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Noninvasive Preclinical and Clinical Imaging of Liver Transporter Function Relevant to Drug-Induced Liver Injury

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Abstract

Imaging technologies can evaluate many different biological processes in vitro (in cell culture models) and in vivo (in animals and humans), and many are used routinely in investigation of human liver diseases. Some of these methods can help understand liver toxicity caused by drugs in vivo in animals, and drug-induced liver injury (DILI) which arises in susceptible humans. Imaging could aid assessment of the relevance to humans in vivo of toxicity caused by drugs in animals (animal/human translation), plus toxicities observed using in vitro model systems (in vitro/in vivo translation). Technologies and probe substrates for quantitative evaluation of hepatobiliary transporter activities are of particular importance. This is due to the key role played by sinusoidal transporter mediated hepatic uptake in DILI caused by many drugs, plus the strong evidence that inhibition of the hepatic bile salt export pump (BSEP) can initiate DILI. Imaging methods for investigation of these processes are reviewed in this chapter, together with their scientific rationale, and methods of quantitative data analysis. In addition to providing biomarkers for investigation of DILI, such approaches could aid the evaluation of clinically relevant drug–drug interactions mediated via hepatobiliary transporter perturbation.

Key words Drug-induced liver injury, Drug labelling, Causality assessment, Hepatotoxicity, Hepatobiliary transporters, Bile salt export pump, Gadoxetate, Drug-drug interactions

1 Introduction

Many hundreds of different drugs cause liver injury in humans which occurs only infrequently and in certain susceptible individuals, and cannot be anticipated from nonclinical safety studies undertaken in vivo in experimental animals [1, 2]. Hence their ability to cause human drug-induced liver injury (DILI) only starts to be appreciated in Phase 2 or Phase 3 clinical trials, or even post-licensing [2, 3]. The consequences in affected patients may be marked symptomatic liver damage, or even acute liver failure, and currently it is not possible to predict and identify “at risk” patients

prior to their exposure to the relevant drugs [1, 2]. Because of this, unexpected “idiosyncratic” human DILI continues to be a leading cause of failed development of new drugs, of withdrawal from use of previously licensed drugs and of cautionary labeling that restricts prescribing [1–5].

The mechanisms by which drugs cause human idiosyncratic DILI are complex, and involve both drug-related adverse biochemical processes and susceptibility factors specific to susceptible patients [1, 2]. Important drug-related adverse processes which can initiate idiosyncratic DILI include formation of chemically reactive metabolites, injury to mitochondria, and inhibition of the activity of the bile salt export pump (BSEP), which mediates efflux of toxic bile salts from hepatocytes into bile [6–8]. The susceptibility factors that explain why only some patients develop DILI are less well defined, although it is clear that these can include activation of both innate and adaptive immune responses [9].

Several of the key drug-related events that initiate idiosyncratic DILI can be quantified using various *in vitro* assays. These assays can be used during drug discovery, to enable early identification and deselection of compounds with high propensity to cause DILI and other serious adverse reactions [6–8]. Many different methodologies have been described, and are used routinely in pharmaceutical companies. They are discussed in Chap. 17 of this volume, by Light et al., and can reduce the likelihood that compounds progressed into clinical trials will cause DILI in humans. However, *in vitro* toxicity assays have several important limitations. The most commonly used *in vitro* toxicity assays fail to reproduce many of the key molecular events that influence hepatic drug uptake, biotransformation and excretion *in vivo*. Furthermore, the assays do not reproduce all of the mechanisms by which DILI occurs *in vivo*. Consequently, the precise relationship between potencies of effects observed in *in vitro* safety assays and functional consequences that may arise within the liver *in vivo*, and may result in DILI in susceptible drug exposed humans, remains poorly understood.

One approach which can help to address this important translational gap is medical imaging. Several imaging modalities can measure the hepatic uptake and clearance of probe substrates. Many are well suited to *in vivo* studies in animals and humans, while others can be utilized in cellular systems *in vitro*. Mechanistically relevant processes include the transporters that mediate drug uptake into and excretion from the liver, plus BSEP and other hepatobiliary transporters that mediate bile flow. Suitable imaging modalities and probe substrates are reviewed in Sect. 2. Key issues that need to be considered when generating reproducible data and undertaking quantitative analyses of imaging data are discussed in Sect. 3. These include assessing interaction between investigational drugs and the probe substrates used in imaging studies. Some of these interactions provide insight into undesired

drug–drug interactions and this aspect is addressed in Sect. 4. An imaging approach to investigate and characterize liver injury with potential to provide novel insight into DILI risk is dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) using the contrast agent gadoxetate. This is reviewed in Sect. 5, which discusses the value and limitations of the approach. Finally, challenges and opportunities in using imaging technologies to understand and risk-manage DILI are considered in Sect. 6.

2 Hepatobiliary Imaging Modalities and Tracers

Imaging techniques can measure the appearance of probe substrates in different compartments (blood, hepatocyte, and bile) and, if imaging is repeated with high temporal resolution (the so-called dynamic imaging), then transporter kinetics can be inferred. The added value of spatially resolving the liver signal (as opposed to simply monitoring tracer disappearance from blood) was recognized over 60 years ago [10], although it is only recently that the uptake and elimination rates or kinetics have been employed to derive absolute kinetic rate constants. Hepatic uptake of drugs is mediated primarily by solute carriers expressed on the sinusoidal plasma membrane domain of hepatocytes and is an essential first step before DILI can be initiated. Consequently, data provided by imaging technologies which provide quantitative insights into hepatic uptake transporter kinetics has the potential to improve interpretation of the *in vivo* DILI relevance of *in vitro* toxicity assay data, which currently poses a major challenge (e.g., see Chaps. 6, 8, and 17). In addition, inhibition of the activity of the biliary efflux transporter BSEP plays a direct role in the mechanism by which numerous drugs can initiate DILI, while upregulation of the activity of other biliary efflux transporters plays an important hepatoprotective role in response to BSEP inhibition by drugs [11] (see also Chap. 15). Furthermore, a recent genetic analysis undertaken in a Chinese patient cohort has revealed an association between genetic variants in the gene encoding BSEP (*ABCB11*) and cholestatic liver injury caused by treatment for between 6 and 9 months with antituberculous drugs (a combination of isoniazid, rifampicin, pyrazinamide, ethambutol, and/or streptomycin) [12]. Hence imaging methods that enable direct quantitative evaluation of drug-induced hepatic uptake and efflux transporter kinetics *in vivo*, and can be used to investigate perturbation of transporter function following administration of test drugs, have the potential to improve understanding of DILI mechanisms, and of DILI risk.

