MINI REVIEW

BCPT
Basic & Clinical Pharmacology & Toxicology

Translational aspects of cytochrome P450-mediated drug–drug interactions: A case study with clopidogrel

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Abstract

Multimorbidity, polypharmacotherapy and drug interactions are increasingly common in the ageing population. Many drug–drug interactions (DDIs) are caused by perpetrator drugs inhibiting or inducing cytochrome P450 (CYP) enzymes, resulting in alterations of the plasma concentrations of a victim drug. DDIs can have a major negative health impact, and in the past, unrecognized DDIs have resulted in drug withdrawals from the market. Signals to investigate DDIs may emerge from a variety of sources. Nowadays, standard methods are widely available to identify and characterize the mechanisms of CYP-mediated DDIs in vitro. Clinical pharmacokinetic studies, in turn, provide experimental data on pharmacokinetic outcomes of DDIs. Physiologically based pharmacokinetic (PBPK) modelling utilizing both in vitro and in vivo data is a powerful tool to predict different DDI scenarios. Finally, epidemiological studies can provide estimates on the health outcomes of DDIs. Thus, to fully characterize the mechanisms, clinical effects and implications of CYP-mediated DDIs, translational research approaches are required. This minireview provides an overview of translational approaches to study CYP-mediated DDIs, going beyond regulatory DDI guidelines, and an illustrative case study of how the DDI potential of clopidogrel was unveiled by combining these different methods.

KEYWORDS
cytochrome P450, drug–drug interaction, drug-metabolizing enzymes, pharmacokinetics, translational research

1 | INTRODUCTION

Drug–drug interactions (DDIs), especially those involving drug metabolism by cytochrome P450 (CYP) enzymes, are a common cause for adverse drug reactions (ADRs) and treatment failure. DDIs are often preventable, but only in cases where the risk of DDI and its clinical consequences have been identified and sufficiently characterized. The ageing population and increasing polypharmacotherapy increase the risk of occurrence of clinically relevant DDIs. During drug development, specific DDI mechanisms are routinely screened in vitro, and clinical DDI studies are performed using standard methods, according to guidelines set by medical authorities.[1–3] At present, these guidelines are undergoing a process of harmonization by the International

Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). As the first detailed DDI guidelines were published in 1997 and have been revised several times, there are, however, many older drugs that have not been examined according to current standards.

A comprehensive translational approach of CYP-mediated DDI investigation requires the input of different research disciplines.[4,5] Furthermore, consideration of other mechanisms such as interplay between CYP enzymes and drug transporters is essential. Both CYP-mediated metabolism and CYP inhibition or induction can be characterized with relatively straightforward in vitro studies, providing a biochemical mechanistic understanding on the potential for CYP-mediated DDIs. Various types of clinical studies can produce in vivo data on the pharmacokinetic, pharmacodynamic and clinical consequences of DDIs, but due to safety and other practical reasons (e.g. costs), it may not be possible to study all relevant DDI situations clinically. However, simulation methods, such as physiologically based pharmacokinetic (PBPK) modelling, can utilize available preclinical and clinical data to predict DDI scenarios that have not been directly studied clinically. Ultimately, epidemiological studies on real-world data, for example, based on mining of medical records or adverse effect reports, can be used to identify signals of potential DDIs, as well as to shed light on the health outcomes of DDIs. Accordingly, to identify previously unknown CYP-mediated DDIs, to characterize their mechanisms, to assess their pharmacokinetic and clinical consequences and to evaluate approaches to avoid or mitigate specific DDI risks, translational approaches utilizing a plethora of methodologies are an intrinsic part of the research of CYP-mediated DDIs.

This minireview summarizes the different approaches to study DDIs and presents a case study on a series of clopidogrel DDI studies demonstrating the importance of translational DDI research in practice and how multidisciplinary approaches can add value to DDI assessment.

2 | SIGNALS AND SCREENING FOR DDIS

Signals or need to study DDIs in detail may arise from different sources (Figure 1). For CYP-mediated interactions, the most obvious source for DDI signals are in vitro findings. Case reports, ADR reporting and other clinical observations may alert for DDI studies at any point during a drug's lifecycle. Register-based epidemiological data, including informatics-driven approaches

utilizing big data from various sources, can provide signals for previously unrecognized DDIs, prompting for their further characterization.[6,7] In addition, computational methods, for example, pharmacophore or docking simulations or other computational approaches, can be useful to infer potential DDIs already during preclinical development phases.[8] Even though animal studies have a distinct role in characterizing the preclinical absorption, distribution, metabolism and elimination (ADME) properties of a drug, they have little value in directly predicting DDIs in humans due to species differences in enzymes.

3 | IN VITRO STUDIES

The in vitro CYP-mediated metabolism and DDI profile of a drug and its main metabolites can be characterized early in the drug development process. The assays are well established and described in regulatory guidelines.[1,3] For older drugs, however, crucial DDI properties may have been missed due to methodological limitations at the time. Such limitations could have included use of limited CYP test palettes, supratherapeutic substrate concentrations or protocols that did not comprise testing for time-dependent inhibition or testing the DDI potential of drug metabolites. For example, the role of CYP2C8 in drug metabolism and the interaction potential of the glucuronide metabolite of gemfibrozil were unrecognized before characterization of the DDI between gemfibrozil and cerivastatin, an inhibitor–substrate pair of CYP2C8.[9]

