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Assessment of Pharmacokinetic Drug–Drug Interactions in Humans: In Vivo Probe Substrates for Drug Metabolism and Drug Transport Revisited

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Abstract

Pharmacokinetic parameters of selective probe substrates are used to quantify the activity of an individual pharmacokinetic process (PKP) and the effect of perpetrator drugs thereon in clinical drug–drug interaction (DDI) studies. For instance, oral caffeine is used to quantify hepatic CYP1A2 activity, and oral dagibatran etexilate for intestinal P-glycoprotein (P-gp) activity. However, no probe substrate depends exclusively on the PKP it is meant to quantify. Lack of selectivity for a given enzyme/transporter and expression of the respective enzyme/transporter at several sites in the human body are the main challenges. Thus, a detailed understanding of the role of individual PKPs for the pharmacokinetics of any probe substrate is essential to allocate the effect of a perpetrator drug to a specific PKP; this is a prerequisite for reliably informed pharmacokinetic models that will allow for the quantitative prediction of perpetrator effects on therapeutic drugs, also in respective patient populations not included in DDI studies.

1. INTRODUCTION

The present article in part updates an article we published in 2007 (1). It should be mentioned that some information on basic pharmacokinetic parameters of individual drugs are taken from the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA) approved summaries of product characteristics and are not referenced to save space.

1.1. General Considerations and Definitions

Inappropriate pharmaceutical dosing is a major reason for both insufficient efficacy and adverse drug reactions (ADRs) requiring medical intervention (2). Up to half of respective ADRs are considered preventable (3). Neglecting major known pharmacokinetic covariates is a frequent reason for inappropriate dosing and preventable ADRs (4, 5). Such covariates include comedication causing pharmacokinetic drug–drug interactions (PK-DDIs), age, body weight, ethnicity, genetic variants for drug metabolizing enzymes and transporters, kidney function, liver function, nutrition, inflammatory processes, severe systemic disease, and extracorporeal elimination procedures.

PK-DDIs represent the most relevant covariates for two reasons: They may occasionally cause very pronounced (more than fivefold) changes in drug concentrations (6), and their effect may change abruptly, depending on both the start and stop of comedication and concentration fluctuations of the causative (perpetrator) drug during therapy. Indeed, unintended PK-DDIs are suspected to cause a major burden of disease. About 10–20% of ADRs may be associated with a DDI (7, 8). Most are pharmacodynamic DDIs, but PK-DDIs can account for about half of all DDIs depending on the patient group (7, 9).

This situation shows that avoiding PK-DDIs that cause ADRs is not a simple task in real patient treatment, although most clinical PK-DDIs have a known mechanism. When two drugs are coprescribed, the available dosing information typically covers potential DDIs, which rarely occur as an actual clinically relevant DDI in the individual patient (2, 7). Despite extensive investigations on PK-DDIs stipulated by regulatory authorities (10, 11), available information does not include a reliable quantitative assessment of changes in pharmacokinetics (PK) and concentrations of the victim drug, a risk assessment, or an actionable recommendation of appropriate measures.

The challenge in quantitatively predicting PK-DDIs is that the PK of any drug (including perpetrator and victim drugs) depends on a large number of individual processes in the human body, with most of them having pronounced inter- and intraindividual variability. These processes mainly include convection of fluids (e.g., gastrointestinal content, blood, bile, urine, cerebrospinal fluid, lymph), diffusion, binding to proteins not directly involved in drug effects (e.g., plasma proteins, structural proteins), binding to target proteins, transport of individual drug molecules across membranes (specifically uptake and excretion by cells involved in drug elimination such as enterocytes, hepatocytes, and renal tubular cells at the basal and apical membranes), and biotransformation. In the following, a respective process and/or the underlying activities are termed pharmacokinetic processes (PKPs). Examples of such PKPs are glomerular filtration, metabolism by intestinal CYP3A4, and hepatic uptake by OATP1B1. Obviously, observable PK parameters and PKPs are the result of a number of individual sub-PKPs. For example, in the case of intestinal extraction mediated by CYP3A4, actual uptake into the enterocyte, intracellular transport to the enzyme, binding to the enzyme, biotransformation by the enzyme, and release from the enzyme are all important sub-PKPs (12). Currently there is no way to conduct an experimental assessment of these individual processes in vivo. Thus, the corresponding observable PKPs reflect only the rate-limiting sub-PKPs.

PK-DDIs occur if a perpetrator drug has an effect on an individual PKP that is rate-limiting for the PK of another drug (the victim drug) in an individual. Thus, a main task for drug developers is to identify the rate-limiting PKP(s) of a new drug (10). This is possible because in a relatively homogeneous population (e.g., healthy adults of a single ethnicity, without concomitant drug intake), major sources of inter- and intraindividual pharmacokinetic variability of a drug are typically limited to a few or just one PKP. However, this may not be the same for all patient groups: For example, those with renal impairment will be more prone to metabolic DDIs.

Another task for drug developers is to then identify whether a new drug is a pharmacokinetic perpetrator itself. If preclinical investigations cannot exclude that this new drug affects PKPs of other drugs, then clinical studies need to be conducted (11, 13). This is done by two approaches: the empirical approach and the mechanistic approach. The empirical approach compares PK of a potential victim drug between the presence and absence of a potential perpetrator drug, in which the selection of the pair is based on clinical relevance, e.g., caused by expected frequent coadministration (concomitant use studies). A standard DDI study design is a cross-over trial in healthy volunteers, comprising a period with a single dose of the potential victim drug only and a period with maximum-dose chronic coadministration of the presumed perpetrator drug (10, 11). This design provides the maximum effect, and results are directly valid irrespective of the interaction mechanism, although they apply only to this drug pair, the dosing schedule, and the population tested. In contrast, the mechanistic approach aims at understanding the PK-DDI by assessing the effect of a potential perpetrator drug on involved PKPs, including the magnitude and exposure dependency of any change. The advantage of the mechanistic approach is that it generally enables a prediction of the magnitude of PK-DDIs to any dosing schedule, population, or even other drugs that fully or partially depend on the altered PKP. Pharmacokinetic models are used for such predictions (13–15), and their performance depends on both the valid assessment of the PKPs and the quality of the model. Mechanistic elements are indispensable in the assessment of a drug's potential to cause DDIs because not all possible drug–drug combinations can be directly tested in clinical studies (16).

Probe substrate drugs are used to quantify the activity of key PKPs in the human body that determine the PK of drugs. According to the 2012 EMA definition, "a probe drug is a drug which is exclusively or almost exclusively eliminated through metabolism catalyzed by one specific enzyme or eliminated through excretion by one specific transporter in vivo" (11, p. 27). In its 2017 DDI guideline, the FDA mostly avoids the term probe drug but refers to "sensitive index substrates" [i.e., "drugs whose area under the concentration-time curve (AUC) values increase 5-fold or more when coadministered with a known strong index inhibitor for a particular pathway" (10, p. 4) or with a similar difference between poor and extensive metabolizers] to assess effects on cytochrome P450 (CYP) enzymes and merely to "(transporter) substrates whose pharmacokinetic profile is markedly altered by coadministration of known inhibitors" (10, p. 16). As these definitions focus on drug elimination, the AUC is considered to reflect the activity of a respective enzyme or transporter in the liver or in the kidney and needs to be reported according to the guidelines. Unfortunately, also the PK of probe drugs depend on PKPs beyond the activity of a single enzyme or transporter. Furthermore, for a thorough understanding of the PK of a drug and the effects of covariates including DDIs thereon, it is in most cases not sufficient to quantify just elimination processes. Therefore, a more widely applicable definition of a probe drug would be a drug that is suitable to quantify the activity of a PKP at a specific site in the human body. Of course, endogenous substances, food components, or other xenobiotics could also be suitable as probe substrates (17, 18), but this is beyond the scope of this article.

1.2. Desirable Properties of Suitable Probe Drugs and Probe Drug Metrics

Finding a suitable probe drug may be very difficult since there is no probe drug valid in all groups of patients for any PKP. Even for highly selective enzymes of transporter substrates, expression of the same target enzyme or transporter at various sites (e.g., gut wall, liver, and kidney) that contribute to the systemic PK of a probe drug is an obstacle for allocation of the respective pharmacokinetic parameters to one site. This may, however, be important for the assessment of PK-DDIs because perpetrators may have different effects on PKPs at individual expression sites. Invasive sampling (e.g., portal vein blood, bile) would enable the measurement of only the desired PKP, but this is not feasible in routine DDI studies. Furthermore, not all rate-limiting (sub-)PKPs governing the PK of a drug are accessible by the probe drug approach in clinical studies (e.g., individual intracellular processes, in vivo binding kinetics). It is also discouraged to design studies that enforce a combination of the (empirical) concomitant use approach and the (mechanistic) probe drug approach with the intention to additionally obtain an immediately clinically relevant result (providing, e.g., the maximal effect on the probe drug) (19), because this dual approach would compromise the decision for the most suitable probe.

The suitability of a probe drug includes the availability of an appropriate probe drug metric reflecting the activity of a PKP, i.e., a pharmacokinetic parameter of the drug for which a specific PKP is rate-limiting and can be determined experimentally. It is essential that (most of) the variability of the metric used to quantify the respective PKP depends on the variability in this PKP. Optimally, this would apply not only in healthy volunteers but also in (even severely ill) patients. The PKP is then quantified by measuring the metric following experimental administration of the drug. The fraction of variability of the metric needed to be determined by the respective rate-limiting process depends on the ability to avoid and/or quantify other confounding processes. Plasma AUC of the probe drug, the primary metric suggested by guidance documents, may be a suitable metric to quantify the activity of a specific enzyme if the primary metabolism of the probe drug is mediated mainly by this enzyme, if the enzyme is expressed predominantly in the liver, and if enzyme activity (as opposed to, e.g., hepatic plasma flow) is rate-limiting for biotransformation. If the enzyme is expressed additionally in the gut wall, the contribution of this site to AUC by first pass metabolism can be avoided by intravenous instead of oral administration of the probe drug, although it is difficult to experimentally quantify the (probably small) contribution of intestinal enzymes to systemic clearance. If more sophisticated approaches are used, such as semiphysiological compartmental models or full physiologically based pharmacokinetic (PBPK) models, taking confounding PKPs into account, information useful to quantify the effect of perpetrator drugs on individual PKPs can also be obtained from less selective probe drugs.

Interestingly, probe drugs that are highly specific for a single PKP and are therefore recommended equally as sensitive index substrates by the respective FDA guidance document (10) may provide distinctly different results in clinical DDI studies if their basic pharmacokinetic properties differ considerably. For instance, the effect of chronic cinacalcet on dextromethorphan AUC was threefold higher than on desipramine AUC (both CYP2D6 probe drugs), while the effect of chronic ciprofloxacin on tizanidine AUC was fivefold higher than on caffeine AUC (both CYP1A2 probe drugs) (all drugs given orally) (20). The reason is that those index drugs with more pronounced effects undergo an extensive first pass metabolism, which takes place early after absorption when the local concentrations of the perpetrator drugs are very high. Thus, sensitivity alone is not sufficient as a requirement for a probe drug to assess the quantitative impact of a perpetrator drug on a PKP, and AUC is not always a reasonable choice as a metric, even for highly specific probe drugs. Ideal metrics should be close to the PKPs. For the above examples, AUC corresponds to clearance over bioavailability. Thus, either clearance or bioavailability is selected as a PKP to be

assessed separately, or hepatic (and intestinal, if applicable) extraction of the drug is used. To assess these parameters, the design of experimental DDI studies would need to include intravenous administration of those drugs with high first pass metabolism.

Obviously, a probe drug should not itself influence PKPs, which would result in DDIs (see below). A probe drug should have a short elimination half-life to avoid the need for prolonged sampling. A limited volume of distribution (e.g., <50 L) and a limited plasma protein binding (e.g., <50%) would ensure that the concentrations typically measured, i.e., total plasma concentrations, would not be too remote from unbound, i.e., systemically relevant, concentrations. Rapid and essentially complete absorption upon oral administration and a limited first pass metabolism (unless used to assess intestinal extraction; e.g., <20%) would also be advantageous. PK should be dose-linear (11). Finally, probe drugs should not have relevant pharmacodynamic effects at the doses tested, and they should be well tolerated (see below), available as therapeutic drugs, easily quantified, and inexpensive.

Despite the limited specificity of probe drugs, in homogenous populations such as healthy volunteers, major sources of their pharmacokinetic variability are typically limited to a few or even just one of all PKPs. Still, the accuracy with which a probe drug metric indeed reflects the process to be characterized needs to be scrutinized in several validation steps, which have been described previously (1, 21–23). These steps ultimately include clinical studies (*a*) showing that there is a close correlation between the respective metric and a reliable method to assess PKP activity directly and/or independently and (*b*) demonstrating the use of covariates with a selective effect on the PKP. Examples for the former include in vitro (tissue samples) versus in vivo correlations in the same individual or correlation to a different but established method (such as other validated probe drugs for enzymes/transporters, or Doppler ultrasonography for hepatic plasma perfusion). For the latter, covariates with maximum specificity are polymorphisms in the genes coding for the respective enzyme/transporter with known effects on activity, where nonfunctional alleles are most helpful; validation by induction and inhibition studies is the second important respective approach, but the information that can be derived depends on the selectivity of the perpetrator used.

A major limitation for existing validation studies is that these are typically conducted in healthy volunteers. In severely diseased patients, other PKPs may become rate-limiting for the PKP metric and render it invalid (24). Furthermore, validation is not always possible because the respective sites in the human body are not accessible at an acceptable risk, independent methods are not available, or neither respective genetic variants nor selective inhibitors or inducers exist. For example, how could the activity of an uptake transporter be measured in clinical studies independently from that of an efflux transporter located at the basolateral and the apical membrane of the same cell?