Several different imaging modalities can be used to assess hepatobiliary transporter kinetics (Table 1) [13]. A suitable imaging modality requires sufficient spatial resolution to resolve compartments, and adequate temporal resolution to enable characterization

Table 1
Imaging modalities used in liver transporter research in rats and humans [13]

	Typical region electromagnetic spectrum ^a	Ionizing radiation	Tissue depth	Typical spatial resolution/mm (human liver; rat liver)
Magnetic resonance imaging (MRI)	63–500 MHz	No	Full	2 × 2 × 4; 0.2 × 0.2 × 2
Fluorescence or optoacoustic	500–1000 nm	No	20 mm	–; 1 × 1
Radiography and X-ray computed tomography (CT)	20–50 keV	Yes	Full	1 × 1 × 1; 0.1 × 0.1 × 0.1
Scintigraphy and single-photon emission computed tomography (SPECT)	141–159 keV	Yes	Full	5 × 5 × 5; 0.5 × 0.5 × 0.5
Positron emission tomography (PET)	511 keV	Yes	Full	2 × 2 × 2

^aIn the electromagnetic spectrum, a wavelength of 1 m corresponds to a frequency of 300 MHz and an energy of 1.24×10^{-6} eV

of kinetics. In addition, its signals must penetrate tissue to the depth of the liver in the species of interest (e.g., rat, human), and the modality must be able to detect exogenous substances which are transporter substrates. Ideally the imaging technology, and the substance detected, must also be widely available and of sufficiently low risk to allow studies to be performed in humans. Exogenous imaging substances used at high doses in imaging studies are called contrast media or contrast agents: these have the potential to saturate transporters. Conversely, exogenous imaging substances used in microdoses are called tracers. Many contrast agents and tracers will be discussed below, with priority given to those approved for human use as such approval demonstrates successful preclinical to clinical translatability for hepatobiliary function assessment.

Each modality exploits the different chemistry of the probe substances it detects [14]. Nuclear medicine modalities, i.e., positron emission tomography (PET) and single-photon emission computed tomography (SPECT)/scintigraphy, detect trace (subnanomolar) amounts of a substance radiolabeled with an isotope possessing a particular emission characteristic. SPECT studies detect gamma-emitting isotopes such as technetium-99m ($t_{1/2} = 6$ h) or iodine-123 ($t_{1/2} = 13$ h). PET studies detect positron-emitting isotopes such as carbon-11 ($t_{1/2} = 20$ min) or fluorine-18 ($t_{1/2} = 110$ min). CT and X-radiography can detect high micromolar or millimolar amounts of a heavy atom such as iodine in organoiodine contrast agents. MR can detect high micromolar or millimolar amounts of substances which accelerate the nuclear

magnetic relaxation of water protons, such as gadolinium-chelate contrast agents. Fluorescence or optoacoustic imaging detects chromophores that emit visible or near-infrared light when excited by light of a specific wavelength. A final imaging modality, ultrasound, will not be discussed further as, although it is used clinically to evaluate hepatobiliary structures, there are no known chemistries that could generate hepatobiliary tracers suitable for ultrasound studies.

In principle, any adequately nontoxic substance with significant clearance through the liver can be used to measure liver transporter kinetics by imaging, provided that the substance is detectable using one of the imaging modalities in Table 1 and the mechanism of clearance is well understood. Since the 1920s, many tracers and contrast agents (Table 2, Fig. 1) have been developed specifically for medical imaging of the hepatobiliary system [15]. This allowed different medical imaging procedures to be devised, including radiographic visualization of the bile duct (cholangiography) and gall bladder (cholecystography), or visualization of the bile duct by SPECT and scintigraphy (cholescintigraphy) [16]. More recently, contrast agents have been developed for liver imaging via MRI, of which gadoxetate is most notable. Such agents are taken up by normal hepatocytes (but not neoplasms), and appear in the biliary tree. Quantitation of their uptake and biliary excretion provides an assessment of liver function [17, 18], and (through imaging) a functional liver volume. These tracers and contrast agents were optimized iteratively by medicinal chemists. For cholangiography, cholecystography, and cholescintigraphy, the ideal molecule reaches the biliary tree as a bolus requiring both very rapid uptake into the hepatocyte and very rapid elimination from the hepatocyte into the bile. However, for more modern applications such as detecting neoplasms and measuring functional liver volume) the ideal molecule is taken up rapidly into the hepatocyte but is eliminated rather slowly, to allow flexibility in the timing of imaging.

Few of the reported tracers and contrast agents are currently marketed. Others are or were investigational, or were formerly marketed then withdrawn (Table 2). From the perspective of an investigator planning a clinical imaging study on transporter function, a marketed agent is much more appealing than an investigational or withdrawn agent. The former (such as gadoxetate or mebrotfenin) can be sourced readily from the pharmacy: ethical review would note off-label use of an approved medicinal product (with due consideration of radiation dose and other potential harms). On the other hand, use of a nonapproved agent (such as gadocoletic acid or arclofenin) would introduce many complications, requiring an IND (investigational new drug) application and establishing production according to Good Manufacturing Practice. It is for this reason that gadoxetate is of particular interest.