The processes of identification of the CYPs that are involved in the metabolism of a drug and determination of the fraction metabolized by a specific CYP ($f_{m,\mathrm{CYP}}$) are a crucial part of preclinical studies.[1,3,10] Briefly, these experiments typically include use of CYP-selective inhibitors or antibodies in human liver microsomal (HLM), human liver S9 fraction or hepatocyte incubations combined with kinetic experiments in recombinant enzymes. Regulatory agencies maintain lists of the recommended CYPs that should be tested and CYP-selective inhibitors that could be used in inhibition experiments. At present, pharmaceutical companies are recommended to evaluate metabolism by CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A, but CYP2A6, CYP2E1 and CYP2J2 are not included in this list. Other useful approaches used to support the involvement of key CYPs in the metabolism of a drug are correlation analysis in a panel of individual HLMs, use of small-interfering RNA to knock down CYPs at gene level in hepatocytes and experiments in microsomes or hepatocytes that have been characterized for CYP polymorphisms. If metabolism is expected to occur in organs other than the liver,
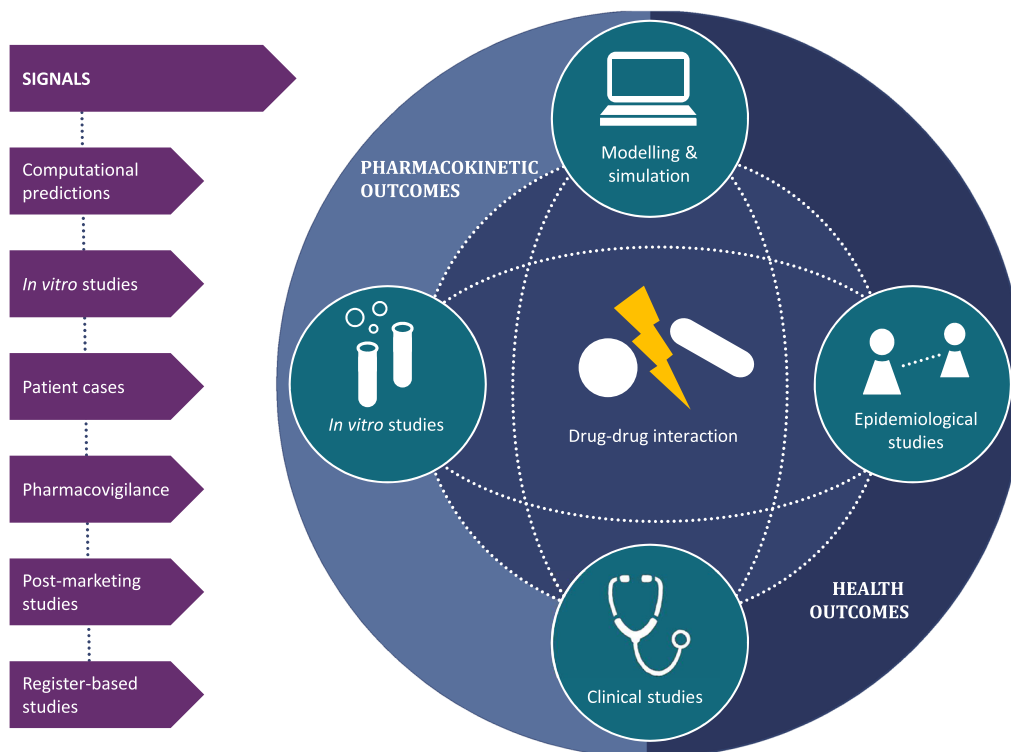
FIGURE 1 Translational investigation of cytochrome P450 (CYP)-mediated drug–drug interactions (DDIs). Signals of a potential DDI may arise from a variety of sources. In vitro assays can be used to identify and characterize the underlying mechanisms of CYP-mediated DDIs. In turn, clinical pharmacokinetic studies provide experimental data on the pharmacokinetic outcomes of DDIs. Physiologically based pharmacokinetic (PBPK) modelling combining in vitro, in silico and in vivo data is a powerful tool to improve mechanistic understanding and predict the outcomes of different DDI scenarios. Finally, epidemiological studies can provide evidence on the health outcomes of DDIs

subcellular fractions or cells from these tissues can be used when available. Selection of the appropriate system for metabolism studies should be carefully considered, as each system has its advantages and limitations.[1,3,11,12] Moreover, in the future, it is likely that novel tools, such as microphysiological 'organ-on-a-chip' models comprising, for example, cultured cells and microfluidic systems, can be used for determination of drug metabolism and DDI parameters.[13]

When evaluating the CYP inhibitory potential of a drug, both reversible and time-dependent (irreversible) inhibition should be tested. Irreversible inhibition is relatively common mechanism of CYP inhibition, which can lead to a more severe and long-lasting inhibition.[14] Use of HLMs or hepatocytes in combination with CYP-specific probe substrates is a good choice,[1,3] especially since recombinant CYPs may display differences in their sensitivity to detect irreversible inhibition.[12] If inhibition of several enzymes should be tested, probe substrate cocktails to assess both reversible and irreversible inhibition could be used in the initial experiments.[15] For instance, inhibition investigations could start with a preliminary screening, followed by determination of half-maximal inhibitory concentration (IC_{50}) for both

inhibition forms and potentially determination of the reversible inhibition constant (K_i) or the irreversible inactivation constant (K_I) and maximal inactivation rate (k_{inact}).

The mechanisms behind CYP induction and particularly downregulation are not as well characterized as those causing inhibition.[1,3] The induction potential of a drug is generally studied in primary human hepatocytes (three individuals), but immortalized hepatic cell lines can also be used. Changes in messenger RNA levels or enzyme activity levels using a suitable CYP probe substrate are measured. From these studies, maximum induction capacity (E_{max}) and the inducer concentration at half-maximal induction (EC_{50}) values are obtained.