The best approach to address all these problems is the following: Get to know both your probe drug and your target PKP in detail! Validation of a suitable probe drug is not an empirical black box. If a new therapeutic drug turns out to be a potential probe drug, not all the information supporting proper validation is available from studies conducted during drug development. Depending on the individual drug, it would be helpful to acquire information on absolute oral bioavailability (this obviously requires an intravenous preparation), biliary excretion and hepatic extraction (both need to be studied in surgical patients), intestinal secretion (e.g., by charcoal studies), rate-limiting transporters of basolateral/apical pairs, and kinetics of binding to plasma protein and blood cells (in vitro and/or in vivo microdialysis studies). Many of these pieces of information are lacking for even established probe drugs, which hinders both our understanding of observed DDIs and our ability to use this information to predict DDIs with other drugs (see below). In addition, the information on expression, function, splice variants, relevance of cofactors, etc., of individual drug metabolizing enzymes and even more so for transporters in various tissues is still incomplete and needs to be developed continuously.

In summary, the probe drug approach is meant to be a mechanistic approach, but in many aspects it still has strong empirical components. It often neglects limited and ill-characterized specificity of probe drugs and probe drug metrics and is based on a number of assumptions that have never been verified (known unknowns) or even explicitly named (unknown unknowns). Furthermore, a number of PKPs that may be rate-limiting for some drugs, such as kinetics of protein binding (12, 25), intra- and transcellular transport (26), and transport via lymph (27, 28), are difficult to assess experimentally in vivo and/or are often not considered in pharmacokinetic evaluations.

2. CURRENT STANDARD PROBE DRUGS

It is beyond the scope of this review to describe and assess all potential probe substrate drugs in detail. The probe substrate approach is well established for glomerular filtration and for a number of CYP enzymes, mainly to assess their hepatic activity, although many limitations apply (see **Tables 1** and **2**). Among the phase 2 enzymes, *N*-acetyltransferase type 2 is the only one with an established probe substrate. For drug transporters (**Table 3**), identifying suitable probe drugs is particularly challenging because several transporters typically contribute to relevant fractions in the variability of pharmacokinetic parameters. It is therefore also questionable whether transporter-based DDIs are as equally clinically relevant as enzyme-based DDIs, although this is suggested by regulatory guidance documents (10, 11). Genetic polymorphisms with pronounced effects on transporter activity are typically less prominent than those affecting drug metabolizing enzymes, and available inhibitors are less selective, making experimental in vivo validation of transporter probes difficult. Furthermore, research on suitable transporter probes started decades after the respective studies were conducted for CYP enzymes, so available clinical information is even more incomplete for many potential probe drugs. Preferred selective probe substrate drugs most frequently used and/or considered as most suitable are briefly summarized in **Tables 1–3**, and four representative examples are described below in detail. For further details of individual probe drugs, including their validation status, readers are referred to References 1, 23, 29, and 30.

2.1. An Established Enzyme Activity Probe: Midazolam for CYP3A-Dependent PKPs

The main PKPs for midazolam are shown in **Figure 1**. Clinically, midazolam is indicated for sedation before or during surgery and in patients requiring long-term sedation. Midazolam is a well-established probe drug for human intestinal and hepatic CYP3A activity with relatively low intraindividual PK variability (1, 10, 11, 31). Still, detailed knowledge of midazolam PK is essential to make appropriate use of the information obtained from its use as a probe drug.

Enzymes of the human subfamily CYP3A are expressed primarily in mature enterocytes and centrilobular hepatocytes (32–34), with some minor expression in the kidney and other organs (35). The effects of perpetrator drugs on enterocyte and hepatocyte CYP3A may differ, and regulation is independent for the two sites (36); thus, the two activities need to be assessed separately. In the liver of Caucasians, about 95% of expressed hepatic CYP3A is CYP3A4, but up to 20% may be CYP3A5 depending on the presence of the functional CYP3A5*1 variant (37). The predominance of CYP3A4 in this population was similar in the small intestine (37). In African Americans, CYP3A5*1 allele frequency and thus the relative abundance of CYP3A5 are much higher (24). Beyond genetic polymorphisms in CYP3A genes, CYP3A activity depends on pregnane X-receptor (PXR), constitutive androstane receptor (CAR), vitamin D receptor, peroxisome proliferator-activated-receptor-alpha(PPARA)–mediated regulation and on activities of P450 oxidoreductase

Table 1 Selected probe drugs for individual pharmacokinetic processes: general processes

Targeted PK process	Probe drug and administration	(Preferred and) Accepted in vivo metrics	Assessment/limitations	Reference(s)	Overall assessment
Hepatic plasma flow	Indocyanine green 0.5 mg/kg as an IV bolus or continuous IV infusion of 0.5–2.0 mg/min	(Systemic clearance of indocyanine green)	Indocyanine green is almost exclusively extracted from plasma by the liver through selective uptake by OATP1B3 and NTCP. As a high clearance drug, hepatic perfusion is rate-limiting for hepatic extraction under standard conditions. However, reduced elimination may be caused by inhibition of these transporters, by genetic OATP1B3 defects, by global hepatocellular dysfunction (e.g., in jaundice), or by hyperbilirubinemia. Available studies are outdated; modern bioanalytical and pharmacokinetic methods are required.	127–132	E
	Sorbitol IV infusion of 300 μmol/min for 120 min	(Nonrenal sorbitol clearance)	Sorbitol has a high hepatic extraction rate (mechanism unknown) exceeding 0.9 in healthy individuals. Renal excretion reaches 5–30% of a dose and has to be accounted for. Available studies are outdated; modern pharmacokinetic methods are required.	133	E
GFR	Iohexol 1.6–3.2 g given as IV infusion as a bolus or 0.16 g/h as continuous infusion	(Renal iohexol clearance); systemic iohexol clearance	Interactions with transporters have not been studied extensively; there are minor unexplained discrepancies to the (unavailable) "gold standard" inulin; it is rarely used in pharmacokinetic studies because estimated GFR either based on plasma concentration of creatinine (an OCT2 substrate) and/or cystatine C or based on measured creatinine clearance is used instead as a standard of convenience.	134–137	+

Table reports selected probe drugs for individual pharmacokinetic processes and assesses whether the method is suitable and recommended (green +) or experimental (E). Abbreviations: GFR, glomerular filtration rate; IV, intravenous; NTCP, Na^+-taurocholate co-transporting polypeptide; OCT2, organic cation transporter 2; OATP1B3, organic anion transporting polypeptide 1B3.

(POR) and cytochrome b_5 (38–41). Thus, any effect of perpetrator drugs on CYP3A activity is not necessarily attributable to direct interaction with CYP3A enzymes. The large substrate binding site of CYP3A4 allows binding to different regions within the site and simultaneous cooperative binding of several substrate molecules (resulting in atypical kinetics), as well as a high promiscuity in substrate (and inhibitor) binding (42, 43). Thus, the extent of CYP3A4 inhibition may depend

Table 2 Selected probe drugs for individual pharmacokinetic processes: activity of drug metabolizing enzymes

Targeted PK process	Probe drug and administration	F	(Preferred and) Accepted in vivo metrics	Assessment/limitations	Reference(s)	Overall assessment
Hepatic NAT2 activity	Caffeine 50–200 mg orally (or from dietary exposure)	1	Ratio of caffeine metabolites in urine	Validated by relationship to genetic polymorphisms but not by inhibition/induction studies; clinical relevance of NAT2 interactions are unclear but minor	138	+
Hepatic CYP1A2 activity	Caffeine 0.025–200 mg orally	1	(Systemic caffeine clearance); caffeine AUC	CYP1A2 mediates > 90% of caffeine metabolism; extensively validated; potential (small) contribution of CYP1A1 and CYP1B1 to caffeine metabolism in vivo unclear; contamination by dietary caffeine possible	1	+
	Theophylline 250–375 mg orally	1	(Systemic theophylline clearance); theophylline AUC	Similar to caffeine, but while CYP1A2 mediates about 80% of theophylline metabolism, perpetrators of other processes (e.g., macrolide antibacterials) caused relevant DDIs with theophylline not attributable to CYP1A2 inhibition	139	–
	Tizanidine 2–4 mg orally	0.3	(Systemic tizanidine clearance); tizanidine AUC	Validated by in vitro data and by inhibition/induction studies; high first pass metabolism, which makes the drug vulnerable to interaction; large effects of inhibitors related to tolerability problems	140, 141	+/–
Hepatic CYP2C9 activity	Tolbutamide 0.025–500 mg orally	Unknown	(Systemic tolbutamide clearance); tolbutamide AUC	Favorable general pharmacokinetic properties; selective among CYPs while recently OAT2 was recognized as a hepatic uptake transporter; unclear role of relevant expression of CYP2C9 in the intestine; tolbutamide becoming irrelevant as a therapeutic drug and thus vanishing from the market	1, 65, 102, 142	+

(*Continued*)

Table 2 (*Continued*)

Targeted PK process	Probe drug and administration	F	(Preferred and) Accepted in vivo metrics	Assessment/limitations	Reference(s)	Overall assessment
Hepatic CYP2C9 activity	Warfarin 10 mg orally	Probably close to 1	(Systemic S-warfarin clearance); S-warfarin AUC	Racemic drug for which only S-warfarin metabolism is attributable to >90% to CYP2C9, requiring enantioselective bioanalysis; slow elimination, requiring prolonged sampling (mean terminal elimination half-life of S-warfarin about 1–1.5 days); unclear role of relevant expression of CYP2C9 in the intestine; transport by OAT2 and BCRP assumed to contribute to S-warfarin PK variability	106, 110, 143, 144	+/–
Hepatic CYP2C19 activity	Omeprazole 0.1–20 mg orally	0.4	(Systemic omeprazole clearance); omeprazole AUC	Omeprazole administered as an acid-fast preparation with complex absorption behavior, which is a challenge for exact assessment of DDIs; some selectivity issues, and limited validation	1, 20, 65, 72, 74, 75; see Section 2.2	+/–
Hepatic CYP2D6 activity	Dextromethorphan-HBr 30 mg orally	0.8 in PMs, 0.01–0.2 in EMs	(Systemic dextromethorphan clearance); dextromethorphan AUC	Dextromethorphan highly selective for the genetically polymorphic CYP2D6, causing DDIs to depend on expression of CYP2D6; discontinuous intestinal absorption; together with a very high first pass metabolism, demonstration of DDIs probably dependent on the local presence of a perpetrator during the short absorption bursts; has a relatively high intraindividual pharmacokinetic variability	20, 72, 76, 79; see Section 2.3	+/–
	Desipramine 50 mg orally	0.4 in mainly EMs	(Systemic desipramine clearance); desipramine AUC	Validated by genotype differences and by DDI studies; unique PK properties (phospholipid binding and lysosomal trapping) and complex metabolism; effects of perpetrators and genotypes less than those for dextromethorphan	145–148	+/–

(*Continued*)

Table 2 (*Continued*)

Targeted PK process	Probe drug and administration	F	(Preferred and) Accepted in vivo metrics	Assessment/limitations	Reference(s)	Overall assessment
Hepatic CYP2D6 activity	Atomoxetine 10 mg orally	0.63 in EMs, 0.94 in PMs	(Systemic atomoxetine clearance); atomoxetine AUC	Validated by genotype differences (9-fold between PMs and EMs) and by DDI studies; additional limited role of CYP2C19 should be taken into account	149, 150	+
Hepatic CYP2E1 activity	Chlorzoxazone 250–500 mg orally	Unknown	(Chlorzoxazone AUC); ratio 6-OH-chlorzoxazone/ chlorzoxazone in a single plasma sample 2–4 h postdose	Extensively validated probe drug; intestinal CYP3A inhibition caused by 500-mg dose, which may have some effect on CYP1A2 and can be avoided by using lower doses	151–153	+
Global CYP3A activity	Triazolam 0.25 mg orally	0.44	(Systemic triazolam clearance); triazolam AUC	Validated by interaction studies, in vitro data, and correlation of other probe drugs; substantial intestinal extraction; variable expression of individual CYP3A enzymes, in part depending on CYP3A5 genotype; unclear but probably small contribution by extrahepatic CYP3A; separate assessment of intestinal and hepatic CYP3A activity by both oral and IV triazolam not established	154–156	–
Hepatic CYP3A activity	Midazolam 0.0001–2 mg given as IV infusion	0.3–0.5	(Fractional systemic midazolam clearance by 1′-hydroxylation); systemic midazolam clearance	Extensively validated probe drug; variable expression of individual CYP3A enzymes, in part depending on CYP3A5 genotype; unclear but probably small contribution by extrahepatic CYP3A	1, 24, 48, 51; see Section 2.1	+
Intestinal CYP3A activity	Midazolam 0.001–2 mg given as IV infusion combined with 0.003–5 mg solution orally	0.3–0.5	Estimated intestinal midazolam extraction	Variable expression of individual CYP3A enzymes, in part depending on CYP3A5 genotype; available models for dissection of intestinal and hepatic activity not experimentally validated	1, 36, 47, 59; see Section 2.1	+

Table reports selected probe drugs for individual pharmacokinetic processes and assesses whether the method is suitable and recommended (green +), acceptable but with relevant limitations (orange +/–), or not recommended (red –). Abbreviations: AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; CYP, cytochrome P450; DDIs, drug–drug interactions; EM, extensive metabolizer; F, average bioavailability for oral administration; IV, intravenous; NAT2, *N*-acetyltransferase type 2; PK, pharmacokinetics; PM, poor metabolizer.

on the specific substrate/inhibitor pair tested (44), making the use of a probe drug approach for CYP3A generally questionable (45). The observed discrepancies between individual probe drugs may, however, be primarily attributable to different degrees of first pass metabolism and CYP3A5 involvement.