Table 2
Imaging tracers and contrast agents which are liver transporter substrates

Modality: Chemical class	Currently used in man	Investigational or formerly used
MRI: Gadolinium chelate	Gadoxetate (Primovist, Eovist) Gadobenate (Multihance)	Gadocoletate
Fluorescence	Indocyanine green Fluorescein	Bromosulfophthalein; tauro-nor-THCA-24-DBD; 5-chloromethylfluorescein diacetate; chloromethylfluorescein; dichlorofluorescein
CT: Triiodophenyl		Bunamiodyl; iobenzamic acid; iocetamic acid; iopanoic acid; ipodic acid; iophenoxic acid; iopronic acid; tyropanote; iosumetic acid; phenobutiodil; RCK-136
CT: Bistriiodophenyl	Iodipamide (Cholografin) Iotroxinate (Biliscopin)	Iodoxamate; ioglycamic acid; iosefamate; iosulamide
SPECT: ^{99m} Tc iminodiacetic acid conjugates	[^{99m} Tc]disofenin (Hepatolite) [^{99m} Tc]mebrofenin (Choletec)	[^{99m} Tc]arclofenin; [^{99m} Tc]bultifenin; [^{99m} Tc]etifenin; [^{99m} Tc]galtifenin; [^{99m} Tc]iprofenin; [^{99m} Tc]lidofenin
SPECT: Radioiodophenyl		[¹³¹ I]iodipamide; [¹²³ I]iodoxamate; [¹³¹ I]ipodate; [¹³¹ I]ioglycamate; [¹³¹ I]rose bengal
SPECT: ^{99m} Tc pyridoxal derivative	[^{99m} Tc]-N-pyridoxyl-5-methyltryptophan	
PET: [¹¹ C]-labeled therapeutic drug or metabolite		[¹¹ C]dehydropravastatin; [¹¹ C]erlotinib; [¹¹ C]metformin; [¹¹ C]rosuvastatin; [¹¹ C]SC-62807; [¹¹ C]telmisartan; [¹¹ C](15R)-16-m-tolyl-17,18,19,20-tetranorisocarbacyclin methyl ester
PET: [¹¹ C]-labeled bile acid derivative		N-[Methyl- ¹¹ C]cholylsarcosine; N-[methyl- ¹¹ C]taurocholic acid; N-[methyl- ¹¹ C]tauroolithocholic acid; N-[methyl- ¹¹ C]tauroursodeoxycholic acid

References are cited in Sect. 2

2.1 Radiography and CT

Radiography and CT have been used clinically to evaluate the hepatobiliary system, employing contrast agents that assess hepatocyte function. The contrast agents for radiography and CT incorporate one or two triiodophenyl moieties, providing respectively three or six heavy atoms (i.e., nonradioactive ¹²⁷I) per molecule. Many such agents have been developed and marketed (see Table 2) in multiple jurisdictions, for cholangiography and cholecystography. These include iodipamide [19–21] and iotroxate [22]. These agents are cleared via the biliary system and, although there were early studies of transport of ipodate, iodipamide [23], and bunamiodyl [24],

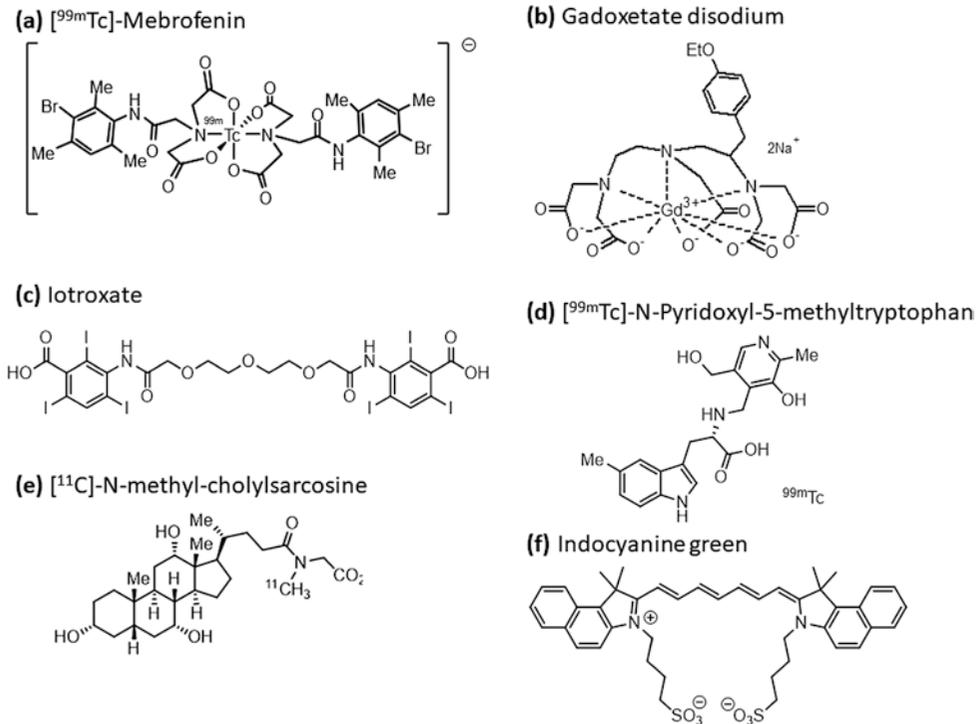


Fig. 1 Tracers and contrast agents used for liver transporter assessment in vivo. (a) $[^{99m}\text{Tc}]$ -Mebrofenin (scintigraphy/SPECT). (b) Gadoxetate disodium (MRI). (c) Iotroxate (radiography/CT). (d) $[^{99m}\text{Tc}]$ -N-Pyridoxyl-5-methyltryptophan (scintigraphy/SPECT). (e) $[^{11}\text{C}]$ -N-Methyl-cholylsarcosine (PET). (f) Indocyanine green (optical)

the clearance of biliary CT agents remains poorly understood [19] and does not yet provide specific information on liver transporters; rather, these tracers are used primarily for gross assessment of the biliary tract. Most have now been withdrawn from the market because of nephrotoxicity or lack of demand. However, iodipamide [25] and iotroxate [26] are still marketed in some jurisdictions.

2.2 MRI

Several gadolinium chelates have been rationally designed for liver MRI. These agents are detected in MRI because they are effective at enhancing the nuclear magnetic relaxation of water protons, i.e., they have high relaxivity.

Gadoxetate [27] and gadobenate [28], which undergo partial hepatocyte-mediated elimination, are approved for use in multiple jurisdictions. Gadoxetate is FDA-approved for detection and characterization of focal liver lesions and exhibits high biliary clearance. Gadoxetate also has affinity for various liver transporters in multiple species (human OATP1B1, OATP1B3, MRP2, and NTCP; rat OATP1a1, Mrp2, Mrp3, and Oatp1A2) [29–32], and has been used preclinically and clinically to investigate liver transporter dysfunction or inhibition that may decrease hepatobiliary function or

cause cholestatic injury [32, 33]. This area of research is developing rapidly and is discussed further in Sect. 4.