Prediction of CYP-mediated DDIs based on these in vitro data can be done by applying static models or by using dynamic modelling as discussed separately. Static models, which can further be divided into basic and mechanistic models, are thoroughly described in regulatory guidelines.[1,3] Basic models only consider one interaction mechanism at a time and are typically applied to project a worst-case scenario in situations where prior knowledge is limited. Mechanistic models may combine several interaction mechanisms and generally provide

more realistic DDI risk estimates by incorporating additional factors such as the interaction at intestinal level, parallel elimination routes of the victim drug and transporter–enzyme interplay as presented in Figure 2. More sophisticated models may also include the DDI effects of perpetrator metabolites.[16] Static models are, however, limited to predictions based on single perpetrator concentrations, and there is considerable debate regarding estimation of the relevant in vivo perpetrator concentration to reflect the concentration available at the enzyme site (e.g. total or unbound average systemic, maximum systemic or hepatic inlet concentration).[16,17] Recent developments in determination of intracellular concentrations have also allowed testing of unbound intracellular hepatocyte concentrations in these models.[18,19] Cut-off values for the DDI risks predicted with static models guide whether further investigation is

necessary.[1,3] However, especially when combining several simultaneous interaction mechanisms, the predicted DDI risk should be interpreted with caution.[1,17] Nevertheless, while static models may be useful for qualitative assessment of DDIs, they rely on steady-state assumptions and hence cannot capture the full dynamic nature of physiologic and pharmacokinetic processes. In addition, they only provide average DDI estimates, and the risk to individuals is not directly evaluated.

In summary, in vitro experiments are necessary to elucidate the exact DDI mechanisms between two drugs. Investigation of CYP-mediated DDIs is fairly straightforward, although careful attention should be paid to accurate experimental conditions. Based on the obtained in vitro parameters, static models can be applied to predict the magnitude of the expected DDI. These predictions are, however, sensitive to the perpetrator

(A) Model combining three interaction mechanisms for evaluation of the overall DDI potential of a perpetrator drug

$$AUC_R = \left[\frac{1}{(A_g \times B_g \times C_g) \times (1 - F_g) + F_g}\right] \times \left[\frac{1}{(A_h \times B_h \times C_h) \times f_m + (1 - f_m)}\right]$$

	Mechanism	Intestine (g)	Liver (h)
A	Reversible inhibition	$A_g = 1/[1 + (I_g/K_i)]$	$A_h = 1/[1 + (I_h/K_i)]$
B	Time-dependent inhibition	$B_g = \dfrac{k_{deg,g}}{k_{deg,g} + (I_g \times k_{inact})/(I_g + K_I)}$	$B_h = \dfrac{k_{deg,h}}{k_{deg,h} + (I_h \times k_{inact})/(I_h + K_I)}$
C	Induction	$C_g = 1 + \dfrac{d \times E_{max} \times I_g}{I_g + EC_{50}}$	$C_h = 1 + \dfrac{d \times E_{max} \times I_h}{I_h + EC_{50}}$

(B) Model based on reversible inhibition for evaluation of the combined DDI potential of a perpetrator drug and its inhibitory metabolite

$$AUC_R = \left[\frac{1}{A_g \times (1 - F_g) + F_g}\right] \times \left[\frac{1}{A_h \times f_m + (1 - f_m)}\right]$$

	Mechanism	Intestine (g)	Liver (h)
A	Reversible inhibition	$A_g = 1/[1 + (I_{g,parent}/K_{i,\text{parent}})]$	$A_h = 1/[1 + (I_{h,parent}/K_{i,\text{parent}}) + (I_{h,met1}/K_{i,\text{met1}})]$

FIGURE 2 Examples of mechanistic static models to predict drug–drug interactions (DDIs) affecting a selected cytochrome P450 (CYP) enzyme. The mechanistic static model recommended by drug authorities[1,3] combines up to three interaction mechanisms (A). If the model estimates a positive result, that is, an area under the plasma concentration time–curve ratio of the victim drug in the presence and in the absence of the perpetrator drug (AUC_R) outside the lack-of-interaction range 0.8–1.25, clinical studies are recommended to investigate the clinical relevance of the DDI. The equation in (B) predicts the effects of simultaneous reversible inhibition by a perpetrator drug and its inhibitory metabolite on the victim AUC_R (adapted from ref.[16]). Here, it is assumed that the metabolite is formed in the liver and does not inhibit the enzyme at intestinal level. d, scaling factor, assumed to be 1; E_{max}, maximum induction effect; EC_{50}, concentration causing half-maximal induction effect; F_g, fraction available after intestinal metabolism; f_m, fraction of hepatic clearance of the substrate mediated by the affected enzyme; I, perpetrator drug concentration at the relevant site; k_{deg}, apparent first-order degradation rate constant of the affected enzyme; K_i, inhibition constant (reversible inhibition); K_I, inactivation constant (time-dependent inhibition); k_{inact}, inactivation rate constant; k_{obs}, observed apparent first order inactivation rate of the affected enzyme

concentrations used. The outcome of the predictions can be used to guide if further (dynamic) modelling or clinical DDI studies are needed.