Midazolam is absorbed rapidly and completely following oral administration (46). Plasma protein binding is 96–98%; the volume of distribution reaches 0.7–1.2 L/kg at steady state; and its terminal elimination half-life is 1.5 to 2.5 h. The bioavailability of oral midazolam is approximately 30–50% due to highly variable CYP3A-mediated first pass metabolism in the gut wall and the liver

Table 3 Selected probe drugs for individual pharmacokinetic processes: activity of drug transporters

Targeted PK process	Probe drug and administration[a]	F	(Preferred and) Accepted in vivo metrics	Assessment/limitations	Reference(s)	Overall assessment
Intestinal P-gp activity	Dabigatran etexilate 0.375–150 mg orally	<0.1	Dabigatran C_{max}; dabigatran AUC	Assessment of systemic DE exposure is indirect by quantification of dabigatran; thus additional processes such as MATE-1 activity, CES-1 activity, and GFR may (also) become rate-limiting; dabigatran PK is highly variable (large sample size needed); the effect of concomitant drugs is higher for DE microdoses, suggesting saturation of P-gp activity; thus, optimal doses need to be assessed; the role of hepatic P-gp is unclear; direct validation is missing.	104, 157–159	+/–
	Digoxin 0.25–1 mg orally	0.6–0.8	C_{max}; partial AUCs	Digoxin is not known to be mediated by other intestinal transporters; intestinal P-gp is correlated with the systemic availability of digoxin following oral administration; however, the effect of even strong P-gp perpetrators, especially inhibitors, on respective metrics secretion is moderate at best.	29, 30, 83, 90; see Section 2.4	+/–
Renal P-gp activity	Digoxin 0.25–1 mg orally or given as IV infusion	0.6–0.8	(Renal secretion); renal clearance	Digoxin secretion accounts only for about one-third of renal clearance, making it relatively insensitive for DDI studies; other renal transporters are also involved (mainly OATP4C1) and may (also?) be rate-limiting; direct validation is lacking.	29, 30, 83, 84, 88; see Section 2.4	–
Hepatic OATP1B1 activity	Pitavastatin 0.01–2 mg orally	0.51	(Hepatic extraction); AUC	Hepatocyte uptake almost exclusively by OATP1B1; efflux from the liver is mediated mainly by BCRP; validation in vivo is mainly by relative large genotype [SLCO1B1*15] effects for OATP1B1 versus no genotype effects [ABCG2 421C>A] for BCRP; other transporters and CYP2C9 may have some limited effect on pitavastatin PK.	23, 93, 104	+

(*Continued*)

Table 3 (*Continued*)

Targeted PK process	Probe drug and administration[a]	F	(Preferred and) Accepted in vivo metrics	Assessment/limitations	Reference(s)	Overall assessment
Renal OAT1 activity	Adefovir dipivoxil 10 mg orally	0.59	(Renal secretion of adefovir); renal clearance of adefovir	Renal clearance of adefovir (45% of a dose) reaches about two to three times that of GFR- and OAT1-mediated secretion is predicted to account for almost 60% of renal secretion; renal cell uptake is essentially mediated by OAT1, while apical efflux may be mediated by MATE1 and MATE2k; adefovir dipivoxil single doses do not cause major interactions with a number of substrates for other transporters (e.g., metformin, digoxin, sitagliptin, pitavastatin).	97, 160, 161	+/−
Renal OAT3 activity	Sitagliptin 100 mg orally	0.87	(Renal secretion); renal clearance	Renal elimination of unchanged sitagliptin (79% of an oral dose) exceeds GFR and renal secretion is predicted to mediate 80% of renal clearance; minor metabolism mainly by CYP3A4 is irrelevant for assessment of renal clearance/secretion; high selectivity for OAT3 is based essentially on in vitro evidence; sitagliptin is also a substrate for OATP4C1 and P-gp, but cyclosporine, a P-gp inhibitor, had no relevant effect on renal elimination of sitagliptin; sitagliptin inhibits OCT1 and OCT2 in vitro but in vivo single doses do not cause major interactions with a number of substrates for other transporters (e.g., metformin, digoxin, adefovir, pitavastatin).	97, 161–163	+/−
Renal OAT3 activity	Benzylpenicillin 240 mg orally	Unclear in adults but sufficient	(Renal secretion); renal clearance	Interaction with benzylpenicillin decay products after oral administration have not been assessed; renal secretion clearly exceeds glomerular filtration; increased renal clearance by p-aminohippurate suggests that other transporters may (also) become rate-limiting in renal excretion of benzylpenicillin.	97, 160, 164	−

Table reports selected probe drugs for individual pharmacokinetic processes and assesses whether the method is suitable and recommended (green +), acceptable but with relevant limitations (orange +/−), or not recommended (red −). Abbreviations: AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; DDI, drug–drug interaction; F, average bioavailability for oral administration; IV, intravenous; OAT, organic anion transporter; OCT, organic cation transporter; OATP, organic anion transporting polypeptide; P-gp, P-glycoprotein; PK, pharmacokinetics.

[a]Transporter probe drugs claimed to be useful to quantify the activity of several transporters in parallel [e.g., metformin (OCT2, MATE1, and MATE2-k), rosuvastatin (OATP1B1, OATP1B3 and BCRP), furosemide (OAT1 and OAT3)] are not included in the table.

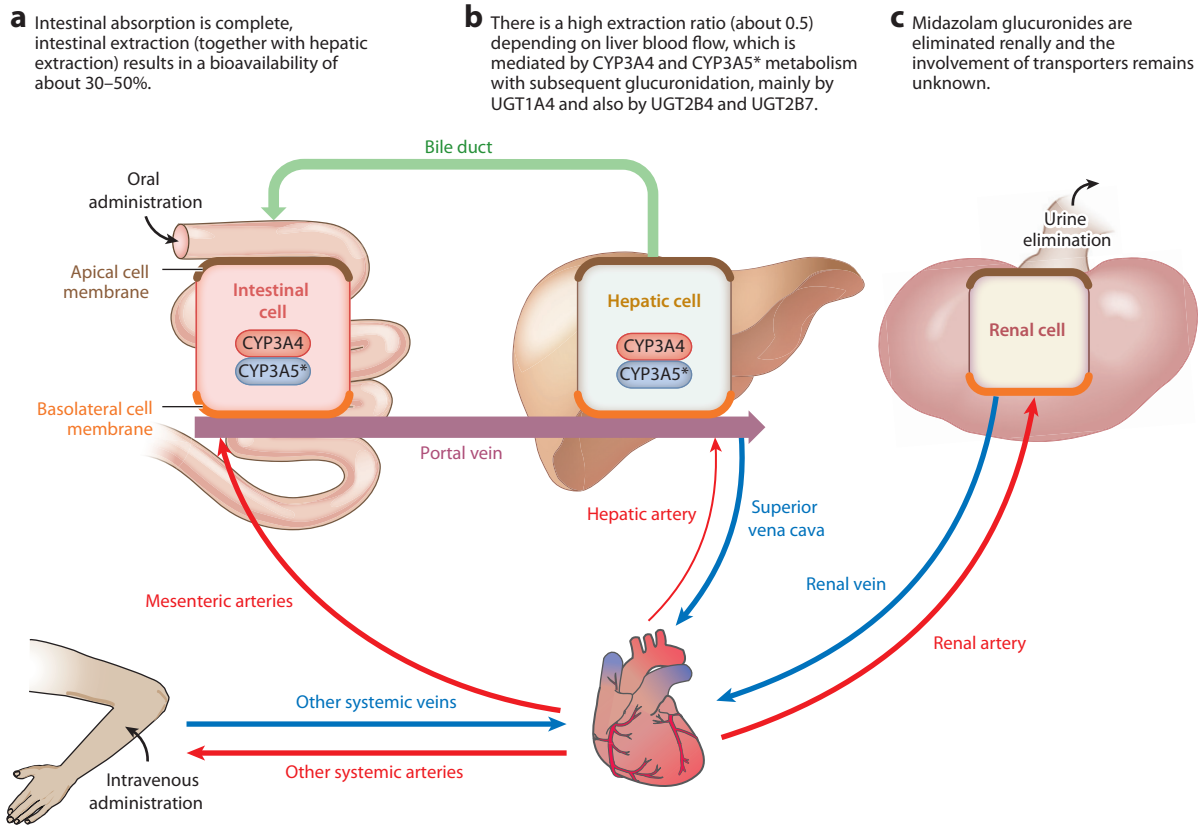

Figure 1

Rate-limiting pharmacokinetic processes of midazolam. The asterisk indicates CYP3A5 is not expressed in homozygous CYP3A5*3 individuals (i.e., most Caucasians) (see Section 2.1 for additional details). Abbreviations: CYP, cytochrome P450; UGT, uridine 5′-diphospho-glucuronosyltransferase.

(47). Both intestinal and hepatic extraction are about 0.5 on average, the latter corresponding to a total systemic clearance of about 300 to 500 mL/min (24, 48). More than 99% of a midazolam dose is eliminated by metabolism, mainly via 1′-hydroxylation (60–80%) and, to a lesser extent, by 4-hydroxylation (mediated primarily by CYP3A4) (3%), followed by glucuronidation (49, 50). Midazolam hydroxylation is carried out nonspecifically by both CYP3A4 and CYP3A5 in vitro and in vivo, while the magnitude of induction and inhibition in vivo may differ between the two CYPs (49, 51, 52). Direct midazolam *N*-glucuronidation via uridine 5′-diphospho-glucuronosyltransferase family 1 member A4 (UGT1A4) accounts for less than 2% of midazolam elimination (53). In contrast to many other CYP3A substrates, midazolam is not a substrate for P-glycoprotein (P-gp) (54). There is no evidence for a role for other transporters in midazolam PKP, but this has not been investigated systematically.

Thus, PKPs with the potential to play a rate-limiting role in midazolam PK include metabolism by intestinal CYP3A4, metabolism by intestinal CYP3A5 (if expressed), hepatic blood flow, metabolism by hepatic CYP3A4, metabolism by hepatic CYP3A5 (if expressed), and possibly binding to and dissociation from plasma proteins. Single midazolam doses used for assessing these PKPs are typically in the 1–7.5-mg range, but much lower doses, including microdoses down

to 100 ng, have also been applied (48, 55). Midazolam PK show dose linearity over the entire range (48, 55) despite substrate cooperativity and the involvement of more than one individual enzyme, while saturation of metabolism in vivo is observed at doses exceeding 15 mg (56). Because of the lack of specificity, midazolam results obtained in Caucasians can be extrapolated to drugs only or mainly metabolized by CYP3A4 but not directly to drugs with a major contribution of CYP3A5, such as tacrolimus (57, 58). Furthermore, the assessment of perpetrator drug effects on CYP3A activity in both the gut wall and the liver using midazolam-based metrics and the respective predictions for other CYP3A substrates requires proper dissection of effects on the two sites. Systemic clearance of midazolam upon intravenous administration is used as a metric for hepatic CYP3A activity. Intestinal extraction of midazolam serves as a metric for intestinal CYP3A activity (1, 47, 59). As there is no ethically justifiable way to directly measure the intestinal metabolism of midazolam, this is estimated by constructing PK models based on clinical studies with both oral and intravenous administration, using parallel (mass-labeled) semi-simultaneous or separate administration via the two routes (1). All respective models, including semiphysiological empirical models (60) and PBPK models (61), have some limitations, including the absence of both a method to directly validate intestinal extraction results by comparison to experimental data and a known probe drug approach to measure CYP3A activity at other sites. Thus these activities are typically neglected in evaluations of intestinal or hepatic activity.

In summary, a wide range of midazolam doses can be used to reliably quantify the sum of hepatic CYP3A4 and CYP3A5 activity and—with some caveats—to quantify the combined activities of intestinal CYP3A4 and CYP3A5. If studying CYP3A5*3 homozygous individuals only, specificity for CYP3A4 can be achieved. The effect of inhibitors and inducers on CYP3A activity is well captured by midazolam while its magnitude may not always be the same for midazolam and other CYP3A substrates (62, 63). The potential role of midazolam protein binding kinetics remains to be assessed. Midazolam AUC following oral administration only was suggested earlier as a global CYP3A metric, assessing the sum of intestinal and hepatic CYP3A4 and CYP3A5 (1, 11). Although this may appear to be a reasonable screening approach, it is not optimal because any perpetrator effect cannot be allocated to the causative PKP; therefore, findings cannot be extrapolated to other drugs.