Mangafodipir, a manganese chelate, was previously used for detection of liver lesions: it releases Mn^{2+} which is taken up by functioning hepatocytes [34, 35] through calcium channels, although the exact transporters and channels are not known. It is no longer marketed.

2.3 Scintigraphy and SPECT

Fortuitously, iodine has several radioactive isotopes suitable for scintigraphy/SPECT (iodine-123, and formerly iodine-131 which is no longer used to high β radiation) or PET (iodine-124). Several of the triiodophenyl-based radiographic contrast media have also been synthesized with radioiodine for nuclear medicine: indeed, the first imaging agent used for hepatobiliary function was [^{131}I]rose bengal [10]. Use of SPECT or PET rather than CT is advantageous, as the high doses of contrast agent required for CT/radiography may be nephrotoxic, while PET and SPECT tracers are used at much lower doses and hence incur no risk beyond the ionizing radiation. Subsequently, ^{99m}Tc -chelate-based cholescintigraphy tracers were rationally designed [36]. The most important series incorporates an iminodiacetic acid ^{99m}Tc -chelate. Of these, [^{99m}Tc]mebrofenin [37] and disofenin [38] are marketed in some jurisdictions. An alternative technetium chelate chemistry uses pyridoxal derivatives [39]: [^{99m}Tc]N-pyridoxyl-5-methyltryptophan ([^{99m}Tc]PMT) [40, 41] is used clinically in Japan. Human hepatic uptake of [^{99m}Tc]PMT is mediated by OATP1B1 and OATP1B3, while its efflux into bile is via MDR1 and MRP2 [42]. [^{99m}Tc]mebrofenin is used in assessment of liver function and functional liver volume before and after surgery and has also been used to investigate hepatobiliary transporter dysfunction in vitro and in vivo [43, 44]. [^{99m}Tc]mebrofenin is almost exclusively taken up into the liver by OATP1B1 and OATP1B3 and is excreted into the bile primarily by MRP2 [44–46].

[^{99m}Tc]sestamibi is marketed in several jurisdictions for assessment of myocardial function, and also has been used in vitro to study basolateral efflux [46]. [^{99m}Tc]sestamibi likely enters hepatocytes passively and undergoes partial fecal clearance, as efflux is modulated by hepatocyte P-gp and the tracer undergoes preferential basolateral efflux into the bile. [^{99m}Tc]galactosyl-human serum albumin is also used in Japan [17, 47] to assess liver function, but is not a known transporter substrate.

Gadolinium has a gamma-emitting isotope (gadolinium-153), so gadolinium-chelates can be detected using scintigraphy/SPECT, although the rather long half-life of this isotope ($t_{1/2} = 270$ d) restricts its use to animal studies [48].

2.4 PET

More recently, specific transporter-targeted positron-emitting tracers have been synthesized and used in animals or man, with particular emphasis on hepatobiliary transporters, although none

has yet gained regulatory approval for use as a diagnostic product. Several of these (Table 2) are carbon-11 versions of small molecule drugs or drug metabolites which are known liver transporter substrates (e.g., erlotinib [49]; metformin [50], rosuvastatin [51], dehydropravastatin [52], telmisartan [53], and celecoxib metabolite [54]). ^{11}C -labeled bile acid derivatives have also been synthesised, such as [^{11}C]cholylsarcosine [55–57]; these have been used to investigate the kinetics of hepatobiliary tracer uptake and secretion in healthy pigs and humans in vivo, and to quantify perturbations that occur in patients with cholestasis.

2.5 Fluorescence

Fluorescent tracers offer the ability to image hepatobiliary processes at a cellular resolution and in real time, thereby granting opportunities to gain insights into detailed mechanistic perturbations of drugs on a high throughput level. Therefore they are well suited to in vitro studies of isolated cells or cell aggregates that can be imaged directly, although in general they are not appropriate for in vivo studies of cells within the liver or other internal organs. Methodologically, direct immunofluorescent antibody-based imaging of multiple transporters can answer whether the total amount of protein or localization (e.g., downregulation or receptor internalization) has occurred (e.g., [58, 59]), whereas studies undertaken with fluorescein analogues and fluorescently tagged bile acid derivative probes enable kinetic measures of uptake and efflux transport rates.

Each fluorescent probe has its own characteristic transporter substrate affinity, which may vary depending on the species and complexity of the transport system that is investigated. Cholyllysyl-fluorescein (CLF), a bile acid analogue, is a particularly widely used probe, with data that have spanned in vitro and in vivo studies involving both animals and humans. CLF is an OATP1B3, MRP2, and MRP3 substrate [60]. Measurement of drug inhibition of CLF transport has been used to discern cholestatic mechanisms [61, 62], and a positive association has been demonstrated between cholestatic DILI in humans and inhibition of apical CLF efflux from rat hepatocytes in sandwich culture [63]. Moreover, CLF has been shown to have 100% sensitivity when used to detect liver cirrhosis in patient cohorts [64, 65].

Additional fluorescent substrates have also been developed for examining specific uptake and efflux transporter routes. Sodium fluorescein is an OATP1B1, OATP1B3, and MRP2 substrate and has been used effectively in vivo to study impaired hepatic transport in animals [66]. Similarly, a fluorescent bile acid derivative (*N*-(24-[7-(4-*N,N*-dimethylaminosulfonyl-2,1,3-benzoxadiazole)]amino-3 α ,7 α ,12 α -trihydroxy-27-nor-5 β -cholestan-26-oyl)-2'-aminoethanesulfonate) may be used to investigate NTCP-mediated uptake in both primary rat and human hepatocyte suspensions, and apical efflux from hepatocytes cultures in sandwich configuration

which is presumed to be mediated by BSEP [67]. 5-Chloromethyl-fluorescein diacetate, a fluorescein analog, is an example of a reagent that can freely diffuse into hepatocytes and is then metabolized to glutathione methylfluorescein, a cell-impermeant fluorescent product and Mrp2 substrate [68]. Finally, 5-(and 6)-carboxy-2',7'-dichlorofluorescein has been used to quantify Mrp2 efflux from primary rat hepatocytes cultured in sandwich configuration, and inhibition of this process by drugs and their metabolites [69]. Interestingly, such reagents can also be used alone or in combination with bright field time course imaging to study the dilation and constriction dynamics of the bile canaliculi, which have recently been shown to be altered by cholestatic drugs [70].