4 | CLINICAL PHARMACOKINETIC STUDIES

Clinical pharmacokinetic DDI studies are most often carried out in healthy volunteers, applying a prospective crossover design with change in the area under the concentration–time curve (AUC) of the CYP substrate and/or its active metabolite as the primary endpoint.[3,20] In cases where the drug under investigation is suspected to be an inhibitor of a certain CYP, a sensitive index substrate is typically administered in a single dose following pretreatment with the perpetrator. Analogously, if the drug is studied as a substrate of a CYP, a strong index inhibitor of the CYP is administered as a pretreatment; in such studies, careful evaluation of relevant beneficial and potentially harmful pharmacodynamic effects can also be warranted to facilitate clinical conclusions. A key issue with clinical DDI studies is that they are interventional studies involving human subjects, and therefore, ethical and/or financial constraints limit the number of different studies or study arms that can be performed. Consequently, clinical studies cannot cover all possible dosing scenarios or drug combinations. Use of well-documented selective index inhibitors and substrates provides the best premises to obtain data that can be extrapolated to other untested scenarios.[21] Drug regulatory authorities list potential substrates and inhibitors for clinical DDI studies in their guidelines.[2,3] For some CYPs such as CYP2B6, however, sensitive substrates and strong inhibitors are lacking.[20]

Design of clinical pharmacokinetic DDI studies is typically informed by in vitro metabolism data, coupled with other preclinical data and clinical findings from early pharmacokinetic studies, such as mass-balance studies that allow estimation of the fraction of the dose eliminated via hepatic metabolism. When the investigational drug is evaluated as a DDI perpetrator, clinical studies are usually performed to test the worst-case scenario with the anticipated highest clinically used doses of the drug, focusing on the CYP that is likely to be affected most. When the drug is investigated as a victim, inhibitors of the most relevant enzymes are studied first. Therefore, negative or weak DDI findings can allow conclusions regarding lack of clinically relevant interactions and may render further DDI studies unnecessary. On the other hand, when a significant interaction is present, the underlying mechanism(s) should be carefully considered. If the victim drug is metabolized by several enzymes or is

a substrate of drug transporters, further studies may be required to evaluate the significance of the different pharmacokinetic pathways in the observed DDI. This may include in vitro studies or PBPK modelling, but sometimes also subsequent clinical studies with inhibitors or substrates with a different CYP enzyme selectivity. For perpetrator studies, an alternative is to use a cocktail approach, where several selective probe substrates are administered simultaneously and thus effects on several CYP enzymes can be quantified in a single study.[22] To account for gene–drug–drug interactions, genotyping of functionally significant CYP gene variants is recommended, since, for example, individuals who are poor metabolizers may present with lack of interaction, risking false negative interpretation of an actual DDI. Ultimately, clinical DDI studies can document the DDI potential of the drug (weak, moderate or strong CYP inhibitor/inducer) and/or its sensitivity to CYP inhibition/induction.

Pharmacokinetic analysis of drug concentrations in samples obtained from clinical trials in patient populations can be a useful addition to formal pharmacokinetic DDI studies. This approach requires a sufficient number of patients using concomitant medications of interest and typically entails population pharmacokinetic analysis of Phase 2 or 3 clinical trials.[23] Such studies require careful planning with respect to data and sample collection, but can be highly valuable, since they represent the consequences of DDIs in a real patient population. Furthermore, some clinical outcomes of DDIs can be evaluated from the clinical data, even in case no pharmacokinetic samples are available. An emerging approach to obtain data on the investigational drug as a perpetrator is to use endogenous compounds as biomarkers of enzyme and transporter activities; this approach can easily be adapted to different study designs, since it does not require administration of index substrates or tedious blood sampling. However, well-documented endogenous biomarkers for CYPs are still scarce.[24]

The results of clinical pharmacokinetic DDI studies need to be translated into clinical situations. In clinical DDI studies, the victim drug is typically administered as a low single dose. If the drug exhibits linear pharmacokinetics, the results can be extrapolated to clinical repeated dosing with confidence. However, in case of dose- and time-dependent pharmacokinetics, and when pharmacokinetics differs between patients and healthy subjects, extrapolation may be challenging. Moreover, as change in drug exposure does not always directly translate into clinical significance, careful consideration of the victim drug's therapeutic index and concentration-dependent ADRs is required. Eventually, after the clinical significance of a DDI is established, it may also be necessary to

carry out prospective clinical studies to investigate clinical approaches to avoid or manage the DDI, for example, by dose adjustments.

5 | MODELLING AND SIMULATION

PBPK modelling methods to predict DDIs have evolved rapidly during the past two decades and are now an integral part of DDI studies.[25,26] PBPK modelling is a computer-based, dynamic translational method that integrates system-dependent parameters (biological and physiological information of the animal/human body) with drug-dependent parameters (physicochemical, ADME and DDI properties). Using a series of linked differential equations, PBPK models can simulate concentration–time profiles of drugs and their metabolites in plasma and selected organs of the chosen system. Thus, they allow for simultaneous modelling of multiple drug disposition processes, providing a range of opportunities, including simulation of CYP-mediated DDIs.[27,28] From a translational perspective, PBPK modelling can be seen as a sophisticated tool to bridge in vitro and in vivo DDI studies. It may also serve as a starting point to investigate novel DDI signals (Figure 1). One of the greatest strengths of PBPK modelling is the possibility to interpret and extend knowledge received from DDI studies to new situations, for example, DDIs in specific patient populations or following clinically untested drug dosing regimens. In addition, PBPK models can incorporate interindividual variability, thus allowing for simulations of population variability of DDIs.