2.2. A Standard Enzyme Activity Probe with Limited Validation: Omeprazole for CYP2C19-Dependent Hepatic (?) Metabolism

Omeprazole, a proton pump inhibitor mainly used for gastroesophageal reflux disease and gastroduodenal ulcer eradication, is administered orally as an enteric coated preparation to avoid degradation in the stomach. Accordingly, absorption may have considerable lag time and is often discontinuous (20). The standard dose for CYP2C19 assessment is 20 mg, but a 100-µg microdose has also been tested and appears to provide similar results (64). Once the drug is released, it is rapidly absorbed and highly (97%) bound to plasma proteins (mainly albumin); systemic bioavailability is about 40% for a single dose and increases (probably by autoinhibition of CYP2C19-dependent first pass metabolism) to 60% for multiple doses. Because CYP2C19 is also present in the gut wall (65), an unknown fraction of omeprazole first pass metabolism may take place there. Elimination of the parent drug occurs completely by metabolism, where omeprazole is metabolized mainly by CYP2C19 to the most important metabolite, i.e., 5-hydroxyomeprazole, and mainly by CYP3A4 to omeprazole sulfone. At low concentrations, CYP2C19 mediated 70% of omeprazole metabolism in vitro (66). Rifampicin, a nonselective inducer (including CYP3A4 and CYP2C19), accelerates omeprazole biotransformation considerably (67) where the contribution of CYP3A may be altered. S-omeprazole, the active enantiomer, is a competitive and

time-dependent inhibitor of CYP2C19 (68). Furthermore, omeprazole is a substrate of P-gp and other membrane transporters and also an inhibitor of P-gp (69). The elimination half-life is about 1 h. Omeprazole and its metabolites may cause further DDIs by direct and time-dependent inhibition of CYPs and by changes of gastric pH (70, 71). The complex PK of omeprazole is reflected by a high interindividual variability of omeprazole PK, with coefficients of variation of about 100% for most parameters, while intraindividual variability is clearly lower (20, 72, 73). Given the involvement of CYP2C19, the AUC of omeprazole is about six- to eightfold higher in carriers of two non-active CYP2C19 variants and about twofold lower in carriers of two high-activity (CYP2C19*17) variant alleles (74, 75). Validation by a direct intraindividual in vivo/in vitro (liver samples) comparison has not been reported, and specific CYP2C19 inhibitors or inducers are not available. A relevant limitation for the use of oral omeprazole as a DDI probe drug appears to be the unpredictable lag time for absorption, making it impossible to synchronize the timing of exposure with that of a potential perpetrator drug. This limitation is significant because a major mechanism of interaction may be the inhibition of omeprazole first pass metabolism. Intravenous omeprazole could be useful to obtain more clear results and also to assess the role of intestinal CYP2C19, but has not been tested as a probe.

In summary, omeprazole can be used to reliably identify an effect of a perpetrator drug on hepatic CYP2C19 activity, but exact quantification of the effect is difficult. Specificity limitations appear to be of minor relevance, but additional validation data (mainly intestinal versus hepatic activity) are needed. Potential DDIs caused by omeprazole are probably negligible for single doses.

2.3. A Standard Enzyme Activity Probe for a Prototype Polymorphic CYP: Dextromethorphan for CYP2D6-Dependent PKPs

Dextromethorphan is used clinically as an antitussive. Establishment of the drug as a CYP2D6 probe was initially based on urinary metabolic ratios to assess genetic metabolizer phenotypes, while plasma exposure is used increasingly in DDI studies (76–78). The selectivity of dextromethorphan as a CYP2D6 substrate at the standard 30-mg dextromethorphan-HBr dose is very high despite some involvement of CYP3A in its metabolic elimination (76, 79), but a microdose of 100 μg did not reflect CYP2D6 genotypes (80). There are no systematic studies on dextromethorphan as a transporter substrate. Dextromethorphan is lipophilic and is probably completely absorbed, although no respective data are available; at least 60% of a dose is absorbed as urinary recovery of oral dextromethorphan is about 60% in CYP2D6 extensive metabolizers but lower in poor metabolizers (81). The drug has a very high first pass metabolism by CYP2D6 (if expressed), with an oral bioavailability of about 1–2% for extensive metabolizers and 80% for poor metabolizers (79). It is unclear to what extent intestinal CYP2D6 expression may contribute to this first pass metabolism because intestinal expression of CYP2D6 is controversial (65, 82). Intestinal absorption appears to be stepwise rather than continuous (20, 72). In CYP2D6 extensive metabolizers, the high first pass metabolism suggests that the demonstration of DDIs depends on the local presence of a perpetrator during the short absorption bursts. The irregular initial concentration versus time profiles of dextromethorphan in plasma, along with a high pharmacokinetic variability, are also a problem for developing pharmacokinetic models. Because of the extensive first pass metabolism, the magnitude of perpetrator effects on dextromethorphan kinetics is larger than that on other CYP2D6 substrates and cannot be used directly to predict respective DDIs (20).

In summary, dextromethorphan is a very sensitive drug to identify CYP2D6 perpetrators, although exact quantification of CYP2D6 activity is difficult and remains controversial.

2.4. A Not-So-Selective Transporter Probe: Digoxin for P-Glycoprotein-Dependent PKPs

Digoxin is an inhibitor of the Na^+/K^+ ATPase and is used for atrial fibrillation and heart failure. The drug is rapidly absorbed following oral administration, with an absolute bioavailability of 60–80% (83). Plasma protein binding is around 20% and the volume of distribution is 510 L/kg in healthy subjects. The drug is metabolized up to 10% in the liver (probably by CYP3A) and in the gut by bacteria. It is excreted unchanged (80% of a dose) by both glomerular filtration and tubular secretion, although secretion only accounts for about one-third of renal clearance, making it relatively insensitive for DDI studies (20). Its elimination half-life in subjects without renal disease is about 2 days (83). Active digoxin disposition appears to depend primarily on P-gp-mediated secretion across the apical cell membranes, and its expression in the gut wall (secretion in the lumen), liver (transport into the bile), and kidney (renal secretion) contributes to the systemic PK of digoxin (29, 30). Digoxin uptake at the basolateral membranes appears to be mediated by OATP4C1 (and maybe OATP1B3) (29, 84). Rifampicin, a P-gp inducer, decreased digoxin oral bioavailability by 30% and C_{max} by 52%, and digoxin AUC correlated with P-gp expression in duodenum (29, 83). On the other hand, fostamatinib, a P-gp inhibitor, increased digoxin C_{max} by 70% and AUC by 37% (85). Both findings support the idea that P-gp activity is the rate-limiting process of digoxin intestinal secretion. No effect of rifampicin was observed on digoxin transport by P-gp in the liver and kidney (83). In contrast, some P-gp inhibitors such as verapamil and clarithromycin reduce systemic (mainly renal) clearance of digoxin (86, 87). A recently discovered DDI, however, shows that for renal secretion of digoxin OATP4C1 may (also?) be rate-limiting (84, 88, 89); the limited selectivity of perpetrators suggests that OATP4C1 might even provide an alternative explanation for observed digoxin DDIs, e.g., by verapamil (86).

The effect of genetic polymorphisms of P-gp on digoxin PK is controversial. In one study, the C3435T polymorphism (a marker SNP) was related to significantly lower expression of duodenal P-gp and higher plasma concentrations of digoxin (90), but P-gp genotype effects were not supported by other studies (20, 29, 30). Therefore, they cannot be used for validation of digoxin as a probe.

Oral doses of digoxin used for phenotyping are 0.25 to 1 mg. The relatively high bioavailability suggests that some saturation of P-gp exists at these doses and that lower doses may be more suitable to assess the effect of perpetrators on intestinal P-gp activity, but this may cause assay sensitivity problems (20). Surprisingly, digoxin AUC has been used as a global metric for systemic P-gp activity in DDI studies, but this is certainly not suitable to allocate the effect of a perpetrator to a P-gp expression site and thus is not useful to predict the respective DDIs (19). More metrics closer to the physiological processes would be C_{max}, partial AUCs, or the absorption rate of digoxin (all not directly reflecting a PKP) for intestinal activity and renal secretion of digoxin for renal P-gp (20, 83), while nonrenal clearance would probably reflect hepatic activity. Direct validation of any digoxin metric for hepatic or renal P-gp by comparison to tissue expression has not been done. Still, its complex PK may lead to results of DDI studies with digoxin that are difficult to interpret, as in the case of tolvaptan (91).

In summary, digoxin can be used to identify an effect of an inducer for intestinal P-gp and to some extent (preferably at low doses) also of an inhibitor at this site. Since it lacks specificity, its use for renal P-gp activity is questionable.

2.5. Use of Nonselective (or Polyspecific) Probe Drugs

In the past, some drugs that underwent metabolism by multiple pathways, such as antipyrine, were used as global drug probes for hepatic metabolism (92). This approach has been abandoned due

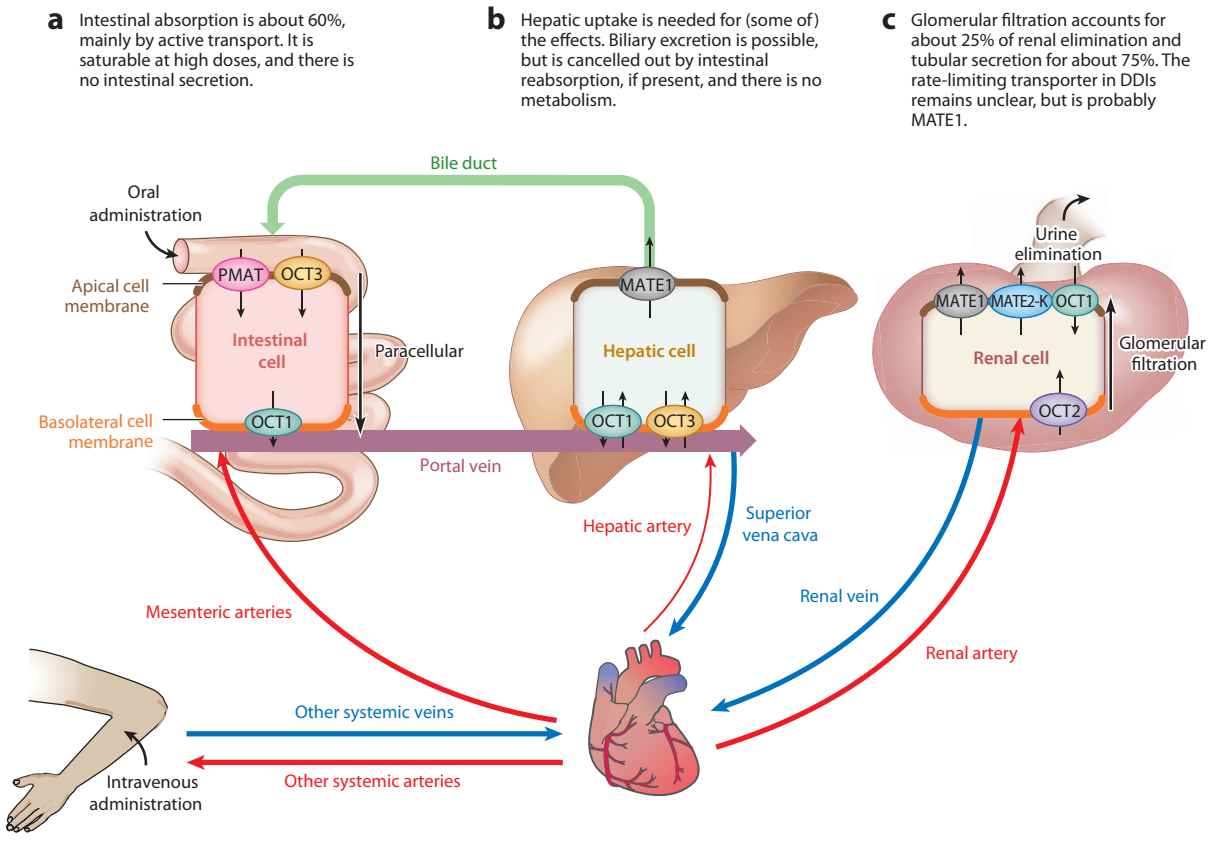

Figure 2

Possibly rate-limiting pharmacokinetic processes of metformin (94, 95) (see Section 2.5 for additional details). Abbreviations: DDIs, drug-drug interactions; MATE, multidrug and toxin extrusion transporter; OCT, organic cation transporter; PMAT, plasma membrane monoamine transporter.

to our increasing knowledge of the identity and role of individual drug metabolizing enzymes. As transporter activities have been recognized as important rate-limiting PKPs, there is a need to assess transporter activity in vivo. For many of the relevant transporters, however, there is no selective substrate available; therefore, nonselective probes are used. Examples of such nonselective probes include the following compounds: Rosuvastatin is a substrate for breast cancer resistance protein (BCRP) and organic anion transporting polypeptides OATP1B1 and OATP1B3 (10, 93); metformin is transported by several organic cation transporters OCT1, OCT2, and OCT3 by the plasma membrane monoamine transporter PMAT, and by the multidrug and toxin extrusion transporters MATE1 and MATE2-k (**Figure 2**) (94, 95); and furosemide is a substrate of organic anion transporters OAT1 and OAT3, among others (96, 97). It is unclear which of the respective processes in the intestine, liver and kidney are rate-limiting for systemic PK of these drugs or even for their handling by individual organs. Without knowing which are rate-limiting, it is impossible to allocate observed DDIs to a single transporter PKP. Thus, the use of nonselective probe drugs, in most cases transporter substrates, is a concomitant use approach rather than a mechanistic approach. Nonselective probes still have important merits, but results cannot be applied to directly predict other DDIs.

3. DESIGN ASPECTS OF PROBE SUBSTRATE DDI STUDIES

Probe drug studies should be maximally informative with regard to the effect of a perpetrator. Thus, intraindividual comparisons are essential (10, 11). Although guidelines suggest maximal exposure to potential perpetrators during studies, lower doses or the use of more than one dose may be more informative if the perpetrator has nonlinear PK. The magnitude of any effect may change considerably with time for inducers, but also if there is time-dependent inhibition, or both inhibition and induction. Thus, in such cases the extent of inhibition may be quantified at both the beginning and during chronic administration of a perpetrator.