Indocyanine Green (ICG) is transported by OATP1B3 and NTCP [45], and is established for estimating global liver function. Feng et al. [71] demonstrated improved accuracy of predicting 3-month mortality in acute liver failure patients, using a combination of ICG clearance measured with a pulse spectrometer and the model-for-end-stage liver disease (MELD) score, when compared to conventional scores. Subsequently, this result was extrapolated further to develop a human ex vivo model for acetaminophen-induced liver injury, in which ICG clearance was used as the outcome measure [72]. While probes that are more specific for liver transporter dysfunction have been reported, ICG is still used relatively routinely and has been approved for in vivo measurement of human hepatic function [73].

3 Quantitative Analyses of Hepatic Imaging Data

3.1 *Absolute Quantification of Hepatocellular Transporter Expression with Dynamic Imaging*

Imaging approaches to quantifying transporter expression follow the principles of standard pharmacokinetic measurements [74]. A suitable probe indicator (Sect. 2) is injected into the bloodstream and its concentration “ c ” in a tissue of interest is measured as a function of time “ t ” (min). The temporal structure of these concentration-time profiles is then interpreted using kinetic models of the motion of indicator molecules through tissue compartments (blood, interstitium, cells, bile, etc.).

Two different types of parameter can be derived from such dynamic imaging experiments [75, 76]. The distribution volumes “ v ” (ml/g) measure the space (ml) occupied by the indicator inside the compartments in a unit (g) of tissue (examples are plasma, interstitial, and intracellular distribution volumes). The transfer constants “ k ” (ml/min/g) measure the indicator flux (mmol/min/g) out of a compartment per unit concentration “ c ” (mM). For instance, if c_i is the indicator concentration in the interstitium, then the indicator flux from interstitium to intracellular space is $k_{hi}c_i$. Physiologically, k_{hi} is the volume of interstitial fluid (ml) in a unit of tissue (g) that is cleared of indicator in a minute. At the low

concentrations that are used in imaging experiments it is assumed that the transfer constants are not concentration-dependent (linearity). Other parameters can be derived from the transfer constants, such as the hepatic extraction fraction (%), i.e., the percentage of indicator molecules that is extracted from the blood stream in one pass through the liver [77].

An accurate measurement requires at least two different concentration-time curves. One is the tissue concentration $c(t)$, which may be measured over the entire liver, an individual lobe, a liver segment, a smaller region within a segment or even a single imaging voxel (=3D pixel). The second curve, the input function, is measured in the arterial or portal venous inlet to the liver. Input functions are required as a reference to eliminate effects of systemic changes in the circulation, or differences in the way the tracer is injected. In measurements that also target perfusion, arterial and portal venous input functions are both required to separate out their individual contributions. In some cases outlet data are needed—in particular for indicators that are excreted from hepatocytes through biliary and interstitial routes.

The technical details of the image acquisition have a strong effect on parameter accuracy, but choosing the right approach involves trade-offs between accuracy, precision, practicality and cost. For instance, at higher sampling rates more rapid processes can be resolved, but this comes at a cost of image resolution and organ coverage. Equivalently, very long data collections (>45 min) are required to characterize slow processes such as biliary excretion, but this has significant implications on patient comfort and scan costs. Another important consideration in the liver is to minimize the effect of breathing motion, which is detrimental to image quality [78]. The best compromise depends critically on the exact purpose of the measurement and requires careful application-specific optimization.

3.2 Semiquantitative Imaging Analysis

Clinical evaluation of gadoxetate-enhanced MRI is based on evaluation of imaging features such as observation size, presence of arterial phase hyper- versus hypo- or iso-enhancement, washout appearance, capsule appearance, and threshold growth [79]. Quantitation of the disposition of the contrast agent can be undertaken by calculation of maximum relative enhancement (RE) when compared with pre-contrast images [29] and measurement of area under the curve (AUC) of the liver enhancement. In addition, curve fitting of the intensity profile in the liver after a bolus injection of contrast agent enables estimation of kinetic parameters which include rate of hepatic wash-in and wash-out, and hepatic extraction fraction [80]. These descriptive analysis techniques can exhibit good signal-to-noise ratio and low variance at individual sites in comparison to the compartmental modeling techniques described in the previous section. However, the results often dis-

play a greater dependence on experimental parameters, making comparisons between sites difficult and they lack direct relationship to transporter function that can provide additional and useful insights into pharmacologically and toxicologically relevant effects which otherwise may be difficult to obtain. For example, inhibition of hepatobiliary efflux transporters at the apical plasma membrane domain of hepatocytes membrane may lead to changes in drug exposure in the hepatocyte that have potential toxicological significance, but result only in minor changes in drug plasma exposure [81] (see also next section).

4 Hepatobiliary Transporter Mediated Drug–Drug Interactions

Hepatocytes express a range of transporter proteins mediating either active uptake of drugs/endogenous compounds from the blood (e.g., OATP1B1, which is expressed on the basolateral plasma membrane domain) or their active secretion into the bile (e.g., BCRP, expressed on the apical plasma membrane domain) [82, 83]. Characterization of drug transporters in the liver (also in the intestine, kidney and brain) and their effect on drug pharmacokinetics and drug–drug interaction (DDI) risk is now an integral part of drug development, and is required by regulatory agencies [84–86]. P-gp (MDR1/ABCB1), BCRP, OATP1B1, OATP1B3, OAT1, OAT3, and OCT2 are currently identified as key transporters for screening in drug development, with increased recognition of the clinical relevance of other transporters (e.g., MATE, BSEP, and OATP2B1) [87, 88]. Increasingly, clinical evidence raises concerns about transporter-mediated DDIs, where changes in drug exposure in blood or plasma (commonly used as metric) may not be reflective of the changes in the tissue/cellular drug exposure (local DDI) that may have consequences on drug safety and efficacy. For example, modulation of OCT/MATE transporters has resulted in minimal or no changes in metformin systemic exposure in a number of cases, yet modified glucose lowering effect was reported (which was attributed to modified liver exposure to metformin) [88].