PBPK modelling is encouraged by regulatory agencies,[2,3] and the pharmaceutical industry is increasingly using it to predict DDIs and to inform drug labelling as a replacement for clinical studies.[29] Assessment of metabolic DDIs was the most common purpose (60%) of PBPK analyses in new drug submissions reviewed by the Food and Drug Administration (FDA) between 2008 and 2017.[29] In regulatory submissions, PBPK modelling has been used to predict different DDI scenarios, support clinical DDI study design and mechanistically explain clinically observed DDIs.[25] There are many examples of successful DDI simulations, particularly in cases where the underlying mechanism is reversible CYP inhibition. Unlike static models, PBPK models can accommodate transient effects on CYPs, as well as the effects of time-dependent inhibition and induction on enzyme activity. However, the modelling of these mechanisms is not as established as that of reversible inhibition.[29,30] Moreover, prediction of complex DDIs and DDIs affecting non-CYP drug-metabolizing enzymes and drug transporters may

be difficult due to lack of selective in vivo data, uncertainties regarding protein expression levels in the relevant cellular localization and enzyme turnover characteristics and scaling of drug concentration levels at the relevant sites.

The predictive performance of PBPK models relies not only on the accuracy of the scaling and physiological parameters but also on the quality and extent of input data.[31] So-called 'bottom-up' PBPK models are typically built at earlier stages of drug development and, as such, primarily depend on in vitro and in silico data. A 'top-down' approach refers to fitting of models to clinical pharmacokinetic data. Usually, top-down models can interpolate data, but extrapolation to outside the data space used to fit the model may be challenging. Verified bottom-up and 'middle-out' models integrating in vitro and clinical data may offer enhanced flexibility by applying 'learn and confirm' cycles of feedback and model optimization.[25] The reliability of these models increases with accumulating clinical data. In PBPK modelling, however, there has been a lack of consistency in model development.[27]

Modelling of CYP-mediated DDIs is most easily carried out using commercially available or freeware PBPK software, which provide the computational and physiological frameworks of the PBPK platform (Figure 3).[28] Accordingly, only building of the drug-dependent component of the model relies on the user. However, PBPK models require considerably more input data than static prediction approaches, and familiarity with the applied equations and assumptions is crucial.[27,28] Ideally, separate sets of clinical data for model building and refinement (training set) and model verification are used, and acceptance criteria and purpose of the modelling are predefined.[25,27] In the ideal case, the models can be cross-verified using data from multiple clinical studies concerning both the perpetrator and the victim drug.

To summarize, PBPK modelling is a promising tool in translational DDI research. At present, PBPK models can be highly valuable in interpretation and extrapolation of clinical DDI findings to different clinical situations, as well as in planning and design of new clinical studies. The present regulatory PBPK guidelines outline the desired format and contents of PBPK reports but provide little guidance regarding model development and quality assessment, indicating a need for best practice guidelines.[25,27]

6 | EPIDEMIOLOGICAL STUDIES

Pharmacoepidemiological studies using healthcare and other register data for DDI research can complement other approaches that are used for characterization of the
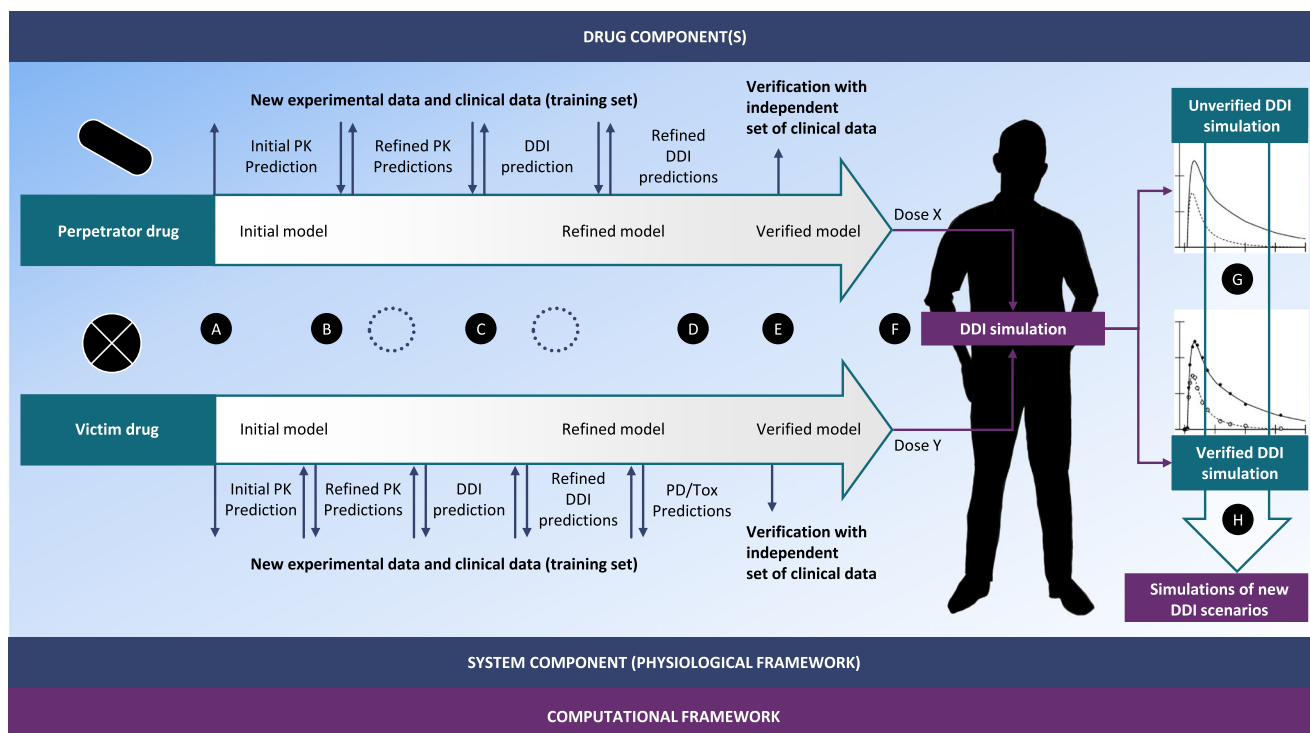
FIGURE 3 Physiologically based pharmacokinetic (PBPK) modelling of cytochrome P450 (CYP)-mediated drug–drug interactions (DDIs) is usually carried out in a stepwise, iterative manner. Firstly, if not already available, separate victim and perpetrator drug models are developed based on the available preclinical and clinical pharmacokinetic data of the drugs (A). Initial predictions are compared with clinical pharmacokinetic data of the training set (B). Subsequently, or after obtaining new clinical/experimental data, there may be a need for adjustment of selected parameters to improve the model. Next, the predictive performance of the model can be confirmed by comparing predictions with additional clinical data that were not included in the previous steps. Here, the DDI profiles of the drugs could be established (C). If the relevant in vitro DDI parameters are known, their legitimacy could be tested by simulating previously documented clinical DDIs. Conversely, these parameters can also be estimated based on former DDIs by use of parameter estimation/sensitivity analysis approaches. In addition, pharmacokinetic modelling in genetic subpopulations, for example, poor metabolizers, could be done. Preliminary simulations to predict the DDI magnitude between the perpetrator and victim drugs could also be carried out (circles with dashed lines). Moreover, victim drug pharmacodynamic (PD) and toxicity (tox) data, when available, could also be included in the model for pharmacokinetic–pharmacodynamic simulations (D). When a good description of the pharmacokinetic and DDI profiles has been established, refined models are verified against independent sets of clinical data (E). Ideally, the DDI profile of both the perpetrator and the victim drug can be cross-verified using data from multiple clinical studies. Finally, the perpetrator and victim drug models are interlinked in DDI predictions (F), verified if possible (G) and extrapolated to new scenarios if necessary (H)