The concentration versus time profiles of both individual perpetrator and probe (victim) drugs fluctuate considerably during DDI studies; thus the extent of interaction would also change over time during a study and—strictly speaking—could not be fully captured in a single value such as a fold change in AUC. Furthermore, direct concomitant administration may have different overall effects than staggered (e.g., separated by 2 h) administration because the maximal effect is achieved when peak concentration of the inhibitor occurs at the site of interaction. A coadministered probe drug with delayed release (such as omeprazole) may not fully capture the extent of enzyme inhibition in the intestinal wall immediately after administration of a perpetrator drug. Beyond the samples required to assess the metric of the probe drug, the time course of exposure to the perpetrator, including its potentially relevant metabolites, should be determined by appropriate blood sampling to understand the interplay between perpetrator and substrate and also for the use of dynamic DDI models (see below). In general, avoiding such fluctuations can also be achieved by continuous infusion of both the perpetrator and the probe drug. In a study conducted in six healthy volunteers, a combination of continuous intravenous and intrajejunal infusions was used to characterize the intestinal and hepatic interaction between voriconazole and midazolam (98). Another approach used continuous infusions of the probe substrate only (99). A major problem for the continuous infusion approach turned out to be the unexpected fluctuations in concentrations despite an apparently constant infusion rate, which is probably attributable to pronounced variability in the delivery rate of current infusion systems under practical conditions (98). If a DDI is expected only with regard to renal elimination of a drug, a simplified approach could be chosen. Renal clearance and/or secretion of a probe can be quantified for parts of the concentration versus time profile by the ratio of the fractional amount excreted in urine and the corresponding partial AUCs. Thus, if the elimination of the probe drug is not too fast, the effect of a perpetrator could be assessed in a single study period by staggered administration of the probe drug and the perpetrator, e.g., 3 h later.

Of course, taking known and potential covariates for the PK of both the potential perpetrator and the probe substrate is reasonable. Thus, genotyping for known variants influencing involved PKPs as well as an assessment of the glomerular filtration rate should be included in the study protocol (see above).

If the metric depends on renal elimination of the drug, renal clearance and renal secretion of the respective moiety should be determined by additional urine sampling. Without continuous control of the volunteers, complete urine sampling is error prone. In our experience, to safeguard proper sampling, study participants must have no access to toilets or even sinks unless study personnel is directly present, although this is ethically problematic. Sufficient fluid administration (e.g., 100 mL/h) should be provided, and it may be wise to check calculated creatinine clearance from each urine sampling period and compare the result to those from the screening examination. In cases of gross deviations, this might indicate a sampling error. Also, extraordinary low urine volumes (or the inability to obtain urine in a 4-h sampling period) are suspicious, and a procedure to address such finding should be defined prior to a DDI study with urine sampling.

3.1. Probe Drug Doses

The lowest possible doses should have preference to avoid adverse effects and pharmacokinetic perpetrator effects by probe drugs. However, if doses lower than established doses are used, these drugs may no longer be suitable as probe drugs (80); thus, it is essential to validate lower doses separately. Very small amounts of drugs are often difficult to administer and quantify because of potential adsorption to plastics and the required sensitivity of the analytical assays (48). Accelerator mass spectrometry has sufficient sensitivity but is very expensive (100); however, ultraperformance liquid chromatography coupled to tandem mass spectrometry is currently approaching the required sensitivity (55). Furthermore, the relative specificity of a drug to be metabolized by a given enzyme/transporter may depend on its concentrations (48). It is reasonable to assume, however, that this applies only to major dose changes (i.e., greater than fivefold) because available validations originate from bolus dose studies, which show pronounced concentration changes during the blood sampling periods anyway.

Practically, doses often depend on the strengths of marketed preparations. For instance, tolbutamide is used as a CYP2C9 probe, and the lowest available strength of 500 mg was used by some investigators (101). To avoid adverse effects possibly related to this dose, we used only a quarter of a tablet (72, 73), and results could be validated by comparison to CYP2C9 genotypes (102). In further studies, Nguyen et al. (103) reduced the dose to 10 mg, and Croft et al. (100) reduced it to a microdose of 25 μg. To date, microdoses (1% of a pharmacologically active dose or 100 μg, whichever is less) have been tested for a limited number of otherwise accepted probe drugs. The combined chronic administration of standard doses of ketoconazole and fluvoxamine had the expected inhibitory effect on the PK of caffeine, tolbutamide, midazolam, and fexofenadine microdoses, but the use of a global inhibition approach did not provide information on potential selectivity shifts (100). Recently, transporter probe drugs have also been tested in microdose cocktails (104, 105) and showed similar behavior as standard doses when coadministered with typical inhibitors (104). Interestingly, known effects of genetic polymorphisms on standard doses were no longer seen for (racemic) warfarin and dextromethorphan microdoses (80), suggesting that these doses also may not be useful to quantify effects of perpetrator drugs on CYP2C9 and CYP2D6, respectively. For both probe drugs, relatively complex PK with multiple metabolites and an involvement of other enzymes (76, 106) would provide possible explanations for this discordance. It is tempting to speculate whether a sufficiently elaborated PBPK model might restore some usefulness of these probe drugs given at microdoses. However, the use of microdoses for probe drugs can be recommended only if validation is available for these doses. This indeed has been done for midazolam as a CYP3A probe, which was shown to have linear PK over a broad dose range, and the lowest dose of 100 ng has been partially validated as a CYP3A probe by induction and inhibition studies (55) (see Section 2.1).

3.2. Limited Sampling Strategies

Assessing a complete PK profile of a probe drug requires that numerous blood samples are taken during a period for which concentrations have typically decreased to 10% or less of C_{max}. To this end, individuals need to remain in an institution for at least several hours, which is often not feasible for patients or in epidemiological studies. Therefore, limited sampling strategies (LSSs) have been tested for many probe drugs (1, 21, 107–110), where a few blood samples or even a single sample is taken, and an estimate of an appropriate metric describing the PKP is derived based on drug concentrations or metabolic metabolite/parent ratios. However, the current DDI guidelines ask for coverage of the complete concentration profile for a reason: Otherwise, effects on different PKPs may no longer be discernible. For instance, a delay in probe drug absorption

may have the same effect on the concentration of the parent drug observed 1 h after administration as induction of the respective metabolizing enzyme. It is debatable how many samples would be needed to cover the complete profile adequately, and in some cases as few as six data points may be sufficient (i.e., not an LSS). The performance of LSSs, especially with very low sample numbers, depends on the type of covariate effects (21, 109) and needs to be validated for all expected types. Thus, the general use of LLSs in DDI probe drug studies with perpetrators with unknown effects (e.g., new drugs) is generally discouraged. Exceptions may apply on a case-by-case basis.

3.3. Use of Dried Blood Spots

Dried blood spots have been tested as a sampling procedure, often in conjunction with LSS, to facilitate sample processing. Given that only unbound concentrations are effective for drug metabolism, transport, and effects, total plasma concentrations (i.e., the standard approach in PK assessment) are not much closer to the effective concentrations than whole blood samples. Respective clinical studies are encouraging (108); however, the benefit of this approach in a standard setting for DDI studies in healthy volunteers is very limited and needs to be balanced against the additional uncertainty introduced by a nonstandard procedure, which may involve additional variability and additional interpretation problems.

4. UNDERSTANDING THE RESULTS OF PROBE SUBSTRATE DDI STUDIES AND APPLYING THESE TO PREDICT DDIs WITH OTHER DRUGS

The immediate result of a probe substrate DDI study, such as a 1.5-fold increase in AUC upon coadministration with a perpetrator drug, requires translation to become clinically applicable. Often, the exact mechanism and the magnitude of the effect on the underlying PKP can be understood only if a detailed PK model of the probe drug (and often also of the perpetrator drug) is available, in particular if the interaction is complex (111, 112). This applies especially for effects taking place at different expression sites of the same enzyme, as for CYP3A4 (20, 60), but also to understand why equally selective probe substrates, such as tizanidine and caffeine for CYP1A2 or dextromethorphan and desipramine for CYP2D6, exhibit completely different magnitudes of effects (20). Furthermore, such models are also able to incorporate the time course of both perpetrator and substrate concentrations and provide a dynamic and realistic description of DDIs. Appropriate approaches include semiphysiological models (60) and full PBPK models (111, 113). Indeed, most published PBPK modeling and simulation approaches deal with the PK of probe drugs or perpetrators (14). In turn, by using a PBPK model for a therapeutic drug depending on the respective PKP, one can then derive the effect of the perpetrator for the PK of this other drug.

PBPK models comprise a structural model and system-specific properties, which together provide a model for the organism, and drug properties, and are able to describe both the drug-related physicochemical processes and the interaction of the drug with endogenous macromolecules (113). Thus, they use quantitative information on PKPs to predict the PK of a drug, and their complexity is designed to a degree that allows useful predictions (114, 115). Typically, predicted PK values, e.g., drug clearance, are within a factor of two compared to measured values during both enzyme induction and enzyme inhibition (116, 117). The magnitude of prediction error, however, can be described only on a posthoc statistical level and is not predictable for the individual drug. Indeed, PBPK modeling has been increasingly used to predict DDIs, and published models perform reasonably well (15). One of the important limitations of PBPK models is that a number of PKPs are not directly accessible. For instance, the activity of enzymes or transporters that interact with drugs may be characterized by in vitro experiments, but the local environment of the macromolecules

influencing their activity as well as local concentrations of drugs in the body is not fully known (118). For these reasons, existing PBPK models still have strong empirical elements (119). Still, PBPK models have the additional benefit that they may put DDIs as a source of variability into a broader perspective with other covariates, such as genetic polymorphisms, age, and impaired renal function (15). Since such models use published population-specific mean values for a number of PKPs (which may not always be suitable), models can probably be improved by measuring values in the individual participants of DDI studies, including but not limited to albumin and total protein plasma concentration, hematocrit, and glomerular filtration rates. Specifically, a probe drug for glomerular filtration could easily be incorporated into cocktail studies (e.g., iohexol, indocyanine green; see **Table 1**).

5. COMBINED ADMINISTRATION OF SEVERAL PROBE DRUGS IN A COCKTAIL

A probe drug cocktail is composed of several probe substrate drugs for each of the PKPs to be quantified, which are coadministered in one study (1). This approach is useful to assess a drug's inhibition or induction potential for multiple PKPs, and it is accepted by regulatory authorities (10, 11) and widely used (120). PKP metrics including the effect of covariates thereon should have identical values when the probe drugs are used in separate experiments or as a cocktail. Thus, an important part of the validation of a cocktail—in addition to the validation of its components—is to show that there is no mutual interaction between any of the individual components (10, 11, 22). This is typically studied by the administration of each probe drug separately and as part of a cocktail in healthy volunteers. The periods are then compared using the standard average bioequivalence approach, excluding a relevant interaction if the 90% confidence intervals of AUC and C_{max} ratios for administrations with and without coadministration are within the 0.8–1.25-fold range. Comprehensive studies successfully confirmed the absence of interaction in healthy volunteers for a number of cocktails used in humans to assess the activity of drug metabolism enzymes (e.g., 121, 122). Depending on the pre-existing information on some of the combinations within a cocktail, a reduced number of study periods may be acceptable (20). A recent attempt to assess a cocktail for drug transporters showed some mutual DDIs (123); thus, the investigators carefully assessed the dose dependency of these interactions in additional clinical investigations and suggested reducing the doses of individual components in order to avoid these DDIs (124, 125). It remains questionable, however, whether the absence of mutual interaction as confirmed empirically by the approach described above would also hold true in a stress test. If the PK of one of the cocktail components and/or its metabolites is fundamentally changed by the administration of a strong inhibitor or inducer or in severe organ dysfunction, new mutual interactions may arise that may confound the PK of the probe drug. For example, let us assume that the true AUC ratio for a cocktail administration over a single administration of a probe drug A is 1.25 (i.e., within the accepted boundary) because one of the cocktail components (B) is a weak competitive inhibitor of A's metabolism. According to the static mechanistic model (13), the ratio of inhibitor concentration (I) over inhibitor constant (K_i) would then be 0.25. If this cocktail is used to assess the effect of another drug as a possible perpetrator (C), an interaction causing a fivefold increased exposure for B would result in an I/K_i value of 1.25, resulting in a 2.25-fold (i.e., $1 + I/K_i$) increased exposure for A. Because of the inherent intraindividual pharmacokinetic variability of any drug, narrowing the 0.8- to 1.25-fold acceptance boundaries to prove no mutual interaction between cocktail components would not be reasonable. As a safety margin, it is therefore suggested that doses of cocktail probe drugs are fivefold lower than the doses that may cause a 1.25-fold increase of exposure. The risk for a true 1.25-fold increase could be determined by in vivo studies and/or

by in vitro information (i.e., by getting to know your probe drug). Using low doses would have the additional benefit that the risk for unexpected tolerability problems would be decreased. Although excellent tolerability has been reported for most cocktails (1), in one case relevant adverse effects were reported in women only, which were tentatively attributed to a mutual interaction involving probe drug metabolites (126).

FUTURE ISSUES

1. In order to fully benefit from the probe drug approach, an extensive pharmacokinetic characterization of existing probe substrates beyond that carried out for registration as a therapeutic drug is recommended, including the development of extensive (semiphysiological or PBPK) models for each probe drug.
2. A search for further probes is recommended. Probes are desired that are able to separate closely linked PKPs such as enterocyte uptake and excretion into the portal vein blood as well as back in the intestinal lumen; hepatocyte uptake and excretion into the bloodstream and into the bile; enterocyte and hepatocyte metabolism; renal cell uptake and excretion into both urine and blood; and, for some drugs, transport to and metabolism in other cells/organs.
3. More extensive cocktails, including low doses or microdoses of probe drugs for general PKPs and enzyme and transporter activities, should be developed and validated. As a side note, the cocktail study approach (using low doses/microdoses) could also be evaluated for concomitant use studies with drugs that are not selective for given PKPs.
4. Collecting all the information on a probe drug is a lengthy process that involves research by many contributors from both pharmaceutical industry and academia. Since probe drugs are registered as therapeutic drugs, they may eventually disappear from the market, which would result in the loss of an important tool in drug development and maybe also individual drug dosing. This is not in the best interest of patient safety. Thus, it would be in the public interest for regulatory authorities to support separate registration of probe drugs as such and support the development of a separate registration process for probe drugs. The respective dossier should contain the most up-to-date PBPK model available for the probe drug.
5. The formation of consortia of pharmaceutical companies to develop, validate, model, and maintain the use of individual probe substrates for DDI studies is encouraged.