In addition to the important role of transporters, hepatic drug exposure may be influenced by passive diffusion through biological membranes, intracellular binding, metabolism and organelle sequestration (Fig. 2) [82]. In recent years, a range of cellular systems have been used to characterize complex interplay of these processes *in vitro*. These range from transporter-transfected cell lines to three-dimensional microphysiological systems; although the utility of the latter as a tool for quantitative *in vitro* metabolism/transporter evaluation and *in vitro/in vivo* translation is yet to be established [89–91]. Characterization of transporter–metabolism interplay in these holistic *in vitro* systems is supported by mechanistic modeling of *in vitro* data, which allows estimation of

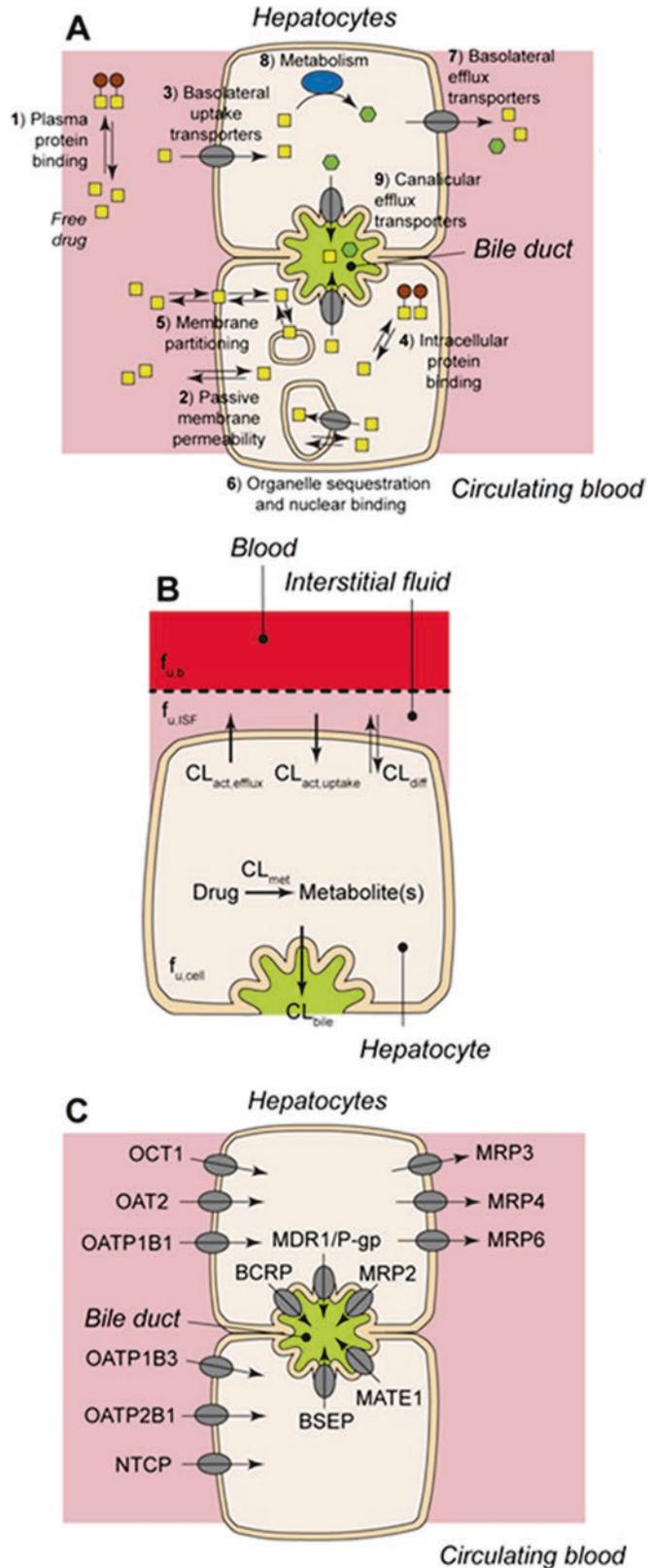


Fig. 2 Processes affecting intracellular drug concentration in the hepatocyte [82]

transporter kinetic parameters (e.g., K_m , V_{max} , and k_i) to be used subsequently for translational purposes in physiologically based pharmacokinetic (PBPK) models [92–94].

One of the key advantages of PBPK modeling is the ability to simulate and interpret concentration–time profiles in the tissues of interest (in addition to plasma). This modeling approach is extremely useful to improve our understanding of the rate-determining process driving hepatic exposure of a drug, i.e., whether this is uptake, efflux/metabolism or a composite of multiple processes. It also provides mechanistic insight into in vivo consequences that arise when individual disposition processes are perturbed [81, 82, 94–97]. For example, active uptake via OATPs from the blood into hepatocytes is the major process leading to high unbound liver–blood concentration ratio of many statins (e.g., simvastatin acid). In such cases, reduced activity in OATP1B1 transporter (due to either transporter inhibition or polymorphism) results in increased systemic exposure of simvastatin acid and increased risk of myopathy [95] (see Fig. 3). For drugs predominantly eliminated via liver, the effect of reduced OATP activity on liver exposure (AUC_{liver}) is expected to be marginal, as this parameter is determined primarily by either metabolic clearance (in case of simvastatin acid) or biliary excretion (BCRP-rosuvastatin, MRP2-pravastatin) [81, 82, 95–98]. In contrast, inhibition of biliary transporters (MRP2, BSEP) or metabolic enzymes (CYP3A4) may lead to changes in drug exposure in the liver and consequently even to hepatotoxicity, with only minor changes in drug plasma exposure. Verification of these PBPK model-predicted changes in tissue exposure is challenging. In the case of statins, clinical data provide indirect evidence to support this, as enhanced cholesterol reduction (associated with higher liver exposure of simvastatin) was reported in DDI studies with CYP3A4 inhibitors [95]. For certain drugs (e.g., repaglinide) delineation of the rate limiting step is