mechanisms and pharmacokinetic outcomes of a DDI. Specifically, unlike other methods, they can offer a tool to evaluate the real-world health outcomes of DDIs. They can also be invaluable in situations, where pharmacokinetic studies are not feasible due to safety issues, such as with many anticancer agents.[32–34] For example, cohort, case–control and case–crossover study designs can be used, depending on the objective of the study.[35]

Generally, in epidemiological DDI studies, the association of concomitant use of a perpetrator with a victim drug is investigated by using a certain health outcome of the victim as an endpoint (e.g. an adverse effect or a laboratory measurement). The source data can vary from electronic health records (EHR) to ADR databases, but in any case, the outcome must be such that it can be reliably

identified from the data. In contrast to clinical trials, data for epidemiological studies are generally collected primarily for other purposes than DDI research, for example, during routine clinical care. In many cases, either positive or negative controls, that is, drugs that are used in a similar clinical situation as the respective perpetrator or victim, may be useful as controls to corroborate the findings.[35] In order to reliably determine concomitant use of interacting drugs, it must be possible to determine the timing of their use from the longitudinal data. Self-evidently, the drugs of interest need to be used clinically to such an extent that an adequate sample of concurrent use can be expected. Fortunately, due to electronic databases, it is increasingly possible to collect large datasets. Even historic cohorts may be useful. A good example of

this is an epidemiological study that focused on the CYP2C8-mediated interactions affecting cerivastatin.[7]

In the context of CYP-mediated DDIs, pharmacoepidemiological studies can serve several purposes. Firstly, they may be used to characterize the health outcomes of a known DDI between a drug–drug pair. Secondly, a suspected effect of a drug on CYP activity or susceptibility to such effects can be studied, especially in cases where it is not feasible to obtain pharmacokinetic or biomarker data. In this case, the selection of suitable perpetrator or victim drug(s) requires additional considerations.

If potential CYP inhibition by drugs is studied or screened in an epidemiological setting, the victim drug should be a documented substrate of the CYP enzyme, and inhibition of its CYP-mediated metabolism should cause a pharmacokinetic change that can be expected to translate into an observable clinical outcome. For example, the CYP2C9 substrate sulfonylureas and warfarin with hypoglycaemia and bleeding as respective outcomes, and CYP3A4 substrate statins with rhabdomyolysis as an outcome have been successfully utilized as victim drugs.[36–38] Some examples for substrates that have been utilized in previous studies are presented in Table S1.

When the drug of interest is studied as a substrate of a CYP enzyme, the aim is usually to focus on the effects of established inhibitors of the enzyme. For this research setting, systematic classification of the inhibitors and their strength of inhibition is essential since inclusion of weak/negligible inhibitors can lead to dilution of the DDI effect. Different databases including both in vitro and in vivo data can be used to extract inhibitor data, and with careful evaluation, inhibitors can be classified as advised, for example, by the FDA.[2] Our recommendation would be to only include strong ($\geq$5-fold increase in AUC of a sensitive index substrate) and moderate (2- to <5-fold increase in AUC) inhibitors.[20] It should be noted that this classification also depends on the sensitivity of the CYP substrates used in the original pharmacokinetic studies, which may be limited particularly in case of CYP2B6 and CYP2C9. A recent example employing this approach is a study examining FDA's Adverse Events Reporting System for pharmacokinetic DDIs.[39] The authors utilized FDA's tables of substrates and inhibitors for clinical studies and drug labelling and the Drug Interactions Flockhart Table to define the inhibitor and substrate status of the drugs and could, for example, identify an increased risk of neuropathy in paclitaxel–clopidogrel combination. It should be noted that when analysing this kind of results, other interaction mechanisms (e.g. pharmacodynamic DDIs or effects on other pharmacokinetic pathways) should be considered when

evaluating how much of the observed effects on outcomes are driven by the inhibition of CYP being studied.

