and pharmacological activity of CYP1A2 are post-transcriptionally regulated by microRNA hsa-miR-132-5p. *Biochem. Pharmacol*. 145, 178–191.

Bernasconi, C., Pelkonen, O., Andersson, T.B., Strickland, J., Wilk-Zasadna, I., Asturiol, D., Cole, T., Liska, R., Worth, A., Müller-Vieira, U. and Richert, L. (2019). Validation of in vitro methods for human cytochrome P450 enzyme induction: Outcome of a multi-laboratory study. *Toxicology in Vitro*, 60, pp.212-228.

Dumond, J.B., Vourvahis, M., Rezk, N.L., Patterson, K.B., Tien, H.C., White, N., Jennings, S.H., Choi, S.O., Li, J., Wagner, M.J. and La-Beck, N.M. (2010). A Phenotype–Genotype Approach to Predicting CYP450 and P-Glycoprotein Drug Interactions with the Mixed Inhibitor/Inducer Tipranavir/Ritonavir. *Clinical Pharmacology & Therapeutics*, 87(6), pp.735-742.

Jiang, W., Wang, L., Zhang, W., Coffee, R., Fazili, I.S. and Moorthy, B. (2009). Persistent induction of cytochrome P450 (CYP) 1A enzymes by 3-methylcholanthrene in vivo in mice is mediated by sustained transcriptional activation of the corresponding promoters. *Biochemical and biophysical research communications*, 390(4), pp.1419-1424.

Stevison F, Kosaka M, Kenny JR, et al. (2019). Does In Vitro Cytochrome P450 Downregulation Translate to In Vivo Drug-Drug Interactions? Preclinical and Clinical Studies With 13-cis-Retinoic Acid. *Clin Transl Sci.* 12(4):350-360.

Ghosh R, Nayan MI, Mitu MM, Nandi T. (2023). Common Approaches of Cytochrome P450 (CYP) Induction Assays. *International Blood Research & Reviews.* Jan 18;14(1):6-14.

Oliva-Vilarnau, N., Vorrink, S.U., Büttner, F.A., Heinrich, T., Sensbach, J., Koscielski, I., Wienke, D., Petersson, C., Perrin, D. and Lauschke, V.M. (2023). Comparative analysis of YAP/TEAD inhibitors in 2D and 3D cultures of primary human hepatocytes reveals a novel non-canonical mechanism of CYP induction. *Biochemical Pharmacology*, 215, p.115755.

Nassar, Y.M., Hohmann, N., Michelet, R., Gottwalt, K., Meid, A.D., Burhenne, J., Huisinga, W., Haefeli, W.E., Mikus, G. and Kloft, C. (2022). Quantification of the time course of CYP3A inhibition, activation, and induction using a population pharmacokinetic model of microdosed midazolam continuous infusion. *Clinical Pharmacokinetics*, 61(11), pp.1595-1607.

Gibson GG, Plant NJ, Swales KE, Ayrton A, El-Sankary W. (2002). Receptor-dependent transcriptional activation of cytochrome P4503A genes: induction mechanisms, species differences and interindividual variation in man. *Xenobiotica*. Jan 1;32(3):165-206.

Zhang R, Wang XX, Xie JF, Yao TT, Guo QW, Wang Q, Ding Z, Zhang JP, Zhang MR, Xu LC. (2024). Cypermethrin induces Sertoli cell apoptosis through endoplasmic reticulum-mitochondrial coupling involving IP3R1-GRP75-VDAC1. *Reproductive Toxicology*. Jan 29:108552.

Si C, Yang H, Wang X, Wang Q, Feng M, Li H, Feng Y, Zhao J, Liao Y. (2024). Toxic effect and mechanism of β-cypermethrin and its chiral isomers on HTR-8/SVneo cells. *Pesticide Biochemistry and Physiology*. May 1; 201:10584.

Carroccio, A.; Wu, D.; Cederbaum, A.I. (1994) Ethanol increases content and activity of human cytochrome P450 2E1 in a transduced HepG2 cell line. *Biochem. Biophys. Res*. Commun. 203, 727–733.

Ingelman-Sundberg, (2004). M. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch*. Pharmacol. 369, 89–104.