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In Vitro Models for Predicting Transporter-Mediated DDIs

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Drug-drug interactions (DDIs) are often caused by the effects of membrane transporters on drug absorption and disposition in the body.[1] Transporters, such as efflux and uptake, can have significant impacts on a drug's safety and efficacy — either alone or in conjunction with drug-metabolizing enzymes.[2] Predicting and understanding DDIs through in vitro studies is vital to gaining insights into their potential causes and consequences. It is therefore necessary that drug developers partner with contract research organizations (CROs) that excel in conducting in vitro studies and generating the required data to determine whether clinical DDI studies are necessary.

The full picture of a drug's DDI potential requires the exploration of a variety of complex factors, including how the drug is absorbed and eliminated, how enzymes and transporters contribute to its disposition, and how to characterize its effects on enzymes and transporters.[2] Well-designed in vitro transporter studies are extremely valuable for predicting the clinical relevancy of a DDI via transporters. High-quality CRO partners should therefore have all the necessary tools and experience to evaluate potential transporter mediated DDIs thoroughly.

Among these tools are the following,[2] which are used to study transporters as outlined in FDA[3] and EMA[4] guidelines:

Caco-2 cells expressing efflux transporters (e.g., P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP))

Caco-2 cells have many of the same transporters and enzymes found in the human intestine and can be used to predict how drugs and other compounds will be absorbed and metabolized in the body

HEK293 cells transfected with uptake or efflux transporters (e.g., OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2k)

HEK293 cells are derived from human embryonic kidney cells; they have high transfection efficiency (i.e., their ability to take up foreign DNA), and they can express recombinant proteins at high levels

MDR1-MDCKII cells

MDR1-MDCKII cells are derived from the Madin-Darby canine kidney (MDCK) cell line and are engineered to express high levels of the MDR1 gene that encodes for P-gp

HEK293 membrane vesicles expressing P-gp, BCRP, and BSEP

HEK293 membrane vesicles are particularly useful for studying membrane transporters that are difficult to express or purify in other systems

These and other tools should be part of the bioanalytical arsenal of a CRO that is well-versed in transporter mediated DDIs. They are the means by which experienced scientists can conduct properly designed in vitro transporter studies, giving drug developers the data they need to determine whether a DDI through transporters is likely to bear consequences for patients.

Without adequate sensitivity, the transporter's ability to detect DDIs may be significantly reduced, potentially leading to false negative results. To ensure that transporter studies provide reliable and accurate results, it is essential to validate the LLOQ of the analytical method used.

The Importance of LLOQ, Stability, and AP in In Vitro Transporter Studies

To determine the potential for a new drug to cause a transporter mediated DDI, numerous factors need to be considered, and one of the most important is the lower limit of quantification (LLOQ). The LLOQ is the lowest concentration of the drug that can be accurately measured with acceptable accuracy and precision. The analytical method should be capable of detecting and quantifying the drug and its metabolites in different matrices at levels below the LLOQ to obtain reliable data. An analytical method's sensitivity is a crucial factor that determines its suitability for transporter studies. Without adequate sensitivity, the transporter's ability to detect DDIs may be significantly reduced, potentially leading to false negative results. To ensure that transporter studies provide reliable and accurate results, it is essential to validate the LLOQ of the analytical method used.

Another important factor in transporter studies is the drug's stability. During the in vitro experiment, the drug's stability in various matrices, including plasma, is crucial to avoid false negative results. A drug that is unstable in plasma may not be detected at the intended concentration, leading to an overestimation of the DDI's effect. Thus, stability studies of the drug should be performed under various conditions to ensure the drug's stability in the matrices used during the transporter study. Transporter studies should use validated analytical methods to ensure that accurate and reliable drug concentrations are measured throughout the experiment.

Alkaline phosphatase (AP) is an enzyme that hydrolyzes phosphate esters at alkaline pH. AP is found in many tissues in the body, including the intestine, liver, and kidney. It can dephosphorylate drugs and metabolites, which can impact their pharmacokinetic (PK) properties. In transporter studies, AP plays a crucial role in assessing the drug's permeability across the intestinal wall. AP is commonly used to identify the extent of the intestinal membrane's enzymatic barrier and assess the extent to which it contributes to the drug's absorption. As a result, AP activity should be assessed during transporter studies to understand the drug's permeability and its contribution to DDIs.