Confounding by indication is an important issue in pharmacoepidemiological research. It is especially relevant in case of DDIs, since many of them are well known and recognizable, likely resulting in changes in drug therapy that cannot be caught from epidemiological data, causing confounding that is difficult to control. To mitigate confounding by indication, a negative control perpetrator may be useful, that is, a drug that does not inhibit the CYP in question and is not expected to cause interaction with the substrate being studied by any other mechanisms either.[35] Similarly, a negative control victim can be used to distinguish between the true pharmacokinetic effect of the perpetrator and any other, such as pharmacodynamic, effects. On the other hand, a perpetrator–victim pair known to interact as a positive control may be useful to evaluate the sensitivity of the study.

7 | A CASE STUDY WITH CLOPIDOGREL

An illustrative example of the power of translational multi-approach research is the sequence of studies that has been carried out to identify and characterize the potential of the antiplatelet agent clopidogrel to cause DDIs via inhibition of CYP2C8 (Figure 4). When clopidogrel was launched for clinical use in 1998, there was little information concerning its CYP-mediated DDI potential, either as a victim or a perpetrator. Moreover, CYP2C8 was still a neglected CYP enzyme.[9] The importance of CYP2C8 started to unfold only after it was implicated in cerivastatin DDIs and rhabdomyolysis risk, which led to withdrawal of cerivastatin from the market in 2001.

It is important to reiterate how the crucial role of CYP2C8 in cerivastatin metabolism was discovered 20 years ago. In 2000, our group had just revealed that the lipid-lowering fibrate gemfibrozil markedly elevated the concentrations of the active acid forms of simvastatin and lovastatin.[40] When cases of rhabdomyolysis in cerivastatin–gemfibrozil combination therapy started to accumulate, we decided to investigate the effect of gemfibrozil on the pharmacokinetics of cerivastatin in healthy volunteers. Gemfibrozil increased the plasma concentrations of cerivastatin more than fivefold, and almost abolished its CYP2C8-dependent M-23 metabolite, indicating that gemfibrozil is a strong CYP2C8 inhibitor in vivo and that cerivastatin is a sensitive CYP2C8 substrate. Years later, the 1-O-beta-glucuronide metabolite of
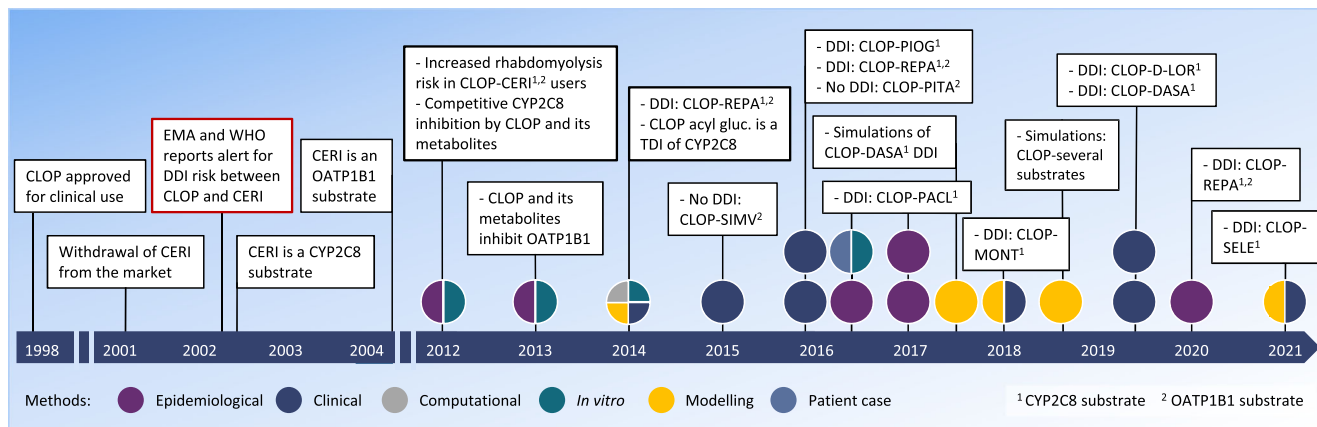
FIGURE 4 Timeline of the sequence of studies to identify and characterize the potential of clopidogrel to cause drug–drug interactions (DDIs) via inhibition of cytochrome P450 (CYP) 2C8 and organic anion transporting polypeptide (OATP) 1B1. References are given in the text. CERI, cerivastatin; CLOP, clopidogrel, DASA, dasabuvir; D-LOR, desloratadine; MONT, montelukast; PACL, paclitaxel; PIOG, pioglitazone; PITA, pitavastatin; REPA, repaglinide; SELE, selexipag; SIMV, simvastatin; TDI, time-dependent inhibitor

gemfibrozil was identified as a mechanism-based inhibitor of CYP2C8, providing a mechanistic explanation for the pharmacokinetic interactions of gemfibrozil with CYP2C8 substrates, and for the first time, incriminating a glucuronide metabolite as a clinically significant inhibitor of CYP2C8.[41]