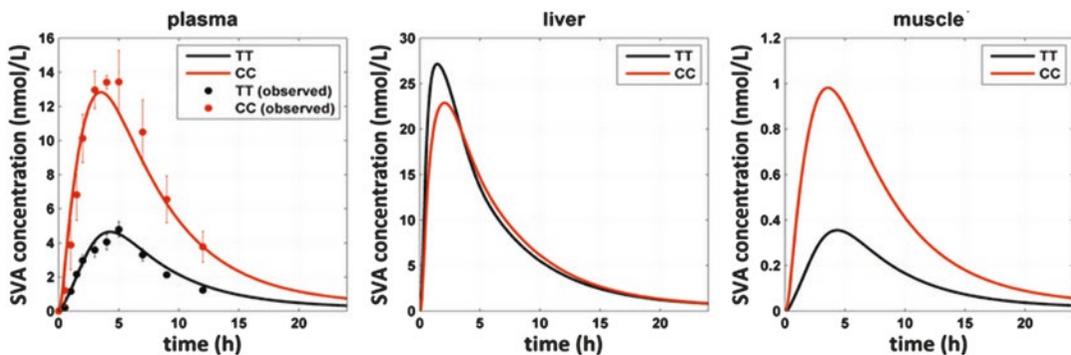


Fig. 3 Simulated concentration–time profiles for simvastatin acid (SVA) in plasma, liver tissue and muscle tissue for individuals with the homozygous wild-type TT (black line) and homozygous variant CC (red line) SLC01B1 c.521T>C. Full symbols represent observed mean \pm SE plasma SVA concentrations for individuals with the TT (black circles) and CC (red circles) genotype [95]

not as straightforward as in the case of simvastatin acid, as hepatic disposition is a composite of multiple contributing processes. Drugs like repaglinide highlight the utility of PBPK modeling to gain mechanistic insight into interplay of processes and prediction of DDI risk [97].

In all the cases above, quantification of drug exposure in the liver (and other tissues) is crucial to support modeling and simulation efforts. Emerging data on tissue concentrations obtained by advanced imaging methods [33, 99], in conjunction with mechanistic PBPK modeling, is envisaged as a powerful tool to improve predictability and understanding of implications of transporter-mediated tissue distribution and interactions.

5 Liver Imaging with Gadoxetate

5.1 Current Clinical Use of Gadoxetate in Liver Disease Diagnosis

The disodium salt of gadoxetic acid (gadoxetate, gadolinium-ethoxybenzyl-diethylenetriaminepentaacetic acid, SH L569 B, Gd-EOB-DTPA) is marketed by Bayer as Primovist® in Europe and Japan and as Eovist® in the USA and has been clinically approved as a liver-specific contrast agent for detection of focal liver lesions by magnetic resonance imaging. Gadoxetate exhibits a favorable safety profile [29, 100–104]. It has been demonstrated that gadoxetate does not trigger nephrogenic systemic fibrosis (NSF), even in the presence of moderate to severe renal impairment [105, 106], as a result of its dual excretion pathway. Recently, trace amounts of gadolinium have been detected in the brain of patients after several injections of the linear gadolinium-containing contrast agents gadodiamide, gadopentetate dimeglumine, gadobenate dimeglumine, and gadoversetamide. For gadoxetate, one report has indicated a correlation between the number of prior gadoxetate administrations and increase signal on nonenhanced T1-weighted images in the dentate nucleus [107], while a second report did not find such a correlation [108]. Currently it is not known whether persistence of gadolinium in the brain following therapeutic administration of contrast agents is associated with adverse health consequences.

Gadoxetate combines the well-established imaging characteristics of extracellular nonspecific gadolinium contrast agents during dynamic phases with further imaging information during the hepatobiliary disposition phase. This enables enhanced detection, classification and characterization of focal liver lesions, as well as improved assessment of liver function in diffuse liver diseases. Gadoxetate-enhanced liver MRI is most commonly used for clinical diagnosis of hepatocellular carcinoma, classification of focal liver lesions and cirrhotic nodules [109], but also is used in a large number of further conditions [110]. The possibility to distinguish between benign and malignant liver tumors, due to differences in accumulation of gadoxetate in hepatocytes, allows imaging based

formulation of the therapeutic strategy [110, 111]. For instance, hemangiomas and focal nodular hyperplasia do not require any therapeutic intervention, whereas adenomas possess a risk for malignant transformation, and therefore require treatment [112]. Dynamic hepatocyte contrast enhanced MRI is a valuable tool for the assessment of liver volume and liver functional capacity in patients with primary sclerosing cholangitis [113]. Gadoxetate uptake and enhancement in patients with diffuse chronic liver disease is generally lower than in healthy individuals, due to differences in transporter number or activity. Therefore it allows differentiation between two subgroups of nonalcoholic fatty liver disease, plus between simple steatosis and nonalcoholic steatohepatitis [114]; and also can be used for assessment and staging of fibrosis [76] and cirrhosis [115], as well as in predicting liver transplant graft survival [116]. In addition, gadoxetate enhanced MR imaging together with T2-weighted MR cholangiography may be a useful tool in providing information about the biliary system, like biliary injury, bile duct obstruction, diagnosis of cholecystitis, and differentiation of biliary from extrabiliary lesions [117, 118].

Hepatocellular uptake of gadoxetate from blood is mediated in humans by the sinusoidal solute carriers OATP1B1, OATP1B3, and NTCP [119]. Its active secretion into bile in rats is via the apical transporter Mrp2 [120] and it is presumed that the human ortholog (MRP2) mediates its biliary excretion in humans. The extensive biliary clearance of gadoxetate (healthy human: 50% of administered dose [101]) facilitates its use for evaluation of hepatobiliary transporter inhibition by drugs as a DILI risk factor [32, 33]. This aspect is discussed further in the next section. In addition, gadoxetate-enhanced MRI was successful in prediction of hyperbilirubinemia which occurred during treatment of hepatitis C patients with a triple therapy of simeprevir, pegylated interferon plus ribavirin [121]. Simeprevir is a substrate of the same transporters for hepatic uptake and excretion as gadoxetate [121]. In rats, a transient impairment of bile flow induced with a single dose of estradiol-17 β D-glucuronide was associated with a sixfold decrease in gadoxetate elimination rate [122]. Prednisolone, doxorubicin hydrochloride, cisplatin, and propranolol hydrochloride can lead to a slight but significant increase in the hepatic MRI enhancement observed following administration of gadoxetate to rats, most likely due to the longer retention of the contrast agent in hepatocytes because of its competition with these drugs for biliary excretion into the bile duct [123].