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LITERATURE CITED

1. **Fuhr U, Jetter A, Kirchheiner J. 2007. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. *Clin. Pharmacol. Ther.* 81(2):270–83**
2. McDonnell PJ, Jacobs MR. 2002. Hospital admissions resulting from preventable adverse drug reactions. *Ann. Pharmacother.* 36(9):1331–36

1. In part a previous version of the present article, still worth reading.

3. Hakkarainen KM, Gyllensten H, Jonsson AK, Andersson Sundell K, Petzold M, Hagg S. 2014. Prevalence, nature and potential preventability of adverse drug events—a population-based medical record study of 4970 adults. *Br. J. Clin. Pharmacol.* 78(1):170–83
4. Obreli-Neto PR, Nobili A, de Oliveira Baldoni A, Guidoni CM, de Lyra Junior DP, et al. 2012. Adverse drug reactions caused by drug-drug interactions in elderly outpatients: a prospective cohort study. *Eur. J. Clin. Pharmacol.* 68(12):1667–76
5. Chang F, O'Hare AM, Miao Y, Steinman MA. 2015. Use of renally inappropriate medications in older veterans: a national study. *J. Am. Geriatr. Soc.* 63(11):2290–97
6. Backman JT, Kivistö KT, Olkkola KT, Neuvonen PJ. 1998. The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. *Eur. J. Clin. Pharmacol.* 54(1):53–58
7. Magro L, Moretti U, Leone R. 2012. Epidemiology and characteristics of adverse drug reactions caused by drug-drug interactions. *Expert Opin. Drug Saf.* 11(1):83–94
8. Dechanont S, Maphanta S, Butthum B, Kongkaew C. 2014. Hospital admissions/visits associated with drug-drug interactions: a systematic review and meta-analysis. *Pharmacoepidemiol. Drug Saf.* 23(5):489–97
9. Subramanian A, Adhimoolam M, Kannan S. 2018. Study of drug-drug interactions among the hypertensive patients in a tertiary care teaching hospital. *Perspect. Clin. Res.* 9(1):9–14
10. U.S. Food Drug Admin. 2017. *Clinical drug interaction studies—study design, data analysis, and clinical implications: guidance for industry*. Draft Guid., US Dep. Health Hum. Serv., Rockville, MD. **https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf**
11. Eur. Med. Agency. 2012. *Guideline on the investigation of drug interactions*. Rep. CPMP/EWP/560/95/Rev. 1 Corr. 2**, Eur. Med. Agency, London
12. Weisiger RA. 1985. Dissociation from albumin: a potentially rate-limiting step in the clearance of substances by the liver. *PNAS* 82(5):1563–67
13. US Food Drug Admin. 2017. *In vitro metabolism- and transporter-mediated drug-drug interaction studies: guidance for industry*. Draft Guid., US Dep. Health Hum. Serv., Rockville, MD. **https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM581965.pdf**
14. Sager JE, Yu J, Ragueneau-Majlessi I, Isoherranen N. 2015. Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications, and model verification. *Drug Metab. Dispos.* 43(11):1823–37
15. Min JS, Bae SK. 2017. Prediction of drug-drug interaction potential using physiologically based pharmacokinetic modeling. *Arch. Pharm. Res.* 40(12):1356–79
16. Fuhr U, Weiss M, Kroemer HK, Neugebauer G, Rameis H, et al. 1996. Systematic screening for pharmacokinetic interactions during drug development. *Int. J. Clin. Pharmacol. Ther.* 34(4):139–51
17. Mao J, Martin I, McLeod J, Nolan G, van Horn R, et al. 2017. Perspective: 4β-hydroxycholesterol as an emerging endogenous biomarker of hepatic CYP3A. *Drug Metab. Rev.* 49(1):18–34
18. Tay-Sontheimer J, Shireman LM, Beyer RP, Senn T, Witten D, et al. 2014. Detection of an endogenous urinary biomarker associated with CYP2D6 activity using global metabolomics. *Pharmacogenomics* 15(16):1947–62
19. Fuhr U. 2018. Pharmacokinetic phenotyping to predict drug-drug interactions: time to divorce the hybrid concept of simultaneous mechanistic-based and exposure-based assessment. *Clin. Pharmacol. Ther.* 103(1):42–42
20. Gazzaz M, Kinzig M, Schaeffeler E, Jübner M, Hsin C, et al. 2018. Drinking ethanol has few acute effects on CYP2C9, CYP2C19, NAT2 and P-glycoprotein activities but somewhat inhibits CYP1A2, CYP2D6 and intestinal CYP3A: so what? *Clin. Pharmacol. Ther.* In press
21. Derungs A, Donzelli M, Berger B, Noppen C, Krahenbuhl S, Haschke M. 2016. Effects of cytochrome P450 inhibition and induction on the phenotyping metrics of the Basel cocktail: a randomized crossover study. *Clin. Pharmacokinet.* 55(1):79–91
22. Ma JD, Nafziger AN, Bertino JS. 2012. Validating phenotyping cocktails: More work needs to be done. *J. Clin. Pharmacol.* 52(11):1772–73
23. Momper JD, Tsunoda SM, Ma JD. 2016. Evaluation of proposed in vivo probe substrates and inhibitors for phenotyping transporter activity in humans. *J. Clin. Pharmacol.* 56:S82–98

24. Kirwan C, MacPhee I, Philips B. 2010. Using drug probes to monitor hepatic drug metabolism in critically ill patients: midazolam, a flawed but useful tool for clinical investigation of CYP3A activity? *Expert Opin. Drug Metab. Toxicol.* 6(6):761–71
25. Berezhkovskiy LM. 2012. Determination of hepatic clearance with the account of drug-protein binding kinetics. *J. Pharm. Sci.* 101(10):3936–45
26. Fung KYY, Fairn GD, Lee WL. 2018. Transcellular vesicular transport in epithelial and endothelial cells: challenges and opportunities. *Traffic* 19(1):5–18
27. Petrovich YA, Yarema IV, Kichenko SM, Urtaev BM. 2009. Study of drug transport between the blood and lymph in the predominant direction. *Bull. Exp. Biol. Med.* 147(3):357–60
28. Yadav P, McLeod VM, Nowell CJ, Selby LI, Johnston APR, et al. 2018. Distribution of therapeutic proteins into thoracic lymph after intravenous administration is protein size-dependent and primarily occurs within the liver and mesentery. *J. Control. Release* 272:17–28
29. Oswald S, Terhaag B, Siegmund W. 2011. In vivo probes of drug transport: commonly used probe drugs to assess function of intestinal P-glycoprotein (ABCB1) in humans. In *Drug Transporters*, ed. M Fromm, R Kim, pp. 403–47. Berlin: Springer
30. Ma JD, Tsunoda SM, Bertino JS, Trivedi M, Beale KK, Nafziger AN. 2010. Evaluation of in vivo P-glycoprotein phenotyping probes. *Clin. Pharmacokinet.* 49(4):223–37
31. Tucker GT, Houston JB, Huang SM. 2001. Optimizing drug development: strategies to assess drug metabolism/transporter interaction potential—towards a consensus. *Pharm. Res.* 18(8):1071–80
32. Peters SA, Jones CR, Ungell A-L, Hatley OJD. 2016. Predicting drug extraction in the human gut wall: assessing contributions from drug metabolizing enzymes and transporter proteins using preclinical models. *Clin. Pharmacokinet.* 55(6):673–96
33. Yokose T, Doy M, Taniguchi T, Shimada T, Kakiki M, et al. 1999. Immunohistochemical study of cytochrome P450 2C and 3A in human non-neoplastic and neoplastic tissues. *Virchows Arch.* 434(5):401–11
34. Palmer CN, Coates PJ, Davies SE, Shephard EA, Phillips IR. 1992. Localization of cytochrome P-450 gene expression in normal and diseased human liver by in situ hybridization of wax-embedded archival material. *Hepatology* 16(3):682–87
35. Daly AK. 2006. Significance of the minor cytochrome P450 3A isoforms. *Clin. Pharmacokinet.* 45(1):13–31
36. Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara EM, Hall SD. 1998. The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. *Clin. Pharmacol. Ther.* 64(2):133–43
37. Koch I, Weil R, Wolbold R, Brockmöller J, Hustert E, et al. 2002. Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. *Drug Metab Dispos.* 30(10):1108–14
38. Klein K, Zanger UM. 2013. Pharmacogenomics of cytochrome P450 3A4: recent progress toward the "missing heritability" problem. *Front. Genet.* 4:12
39. Tomalik-Scharte D, Maiter D, Kirchheiner J, Ivison HE, Fuhr U, Arlt W. 2010. Impaired hepatic drug and steroid metabolism in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *Eur. J. Endocrinol.* 163(6):919–24
40. Thirumaran RK, Lamba JK, Kim RB, Urquhart BL, Gregor JC, et al. 2012. Intestinal CYP3A4 and midazolam disposition in vivo associate with VDR polymorphisms and show seasonal variation. *Biochem. Pharmacol.* 84(1):104–12
41. Henderson CJ, McLaughlin LA, Scheer N, Stanley LA, Wolf CR. 2015. Cytochrome b5 is a major determinant of human cytochrome P450 CYP2D6 and CYP3A4 activity in vivo. *Mol. Pharmacol.* 87(4):733–39
42. Sevrioukova IF, Poulos TL. 2013. Understanding the mechanism of cytochrome P450 3A4: recent advances and remaining problems. *Dalton Trans.* 42(9):3116–26
43. Foti RS, Rock DA, Wienkers LC, Wahlstrom JL. 2010. Selection of alternative CYP3A4 probe substrates for clinical drug interaction studies using in vitro data and in vivo simulation. *Drug Metab. Dispos.* 38(6):981–87
44. Kenworthy KE, Bloomer JC, Clarke SE, Houston JB. 1999. CYP3A4 drug interactions: correlation of 10 in vitro probe substrates. *Br. J. Clin. Pharmacol.* 48(5):716–27
45. Benet LZ. 2005. There are no useful CYP3A probes that quantitatively predict the in vivo kinetics of other CYP3A substrates and no expectation that one will be found. *Mol. Interv.* 5(2):79–83

46. Heizmann P, Eckert M, Ziegler WH. 1983. Pharmacokinetics and bioavailability of midazolam in man. *Br. J. Clin. Pharmacol.* 16(Suppl. 1):43S–49S
47. Thummel KE, O'Shea D, Paine MF, Shen DD, Kunze KL, et al. 1996. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin. Pharmacol. Ther.* 59(5):491–502
48. Hohmann N, Kocheise F, Carls A, Burhenne J, Haefeli WE, Mikus G. 2015. Midazolam microdose to determine systemic and pre-systemic metabolic CYP3A activity in humans. *Br. J. Clin. Pharmacol.* 79(2):278–85
49. Floyd MD, Gervasini G, Masica AL, Mayo G, George AL, et al. 2003. Genotype-phenotype associations for common CYP3A4 and CYP3A5 variants in the basal and induced metabolism of midazolam in European- and African-American men and women. *Pharmacogenetics* 13(10):595–606
50. Vossen M, Sevestre M, Niederalt C, Jang IJ, Willmann S, Edginton AN. 2007. Dynamically simulating the interaction of midazolam and the CYP3A4 inhibitor itraconazole using individual coupled whole-body physiologically-based pharmacokinetic (WB-PBPK) models. *Theor. Biol. Med. Model.* 4:13
51. Yu K-S, Cho J-Y, Jang I-J, Hong K-S, Chung J-Y, et al. 2004. Effect of the CYP3A5 genotype on the pharmacokinetics of intravenous midazolam during inhibited and induced metabolic states. *Clin. Pharmacol. Ther.* 76(2):104–12
52. Williams JA, Ring BJ, Cantrell VE, Jones DR, Eckstein J, et al. 2002. Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab. Dispos.* 30(8):883–91
53. Hyland R, Osborne T, Payne A, Kempshall S, Logan YR, et al. 2009. In vitro and in vivo glucuronidation of midazolam in humans. *Br. J. Clin. Pharmacol.* 67(4):445–54
54. Kim RB, Wandel C, Leake B, Cvetkovic M, Fromm MF, et al. 1999. Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm. Res.* 16(3):408–14
55. Halama B, Hohmann N, Burhenne J, Weiss J, Mikus G, Haefeli WE. 2013. A nanogram dose of the CYP3A probe substrate midazolam to evaluate drug interactions. *Clin. Pharmacol. Ther.* 93(6):564–71
56. Bornemann LD, Min BH, Crews T, Rees MM, Blumenthal HP, et al. 1985. Dose dependent pharmacokinetics of midazolam. *Eur. J. Clin. Pharmacol.* 29(1):91–95
57. de Jonge H, de Loor H, Verbeke K, Vanrenterghem Y, Kuypers DR. 2013. Impact of *CYP3A5* genotype on tacrolimus versus midazolam clearance in renal transplant recipients: new insights in CYP3A5-mediated drug metabolism. *Pharmacogenomics* 14(12):1467–80
58. Vanhove T, Bouillon T, de Loor H, Annaert P, Kuypers D. 2017. Fexofenadine, a putative in vivo P-glycoprotein probe, fails to predict clearance of the substrate tacrolimus in renal recipients. *Clin. Pharmacol. Ther.* 102(6):989–96
59. Lee J, Chaves-Gnecco D, Amico JA, Kroboth PD, Wilson JW, Frye RF. 2002. Application of semisimultaneous midazolam administration for hepatic and intestinal cytochrome P450 3A phenotyping. *Clin. Pharmacol. Ther.* 72(6):718–28
60. Frechen S, Junge L, Saari TI, Suleiman AA, Rokitta D, et al. 2013. A semiphysiological population pharmacokinetic model for dynamic inhibition of liver and gut wall cytochrome P450 3A by voriconazole. *Clin. Pharmacokinet.* 52(9):763–81
61. Gertz M, Harrison A, Houston JB, Galetin A. 2010. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab. Dispos.* 38(7):1147–58
62. Ohno Y, Hisaka A, Suzuki H. 2007. General framework for the quantitative prediction of CYP3A4-mediated oral drug interactions based on the AUC increase by coadministration of standard drugs. *Clin. Pharmacokinet.* 46(8):681–96
63. Ohno Y, Hisaka A, Ueno M, Suzuki H. 2008. General framework for the prediction of oral drug interactions caused by CYP3A4 induction from in vivo information. *Clin. Pharmacokinet.* 47(10):669–80
64. Park G-J, Bae SH, Park W-S, Han S, Park M-H, et al. 2017. Drug-drug interaction of microdose and regular-dose omeprazole with a CYP2C19 inhibitor and inducer. *Drug Des. Dev. Ther.* 11:1043–53
65. Gröer C, Busch D, Patrzyk M, Beyer K, Busemann A, et al. 2014. Absolute protein quantification of clinically relevant cytochrome P450 enzymes and UDP-glucuronosyltransferases by mass spectrometry-based targeted proteomics. *J. Pharm. Biomed. Anal.* 100:393–401