Already in 2002, the Europe Medicines Agency (EMA) concluded in their cerivastatin report that the risk of rhabdomyolysis is greatly increased when cerivastatin is used in combination with clopidogrel,[42] but due to withdrawal of cerivastatin, these early observations were left largely unexplored for a decade (Figure 4). In 2012, Floyd et al.[7] used an epidemiological approach to identify medications that increased the risk of cerivastatin-induced rhabdomyolysis in order to pinpoint potential inhibitors of CYP2C8. When they compared rhabdomyolysis cases using cerivastatin with controls using atorvastatin, they found that in addition to gemfibrozil, clopidogrel use was associated with rhabdomyolysis with an odds ratio (OR) of almost 30, and a similar finding was observed in cases reported in FDA's Adverse Events Reporting System. Additionally, the authors tested the inhibitory effects of clopidogrel and its metabolites on cerivastatin M-23 formation using CYP2C8 Supersomes in vitro and found that their competitive inhibition constants were between 2 and 70 μM. In further in vitro studies, clopidogrel and its metabolites inhibited OATP1B1-mediated cellular uptake of cerivastatin with IC_{50} values in the low micromolar range.[43]

These reports caught our interest. It was particularly intriguing that just like gemfibrozil, also clopidogrel has a major glucuronide metabolite. Of note, in addition to gemfibrozil 1-O-beta-glucuronide, also certain other glucuronides had been shown to have affinity for CYP2C8.[9]

Moreover, the active metabolite of clopidogrel is a reactive species, which irreversibly affects its target receptor in platelets, raising the likelihood of mechanism-based inactivation by the glucuronide metabolite of clopidogrel. In addition, it seemed that the competitive inhibitory effects of clopidogrel and its metabolites on CYP2C8 and OATP1B1[7,43] were not potent enough to substantiate a strong interaction with cerivastatin. Accordingly, we initiated clinical, in vitro and modelling studies to find out if clopidogrel is a CYP2C8 inhibitor.

In our clinical study, we chose repaglinide as the index victim drug, because it resembles cerivastatin by being a sensitive CYP2C8 substrate and a substrate of both CYP3A4 and OATP1B1.[44] Repaglinide exposure was increased about fivefold by a 300 mg loading dose of clopidogrel and fourfold by continued administration of 75 mg clopidogrel daily. In HLMs, clopidogrel acyl-β-D-glucuronide turned out to be a time-dependent inhibitor of CYP2C8. A PBPK model suggested that inactivation of CYP2C8 by clopidogrel acyl-β-D-glucuronide leads to continuous 60%–85% inhibition of CYP2C8 during daily clopidogrel treatment and that transient inhibition of OATP1B1 is possible. Further, molecular docking simulations resulted in consistent docking of clopidogrel acyl-β-D-glucuronide at the CYP2C8 active site in an orientation allowing metabolism of its thiophene moiety, suggesting that this moiety is converted by CYP2C8 to a reactive species that inactivates the enzyme.

Collectively, these results provided strong evidence that treatment with typical doses of clopidogrel leads to moderate-to-strong CYP2C8 inhibition and suggested that clopidogrel may have a smaller effect on OATP1B1. In consequent studies, however, clopidogrel had no significant effects on the pharmacokinetics of simvastatin or

pitavastatin, excluding a clinically significant inhibitory effect on OATP1B1.[45,46] Furthermore, more recent clinical studies showed that clopidogrel can significantly raise the concentrations of the CYP2C8 substrates pioglitazone, montelukast, desloratadine, dasabuvir, paclitaxel and active metabolite of selexipag.[46–52] In addition, further epidemiological studies provided real-life evidence that clopidogrel treatment can increase the adverse effect risks of the CYP2C8 substrates repaglinide and paclitaxel[32–34,39,53] Finally, a follow-up in vitro study revealed that unlike in HLMs, time-dependent inactivation of CYP2C8 by clopidogrel glucuronide cannot be consistently detected in recombinant CYP2C8 Supersomes, explaining why inactivation of CYP2C8 was not observed in the initial 2012 in vitro studies.[12] Taken together, this example shows how epidemiological studies can trigger detailed in vitro, modelling and clinical studies to explain DDI mechanisms and to identify a new DDI perpetrator (clopidogrel) and how PBPK modelling can be used as a translational method to improve mechanistic understanding and prediction of DDIs, leading to further clinical studies and even new epidemiological studies to fully understand the DDI potential of the perpetrator and the clinical consequences of its DDIs.[54,55]

8 | CONCLUSIONS AND FUTURE PROSPECTS

Translational multidisciplinary research, including molecular modelling, in vitro studies, mechanistic, physiologically based and systems modelling and various types of clinical and epidemiological approaches, has shown its effectiveness to recognize DDIs, to characterize their mechanisms and pharmacokinetic and clinical outcomes and to demonstrate how to best manage DDI risks in the clinic. In such research, it is crucial to pass hypotheses and knowledge back and forth between different disciplines, as well as between research and clinical care.

Methods to investigate CYP-mediated DDIs in vitro and in vivo are well established. In case of simple CYP-mediated DDIs, it is possible to utilize, for example, PBPK modelling to translate in vitro data to clinical DDI predictions with a high degree of confidence. The key forthcoming developments in this area are the evolving regulatory guidelines and their harmonization between agencies. Novel methodologies such as organ-on-a-chip approaches are emerging in the DDI research field. Endogenous biomarkers and cocktail studies are likely to be used increasingly in pharmacokinetic studies, with a potential to provide supplemental information compared to typical perpetrator–victim pair studies. In the future,

we will see further improvements in PBPK modelling, for example, on predicting and simulating complex DDI scenarios, such as those including multiple simultaneous mechanisms (e.g. CYP enzyme–drug transporter interplay) and individual factors (e.g. genetic variability). Moreover, epidemiological DDI research will benefit from increasing availability and usability of EHR data. Finally, clinical decision support algorithms that comprehensively integrate DDI information with other patient-specific data (e.g. genotypes) will facilitate translation of the acquired knowledge to optimize drug treatments and improve patient safety.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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