5.2 Effects on Gadoxetate Hepatic Clearance of an Investigational Drug Which Cause DILI in Rats

The investigational drug used in these studies [33] is a chemokine receptor antagonist (CKA) whose intended clinical use was in the treatment of systemic inflammatory diseases. Livers from rats dosed orally with the compound for 7 days exhibited dose dependent centrilobular degeneration and necrosis, which was accompanied by neutrophil infiltration and associate sinusoidal congestion. These

abnormalities in liver histopathology were accompanied by marked elevations in plasma levels of alanine aminotransferase activity, bilirubin and bile acids, which also exhibited clear dose dependency. In vitro studies revealed that the CKA was a potent inhibitor of human OATP1B1 ($IC_{50} < 3 \mu M$), plus a less potent inhibitor of both rat Mrp2 ($IC_{50} 69 \mu M$) and rat BSEP ($IC_{50} 130 \mu M$).

To investigate whether these in vitro findings might have in vivo functional relevance, anaesthetized rats were given either a single dose of gadoxetate alone, or CKA plus gadoxetate, then were evaluated by DCE-MRI [33]. Imaging data are shown in Fig. 4. The data were quantified following development of a non-linear two-compartment model. This provided a good description of gadoxetate disposition in animals dosed with the contrast agent alone, and yielded a rate constant for its hepatic uptake and Michaelis–Menten constants (K_m and V_{max}) for biliary secretion. Coadministration of the CKA with gadoxetate resulted in marked inhibition of the rate of hepatic uptake of gadoxetate, plus resulted in a reduced V_{max} and increased K_m for biliary gadoxetate excretion. These effects were dose dependent and correlated well with the abnormalities in plasma bilirubin and bile acids observed in rats dosed for multiple days with the CKA.

These findings suggest that gadoxetate DCE-MRI can characterize functional consequences in vivo of compounds that perturb hepatobiliary transporters. Furthermore, since gadoxetate is

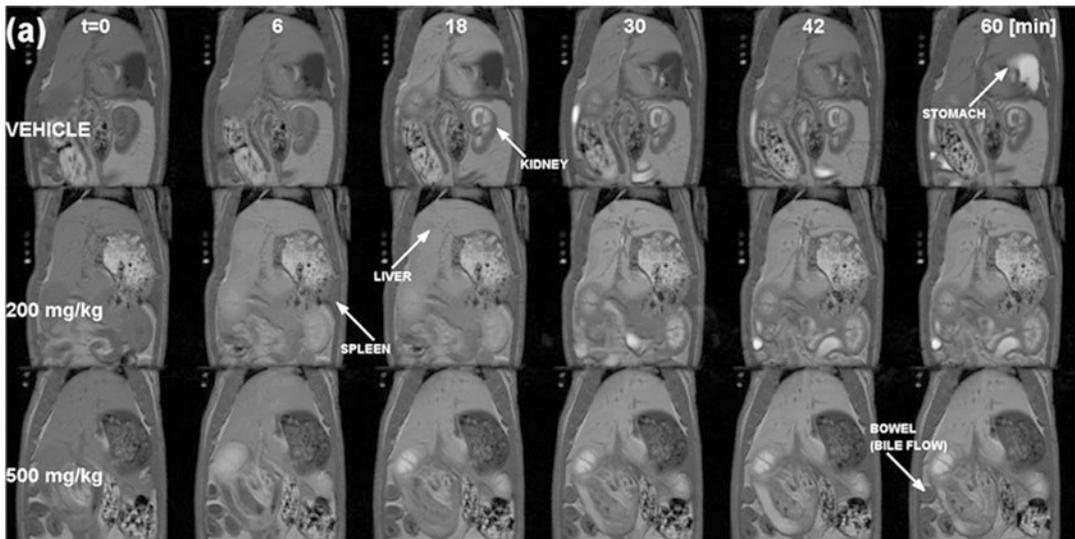


Fig. 4 Examples of dynamic images for animals treated with vehicle (top), 200 mg/kg (middle) or 500 mg/kg (bottom) CKA at $t = 0, 6, 18, 30, 42,$ and 60 min after contrast injection. Note the enhancement of the small bowel lumen at about 30 min after contrast injection and also the reflux of gadoxetate into the stomach at the end of the acquisition in the vehicle treated animal. No enhancement was observed in the bowel of the animal treated with 500 mg/kg CKA. From [33]. Reproduced by permission of John Wiley & Sons

already used clinically to aid the assessment and management of liver disease in patients, this may be a translatable biomarker that can aid human risk assessment of new investigational drugs during clinical trials [33].

6 Future Opportunities and Challenges

Many different imaging methodologies and probe substrates have been used to explore processes in the liver that may be relevant to DILI. These methods could provide important additional tools to detect and investigate DILI, and to gain new insight into underlying mechanisms and susceptibility factors.

An especially promising method is gadoxetate DCE-MRI. This is due to the current widespread use of MRI in human liver disease diagnosis and management, plus the very promising data already obtained in rats dosed with an investigational drug that inhibited several hepatobiliary transporters and caused liver injury [33] (see Sect. 5). Nonetheless, substantial further work is required to develop and validate the use of gadoxetate DCE-MRI for DILI risk assessment in animals, and also as a translational biomarker technology that can be used during clinical trials. It is intended that the necessary additional work will be undertaken as part of the TRISTAN project, which is a large collaborative public-private partnership that is cofunded jointly by the European Union and industry via the Innovative Medicines Initiative [124]. The TRISTAN project will also investigate the potential value of gadoxetate DCE-MRI for the assessment of undesired and clinically important drug–drug interactions that occur via hepatobiliary transporter perturbations (see Sect. 4).

A limitation of gadoxetate DCE-MRI is that, since gadoxetate is not transported by BSEP, it does not enable direct investigation of drug-induced inhibition of BSEP activity, which is considered to play an important role in DILI caused by many drugs [11]. Other probe substrates that are transported by BSEP, and ideally are BSEP-specific, are needed. The chemistries described in Sect. 2 might provide compounds suitable for this purpose. Imaging studies undertaken using a BSEP probe substrate could improve our understanding the *in vivo* significance of *in vitro* BSEP inhibition data, and in particular the relationship between BSEP inhibition and DILI.

The present chapter covers hepatobiliary transporter interactions, and their role in DILI and drug–drug interactions. Imaging probes and technologies that evaluate other processes relevant to DILI would be complementary. Processes meriting particular attention include oxidative stress and inflammation, in view of their known role in DILI pathogenesis [1, 9].

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