55. An amazing example of probe drug microdosing.

60. An example of a semiphysiological midazolam model to improve understanding of CYP3A-based PK interaction.

66. Karam WG, Goldstein JA, Lasker JM, Ghanayem BI. 1996. Human CYP2C19 is a major omeprazole 5-hydroxylase, as demonstrated with recombinant cytochrome P450 enzymes. *Drug Metab. Dispos.* 24(10):1081–87
67. Kanebratt KP, Diczfalusy U, Bäckström T, Sparve E, Bredberg E, et al. 2008. Cytochrome P450 induction by rifampicin in healthy subjects: determination using the Karolinska cocktail and the endogenous CYP3A4 marker 4β-hydroxycholesterol. *Clin. Pharmacol. Ther.* 84(5):589–94
68. Ogilvie BW, Yerino P, Kazmi F, Buckley DB, Rostami-Hodjegan A, et al. 2011. The proton pump inhibitor, omeprazole, but not lansoprazole or pantoprazole, is a metabolism-dependent inhibitor of CYP2C19: implications for coadministration with clopidogrel. *Drug Metab. Dispos.* 39(11):2020–33
69. Pauli-Magnus C, Rekersbrink S, Klotz U, Fromm MF. 2001. Interaction of omeprazole, lansoprazole and pantoprazole with P-glycoprotein. *Naunyn. Schmiedebergs. Arch. Pharmacol.* 364(6):551–57
70. Ogawa R, Echizen H. 2010. Drug-drug interaction profiles of proton pump inhibitors. *Clin. Pharmacokinet.* 49(8):509–33
71. Shirasaka Y, Sager JE, Lutz JD, Davis C, Isoherranen N. 2013. Inhibition of CYP2C19 and CYP3A4 by omeprazole metabolites and their contribution to drug-drug interactions. *Drug Metab. Dispos.* 41(7):1414–24
72. Doroshyenko O, Rokitta D, Zadoyan G, Klement S, Schläfke S, et al. 2013. Drug cocktail interaction study on the effect of the orally administered lavender oil preparation silexan on cytochrome P450 enzymes in healthy volunteers. *Drug Metab. Dispos.* 41(5):987–93
73. Zadoyan G, Rokitta D, Klement S, Dienel A, Hoerr R, et al. 2012. Effect of *Ginkgo biloba* special extract EGb 761® on human cytochrome P450 activity: a cocktail interaction study in healthy volunteers. *Eur. J. Clin. Pharmacol.* 68(5):553–60
74. Zhao W, Leroux S, Biran V, Jacqz-Aigrain E. 2018. Developmental pharmacogenetics of CYP2C19 in neonates and young infants: omeprazole as a probe drug. *Br. J. Clin. Pharmacol.* 84(5):997–1005
75. Baldwin RM, Ohlsson S, Pedersen RS, Mwinyi J, Ingelman-Sundberg M, et al. 2008. Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers. *Br. J. Clin. Pharmacol.* 65(5):767–74
76. Frank D, Jaehde U, Fuhr U. 2007. Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. *Eur. J. Clin. Pharmacol.* 63(4):321–33
77. Nakashima D, Takama H, Ogasawara Y, Kawakami T, Nishitoba T, et al. 2007. Effect of cinacalcet hydrochloride, a new calcimimetic agent, on the pharmacokinetics of dextromethorphan: in vitro and clinical studies. *J. Clin. Pharmacol.* 47(10):1311–19
78. Storelli F, Matthey A, Lenglet S, Thomas A, Desmeules J, Daali Y. 2018. Impact of *CYP2D6* functional allelic variations on phenoconversion and drug-drug interactions. *Clin. Pharmacol. Ther.* 104(1):148–57
79. Capon DA, Bochner F, Kerry N, Mikus G, Danz C, Somogyi AA. 1996. The influence of CYP2D6 polymorphism and quinidine on the disposition and antitussive effect of dextromethorphan in humans. *Clin. Pharmacol. Ther.* 60(3):295–307
80. Ieiri I, Fukae M, Maeda K, Ando Y, Kimura M, et al. 2012. Pharmacogenomic/pharmacokinetic assessment of a four-probe cocktail for CYPs and OATPs following oral microdosing. *Int. J. Clin. Pharmacol. Ther.* 50(10):689–700
81. Schadel M, Wu D, Otton SV, Kalow W, Sellers EM. 1995. Pharmacokinetics of dextromethorphan and metabolites in humans: influence of the CYP2D6 phenotype and quinidine inhibition. *J. Clin. Psychopharmacol.* 15(4):263–69
82. Miyauchi E, Tachikawa M, Declèves X, Uchida Y, Bouillot J-L, et al. 2016. Quantitative atlas of cytochrome P450, UDP-glucuronosyltransferase, and transporter proteins in jejunum of morbidly obese subjects. *Mol. Pharm.* 13(8):2631–40

83. Application of suitable validation procedures for a transporter probe drug.

83. Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, et al. 1999. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J. Clin. Investig.* 104(2):147–53

84. He J, Yu Y, Prasad B, Chen X, Unadkat JD. 2014. Mechanism of an unusual, but clinically significant, digoxin-bupropion drug interaction. *Biopharm. Drug Dispos.* 35(5):253–63

85. Martin P, Gillen M, Millson D, Oliver S, Brealey C, et al. 2015. Effects of fostamatinib on the pharmacokinetics of digoxin (a P-glycoprotein substrate): results from in vitro and phase I clinical studies. *Clin. Ther.* 37(12):2811–22
86. Pedersen KE, Dorph-Pedersen A, Hvidt S, Klitgaard NA, Pedersen KK. 1982. The long-term effect of verapamil on plasma digoxin concentration and renal digoxin clearance in healthy subjects. *Eur. J. Clin. Pharmacol.* 22(2):123–27
87. Rengelshausen J, Göggelmann C, Burhenne J, Riedel K-D, Ludwig J, et al. 2003. Contribution of increased oral bioavailability and reduced nonglomerular renal clearance of digoxin to the digoxin-clarithromycin interaction. *Br. J. Clin. Pharmacol.* 56(1):32–38
88. Scotcher D, Jones CR, Galetin A, Rostami-Hodjegan A. 2017. Delineating the role of various factors in renal disposition of digoxin through application of physiologically based kidney model to renal impairment populations. *J. Pharmacol. Exp. Ther.* 360(3):484–95
89. Kirby BJ, Collier AC, Kharasch ED, Whittington D, Thummel KE, Unadkat JD. 2012. Complex drug interactions of the HIV protease inhibitors 3: effect of simultaneous or staggered dosing of digoxin and ritonavir, nelfinavir, rifampin, or bupropion. *Drug Metab. Dispos.* 40(3):610–16
90. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmöller J, et al. 2000. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *PNAS* 97(7):3473–78
91. Shoaf SE, Ohzone Y, Ninomiya S, Furukawa M, Bricmont P, et al. 2011. In vitro P-glycoprotein interactions and steady-state pharmacokinetic interactions between tolvaptan and digoxin in healthy subjects. *J. Clin. Pharmacol.* 51(5):761–69
92. Vesell ES. 1984. Noninvasive assessment in vivo of hepatic drug metabolism in health and disease. *Ann. N. Y. Acad. Sci.* 428:293–307
93. Prueksaritanont T, Chu X, Evers R, Klopfer SO, Caro L, et al. 2014. Pitavastatin is a more sensitive and selective organic anion-transporting polypeptide 1B clinical probe than rosuvastatin. *Br. J. Clin. Pharmacol.* 78(3):587–98
94. Graham GG, Punt J, Arora M, Day RO, Doogue MP, et al. 2011. Clinical pharmacokinetics of metformin. *Clin. Pharmacokinet.* 50(2):81–98
95. Elsby R, Chidlaw S, Outteridge S, Pickering S, Radcliffe A, et al. 2017. Mechanistic in vitro studies confirm that inhibition of the renal apical efflux transporter multidrug and toxin extrusion (MATE) 1, and not altered absorption, underlies the increased metformin exposure observed in clinical interactions with cimetidine, trimethoprim or pyrimethamine. *Pharmacol. Res. Perspect.* 5(5):e00357
96. Ebner T, Ishiguro N, Taub ME. 2015. The use of transporter probe drug cocktails for the assessment of transporter-based drug–drug interactions in a clinical setting—proposal of a four component transporter cocktail. *J. Pharm. Sci.* 104(9):3220–28
97. Mathialagan S, Piotrowski MA, Tess DA, Feng B, Litchfield J, Varma MV. 2017. Quantitative prediction of human renal clearance and drug-drug interactions of organic anion transporter substrates using in vitro transport data: a relative activity factor approach. *Drug Metab. Dispos.* 45(4):409–17
98. Dieterich A. 2017. *Mechanistische quantitative Bestimmung der Hemmung des Enzyms CYP3A in Leber und Darmwand mit einem neuartigen Studiendesign [Mechanistic quantitative assessment of the inhibition of the enzyme CYP3A in liver and gut wall using a novel study design]*. MD Thesis, Univ. Cologne, Cologne, Ger.
99. Hohmann N, Gottwald K, Czock D, Burhenne J, Haefeli WE, Mikus G. 2016. Effects of the CYP3A perpetrators voriconazole, efavirenz and rifampin on midazolam plasma concentrations during continuous microdose infusion in healthy volunteers. *Clin. Pharmacol. Ther.* 99(Suppl. 1):S40 (Abstr.)
100. Croft M, Keely B, Morris I, Tann L, Lappin G. 2012. Predicting drug candidate victims of drug-drug interactions, using microdosing. *Clin. Pharmacokinet.* 51(4):237–46
101. Nordmark A, Andersson A, Baranczewski P, Wanag E, Ståhle L. 2014. Assessment of interaction potential of AZD2066 using in vitro metabolism tools, physiologically based pharmacokinetic modelling and in vivo cocktail data. *Eur. J. Clin. Pharmacol.* 70(2):167–78
102. Jetter A, Kinzig-Schippers M, Skott A, Lazar A, Tomalik-Scharte D, et al. 2004. Cytochrome P450 2C9 phenotyping using low-dose tolbutamide. *Eur. J. Clin. Pharmacol.* 60(3):165–71
103. Nguyen TT, Bénech H, Delaforge M, Lenuzza N. 2016. Design optimisation for pharmacokinetic modeling of a cocktail of phenotyping drugs. *Pharm. Stat.* 15(2):165–77

104. Prueksaritanont T, Tatosian D, Chu X, Railkar R, Evers R, et al. 2017. Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A. *Clin. Pharmacol. Ther.* 101(4):519–30
105. Chavez-Eng CM, Lutz RW, Goykhman D, Bateman KP. 2018. Microdosing cocktail assay development for drug-drug interaction studies. *J. Pharm. Sci.* 107(7):1973–86
106. Stehle S, Kirchheiner J, Lazar A, Fuhr U. 2008. Pharmacogenetics of oral anticoagulants: a basis for dose individualization. *Clin. Pharmacokinet.* 47(9):565–94
107. de Andrés F, Terán S, Bovera M, Fariñas H, Terán E, et al. 2016. Multiplex phenotyping for systems medicine: a one-point optimized practical sampling strategy for simultaneous estimation of CYP1A2, CYP2C9, CYP2C19, and CYP2D6 activities using a cocktail approach. *OMICS* 20(2):88–96
108. Bosilkovska M, Samer C, Déglon J, Thomas A, Walder B, et al. 2016. Evaluation of mutual drug-drug interaction within Geneva cocktail for cytochrome P450 phenotyping using innovative dried blood sampling method. *Basic Clin. Pharmacol. Toxicol.* 119(3):284–90
109. Masters JC, Harano DM, Greenberg HE, Tsunoda SM, Jang I-J, Ma JD. 2015. Limited sampling strategy of partial area under the concentration-time curves to estimate midazolam systemic clearance for cytochrome P450 3A phenotyping. *Ther. Drug Monit.* 37(1):84–89
110. Chang AT, Bertino JS, Nafziger AN, Kashuba ADM, Turpault S, et al. 2016. *S*-Warfarin limited sampling models to estimate area under the concentration versus time curve for cytochrome P450 2C9 baseline activity and after induction. *Ther. Drug Monit.* 38(3):383–87
111. Varma MV, Pang KS, Isoherranen N, Zhao P. 2015. Dealing with the complex drug-drug interactions: towards mechanistic models. *Biopharm. Drug Dispos.* 36(2):71–92
112. Asaumi R, Toshimoto K, Tobe Y, Hashizume K, Nunoya K, et al. 2018. Comprehensive PBPK model of rifampicin for quantitative prediction of complex drug-drug interactions: CYP3A/2C9 induction and OATP inhibition effects. *CPT Pharmacomet. Syst. Pharmacol.* 7(3):186–96