Making the Right Decisions at the Right Time

Drug developers need to know whether their drug candidate is a substrate or inhibitor of a transporter and whether this is clinically relevant. Properly designed in vitro transporter studies can support the prediction of both.

It is also essential to understand (a) when to do these experiments and (b) how comprehensive they must be — decisions that are based on the therapeutic indication addressed by the drug, the clinical study design, and when the in vitro evaluation occurs. A prime example would be a population that has a high rate of statin use, in which case the investigational drug's potential to interact with OATP1B1/1B3 must precede clinical use.[5]

Co-medications are clearly a major factor in these decisions, so inclusion and exclusion criteria are important considerations. Transporter studies can, of course, be avoided early on (Phase I) by only enrolling healthy volunteers with no co-medications; although, when the time comes to administer a drug to

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patients, concurrent medications and factors such as renal impairment must be considered. To elaborate on this example, when the kidneys are not functioning properly, the drug may accumulate in the body and reach toxic levels, leading to serious side effects and even life-threatening complications. Certain medications can further damage the kidneys, worsening the existing impairment and creating a vicious cycle of ever-increasing toxicity.

As for the comprehensiveness of a transporter study, this hinges on the correlation between in vivo drug concentrations and the concentrations at which the drug interacts with the transporter in vitro. To illustrate, if a drug acts as an inhibitor at an exceedingly high concentration that is not expected to be achieved in a clinical setting, further testing may not be necessary.[6] Characterizing the free fraction of a drug in the in vitro system, as well as in vivo protein binding, are critical data points to consider.

Choosing the Right In Vitro Model

Selecting the appropriate in vitro model for testing depends on the desired outcome. If a quick turnaround time is the priority and a detailed characterization of a transporter interaction is not required, rapid screening assays are adequate. For a more thorough characterization (e.g., deciding whether to conduct a DDI study), HEK293 singly transfected cells or the Caco-2 cell line are highly valuable for analyzing the kinetics of an interaction in detail and generating essential output.

In the case of Caco-2, its ability to mimic the human intestinal epithelium barrier makes it extremely useful. The cells form a polarized monolayer simulating the intestinal membrane, allowing scientists to assess the potential for DDIs to occur by measuring the impact of one drug on the transport of another drug across the Caco-2 monolayer. In all cases, however, it is crucial to choose the appropriate model that can provide the most precise prediction of a new drug's behavior in vivo.

Validating Models and Experimental Conditions

To establish an assay initially, well-characterized substrates can be used to validate the transporter study models and experimental conditions, thus allowing one to characterize assay performance. Thereafter, assay quality must be consistently evaluated to ensure that the assays are operating properly. The assay validation process is an ongoing effort, with frequent reevaluation and optimization to ensure that the assays continue to perform at the highest level possible.

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Conclusion

Transporter studies play a critical role in predicting DDIs in vitro, and high-level CROs have the expertise and experience needed to design and execute comprehensive in vitro transporter studies that provide drug developers with the data they need to make informed decisions about their candidates. DDI studies should be tailored to the specific needs of each project, determined through detailed and fully transparent communication and taking into account factors such as the timing of clinical studies, the intended patient population, and the potential for co-medications.[2]

Optimal CRO partners should have expertise using a variety of in vitro models to evaluate the kinetics of transporter interactions, validating their assays using well-characterized substrates and inhibitors to ensure the highest level of performance. By conducting thorough in vitro testing, a CRO can provide drug developers with the data they need to determine whether clinical DDI studies are necessary, and if so, what parameters should be evaluated in those studies.

References

1 Bentz, J., et al. Variability in P-glycoprotein inhibitory potency (IC50) using various in vitro experimental systems: implications for universal digoxin drug-drug interaction risk assessment decision criteria. Drug Metab Dispos. Published 2013 July.

2 International Transporter Consortium. Membrane transporters in drug development. Nat Rev Drug Discov. Published 2010 March.

3 U.S. Food and Drug Administration. Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry. Published 2020 January.

4 European Medicines Agency. Guideline on the investigation of drug interactions. Published 2012 June 21.

5 Wiggins, B., et al. Recommendations for Management of Clinically Significant Drug-Drug Interactions With Statins and Select Agents Used in Patients With Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation. Published in 2016 October 17.

6 U.S. Food and Drug Administration. Drug Development and Drug Interactions: Possible Models for Decision-Making. Published 2015 Feb. 2.

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