113. Comprehensive description of the method and application of physiologically based pharmacokinetic models.

113. Rowland M, Peck C, Tucker G. 2011. Physiologically-based pharmacokinetics in drug development and regulatory science. *Annu. Rev. Pharmacol. Toxicol.* 51:45–73

114. Wendling T, Tsamandouras N, Dumitras S, Pigeolet E, Ogungbenro K, Aarons L. 2016. Reduction of a whole-body physiologically based pharmacokinetic model to stabilise the bayesian analysis of clinical data. *AAPS J.* 18(1):196–209
115. Tsamandouras N, Rostami-Hodjegan A, Aarons L. 2015. Combining the "bottom up" and "top down" approaches in pharmacokinetic modelling: fitting PBPK models to observed clinical data. *Br. J. Clin. Pharmacol.* 79(1):48–55
116. Wagner C, Pan Y, Hsu V, Sinha V, Zhao P. 2016. Predicting the effect of CYP3A inducers on the pharmacokinetics of substrate drugs using physiologically based pharmacokinetic (PBPK) modeling: an analysis of PBPK submissions to the US FDA. *Clin. Pharmacokinet.* 55(4):475–83
117. Wagner C, Pan Y, Hsu V, Grillo JA, Zhang L, et al. 2015. Predicting the effect of cytochrome P450 inhibitors on substrate drugs: analysis of physiologically based pharmacokinetic modeling submissions to the US Food and Drug Administration. *Clin. Pharmacokinet.* 54(1):117–27
118. Pelkonen O, Kapitulnik J, Gundert-Remy U, Boobis AR, Stockis A. 2008. Local kinetics and dynamics of xenobiotics. *Crit. Rev. Toxicol.* 38(8):697–720
119. Korzekwa K, Nagar S. 2017. On the nature of physiologically-based pharmacokinetic models—*a priori* or *a posteriori*? Mechanistic or empirical? *Pharm. Res.* 34(3):529–34

122. Straightforward validation of a probe substrate cocktail.

120. de Andrés F, Llerena A. 2016. Simultaneous determination of cytochrome P450 oxidation capacity in humans: a review on the phenotyping cocktail approach. *Curr. Pharm. Biotechnol.* 17(13):1159–80
121. Donzelli M, Derungs A, Serratore MG, Noppen C, Nezic L, et al. 2014. The Basel cocktail for simultaneous phenotyping of human cytochrome P450 isoforms in plasma, saliva and dried blood spots. *Clin. Pharmacokinet.* 53(3):271–82

122. Turpault S, Brian W, Van Horn R, Santoni A, Poitiers F, et al. 2009. Pharmacokinetic assessment of a five-probe cocktail for CYPs 1A2, 2C9, 2C19, 2D6 and 3A. *Br. J. Clin. Pharmacol.* 68(6):928–35

123. A description of the first cocktail of transporter probe substrates.

123. Stopfer P, Giessmann T, Hohl K, Sharma A, Ishiguro N, et al. 2016. Pharmacokinetic evaluation of a drug transporter cocktail consisting of digoxin, furosemide, metformin, and rosuvastatin. *Clin. Pharmacol. Ther.* 100(3):259–67

124. Stopfer P, Giessmann T, Hohl K, Sharma A, Ishiguro N, et al. 2018. Effects of metformin and furosemide on rosuvastatin pharmacokinetics in healthy volunteers: implications for their use as probe drugs in a transporter cocktail. *Eur. J. Drug Metab. Pharmacokinet.* 43(1):69–80
125. Stopfer P, Giessmann T, Hohl K, Hutzel S, Schmidt S, et al. 2018. Optimization of a drug transporter probe cocktail: potential screening tool for transporter-mediated drug-drug interactions. *Br. J. Clin. Pharmacol.* 84:1941–49
126. Pedersen RS, Damkier P, Christensen MM, Brosen K. 2013. A cytochrome P450 phenotyping cocktail causing unexpected adverse reactions in female volunteers. *Eur. J. Clin. Pharmacol.* 69(12):1997–99
127. Chow PKH, Yu W-K, Soo K-C, Chan STF. 2003. The measurement of liver blood flow: a review of experimental and clinical methods. *J. Surg. Res.* 112(1):1–11
128. van Griensven JM, Koster RW, Hopkins GR, Beier H, Günzler WA, et al. 1997. Effect of changes in liver blood flow on the pharmacokinetics of saruplase in patients with acute myocardial infarction. *Thromb. Haemost.* 78(3):1015–20
129. de Graaf W, Häusler S, Heger M, van Ginhoven TM, van Cappellen G, et al. 2011. Transporters involved in the hepatic uptake of ^{99m}Tc-mebrofenin and indocyanine green. *J. Hepatol.* 54(4):738–45
130. Kagawa T, Adachi Y, Hashimoto N, Mitsui H, Ohashi T, et al. 2017. Loss of organic anion transporting polypeptide 1B3 function causes marked delay in indocyanine green clearance without any clinical symptoms. *Hepatology* 65(3):1065–68
131. Burns E, Triger DR, Tucker GT, Bax ND. 1991. Indocyanine green elimination in patients with liver disease and in normal subjects. *Clin. Sci.* 80(2):155–60
132. Wissler EH. 2011. Identifying a long standing error in single-bolus determination of the hepatic extraction ratio for indocyanine green. *Eur. J. Appl. Physiol.* 111(4):641–46
133. Keiding S, Engsted E, Ott P. 1998. Sorbitol as a test substance for measurement of liver plasma flow in humans. *Hepatology* 28(1):50–56
134. Seegmiller JC, Burns BE, Schinstock CA, Lieske JC, Larson TS. 2016. Discordance between iothalamate and iohexol urinary clearances. *Am. J. Kidney Dis.* 67(1):49–55
135. Schaeffner ES, Ebert N, Delanaye P, Frei U, Gaedeke J, et al. 2012. Two novel equations to estimate kidney function in persons aged 70 years or older. *Ann. Intern. Med.* 157(7):471–81
136. Koteff J, Borland J, Chen S, Song I, Peppercorn A, et al. 2013. A phase 1 study to evaluate the effect of dolutegravir on renal function via measurement of iohexol and para-aminohippurate clearance in healthy subjects. *Br. J. Clin. Pharmacol.* 75(4):990–96
137. Delanaye P, Melsom T, Ebert N, Bäck S-E, Mariat C, et al. 2016. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 2: Why to measure glomerular filtration rate with iohexol? *Clin. Kidney J.* 9(5):700–4
138. Jetter A, Kinzig-Schippers M, Illauer M, Hermann R, Erb K, et al. 2004. Phenotyping of *N*-acetyltransferase type 2 by caffeine from uncontrolled dietary exposure. *Eur. J. Clin. Pharmacol.* 60(1):17–21
139. Polasek TM, Miners JO. 2008. Macrolide-theophylline interactions: no role for the inhibition of cytochrome P4501A2. *Br. J. Clin. Pharmacol.* 66(6):898–900
140. Granfors M, Backman J, Neuvonen M, Neuvonen P. 2004. Ciprofloxacin greatly increases concentrations and hypotensive effect of tizanidine by inhibiting its cytochrome P450 1A2-mediated presystemic metabolism. *Clin. Pharmacol. Ther.* 76(6):598–606
141. Momo K, Homma M, Osaka Y, Inomata S, Tanaka M, Kohda Y. 2010. Effects of mexiletine, a CYP1A2 inhibitor, on tizanidine pharmacokinetics and pharmacodynamics. *J. Clin. Pharmacol.* 50(3):331–37
142. Bi Y, Mathialagan S, Tylaska L, Fu M, Keefer J, et al. 2018. Organic anion transporter 2 mediates hepatic uptake of tolbutamide, a CYP2C9 probe drug. *J. Pharmacol. Exp. Ther.* 364(3):390–98
143. Yang M-S, Yu C-P, Chao P-DL, Lin S-P, Hou Y-C. 2017. *R*- and *S*-warfarin were transported by breast cancer resistance protein: from in vitro to pharmacokinetic-pharmacodynamic studies. *J. Pharm. Sci.* 106(5):1419–25
144. Bi Y, Lin J, Mathialagan S, Tylaska L, Callegari E, et al. 2018. Role of hepatic organic anion transporter 2 in the pharmacokinetics of *R*- and *S*-warfarin: in vitro studies and mechanistic evaluation. *Mol. Pharm.* 15(3):1284–95

145. Harris RZ, Salfi M, Posvar E, Hoelscher D, Padhi D. 2007. Pharmacokinetics of desipramine HCl when administered with cinacalcet HCl. *Eur. J. Clin. Pharmacol.* 63(2):159–63
146. Brøsen K, Gram LF. 1988. First-pass metabolism of imipramine and desipramine: impact of the sparteine oxidation phenotype. *Clin. Pharmacol. Ther.* 43(4):400–6
147. Samant T, Lukacova V, Schmidt S. 2017. Development and qualification of physiologically based pharmacokinetic models for drugs with atypical distribution behavior: a desipramine case study. *CPT Pharmacomet. Syst. Pharmacol.* 6(5):315–21
148. Ciraulo DA, Barnhill JG, Jaffe JH. 1988. Clinical pharmacokinetics of imipramine and desipramine in alcoholics and normal volunteers. *Clin. Pharmacol. Ther.* 43(5):509–18
149. Choi CI, Bae JW, Lee YJ, Lee HI, Jang CG, Lee SY. 2014. Effects of CYP2C19 genetic polymorphisms on atomoxetine pharmacokinetics. *J. Clin. Psychopharmacol.* 34(1):139–42
150. Yu G, Li GF, Markowitz JS. 2016. Atomoxetine: a review of its pharmacokinetics and pharmacogenomics relative to drug disposition. *J. Child. Adolesc. Psychopharmacol.* 26(4):314–26
151. Lucas D, Ferrara R, Gonzalez E, Bodenez P, Albores A, et al. 1999. Chlorzoxazone, a selective probe for phenotyping CYP2E1 in humans. *Pharmacogenetics* 9(3):377–88
152. Palmer JL, Scott RJ, Gibson A, Dickins M, Pleasance S. 2001. An interaction between the cytochrome P450 probe substrates chlorzoxazone (CYP2E1) and midazolam (CYP3A). *Br. J. Clin. Pharmacol.* 52(5):555–61
153. Blakey GE, Lockton JA, Perrett J, Norwood P, Russell M, et al. 2004. Pharmacokinetic and pharmacodynamic assessment of a five-probe metabolic cocktail for CYPs 1A2, 3A4, 2C9, 2D6 and 2E1. *Br. J. Clin. Pharmacol.* 57(2):162–69
154. Masica A, Mayo G, Wilkinson G. 2004. In vivo comparisons of constitutive cytochrome P450 3A activity assessed by alprazolam, triazolam, and midazolam. *Clin. Pharmacol. Ther.* 76(4):341–49
155. Kroboth PD, McAuley JW, Kroboth FJ, Bertz RJ, Smith RB. 1995. Triazolam pharmacokinetics after intravenous, oral, and sublingual administration. *J. Clin. Psychopharmacol.* 15(4):259–62
156. Patki KC, Von Moltke LL, Greenblatt DJ. 2003. In vitro metabolism of midazolam, triazolam, nifedipine, and testosterone by human liver microsomes and recombinant cytochromes P450: role of CYP3A4 and CYP3A5. *Drug Metab. Dispos.* 31(7):938–44
157. Zhao Y, Hu Z-Y. 2014. Physiologically based pharmacokinetic modelling and in vivo [I]/K_i accurately predict P-glycoprotein-mediated drug-drug interactions with dabigatran etexilate. *Br. J. Pharmacol.* 171(4):1043–53
158. Stangier J, Eriksson BI, Dahl OE, Ahnfelt L, Nehmiz G, et al. 2005. Pharmacokinetic profile of the oral direct thrombin inhibitor dabigatran etexilate in healthy volunteers and patients undergoing total hip replacement. *J. Clin. Pharmacol.* 45(5):555–65
159. Paré G, Eriksson N, Lehr T, Connolly S, Eikelboom J, et al. 2013. Genetic determinants of dabigatran plasma levels and their relation to bleeding. *Circulation* 127(13):1404–12
160. Maeda K, Tian Y, Fujita T, Ikeda Y, Kumagai Y, et al. 2014. Inhibitory effects of *p*-aminohippurate and probenecid on the renal clearance of adefovir and benzylpenicillin as probe drugs for organic anion transporter (OAT) 1 and OAT3 in humans. *Eur. J. Pharm. Sci.* 59:94–103
161. Stoffel M, Hsin C, Kinzig M, Schäffeler E, Lenssen R, et al. 2017. *Plasma pharmacokinetics in healthy volunteers does not suggest major changes in OATP1B1, OCT1/2, MATE1/2K, or OAT1/3 activities by a transporter phenotyping cocktail.* Presented at 14th European ISSX Meeting
162. Chu X-Y, Bleasby K, Yabut J, Cai X, Chan GH, et al. 2007. Transport of the dipeptidyl peptidase-4 inhibitor sitagliptin by human organic anion transporter 3, organic anion transporting polypeptide 4C1, and multidrug resistance P-glycoprotein. *J. Pharmacol. Exp. Ther.* 321(2):673–83
163. Klatt S, Fromm MF, König J. 2011. Transporter-mediated drug-drug interactions with oral antidiabetic drugs. *Pharmaceutics* 3(4):680–705
164. Fujita K, Sakata H, Murono K, Hasegawa H, Takimoto M, Yoshioka H. 1983. Comparative pharmacological evaluation of oral benzathine penicillin G and phenoxymethyl penicillin potassium in children. *Pediatr. Pharmacol.* 3(1):37–41