Evidence of Drug-Drug Interactions through Uptake and Efflux Transport Systems in Rat Hepatocytes: Implications for Cellular Concentrations of Competing Drugs

DAALI, Youssef, et al.

Abstract

For drugs with hepatobiliary transport across hepatocytes, the interplay between uptake and efflux transporters determines hepatic concentrations of drugs, but the evolution over time of these concentrations is difficult to measure in humans other than with magnetic resonance imaging contrast agents in the liver. Gadobenate dimeglumine (BOPTA) is a contrast agent used in liver magnetic resonance imaging that enters into human hepatocytes through organic anion transporting polypeptides (OATP) and exits unchanged into bile through the multiple resistance-associated protein 2 (MRP2). Rifampicin (RIF) is transported by the same membrane proteins and may compete with BOPTA for hepatic uptake. Simultaneous drug-drug interactions through uptake and efflux transport systems in hepatocytes according to the cellular concentrations of competing drugs were never investigated. In perfused rat liver preparations, we demonstrate how the drug-drug interactions through transporters determine cellular concentrations of the competing drugs BOPTA and RIF, and we show that the cellular concentrations by modulating transport through membranes [...]
Evidence of Drug-Drug Interactions through Uptake and Efflux Transport Systems in Rat Hepatocytes: Implications for Cellular Concentrations of Competing Drugs

Youssef Daali, Philippe Millet, Pierre Dayer, and Catherine M. Pastor

Service de Pharmacologie et Toxicologie Clinique (Y.D., P.D.), Unité de Neurophysiologie Clinique et Neuroimagerie (P.M.), Laboratoire de Physiopathologie Hépatique et Imagerie Moléculaire, Hôpitaux Universitaires de Genève, Geneva, Switzerland; and INSERM U 773, Département de Radiologie, Hôpital Universitaire de Beaujon, Clichy, France (C.M.P.)

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ABSTRACT

For drugs with hepatobiliary transport across hepatocytes, the interplay between uptake and efflux transporters determines hepatic concentrations of drugs, but the evolution over time of these concentrations is difficult to measure in humans other than with magnetic resonance imaging contrast agents in the liver. Gadobenate dimeglumine (BOPTA) is a contrast agent used in liver magnetic resonance imaging that enters into human hepatocytes through organic anion transporting polypeptides (OATP) and exits unchanged into bile through the multiple resistance-associated protein 2 (MRP2). Rifampicin (RIF) is transported by the same membrane proteins and may compete with BOPTA for hepatic uptake. Simultaneous drug-drug interactions through uptake and efflux transport systems in hepatocytes according to the cellular concentrations of competing drugs were never investigated. In perfused rat liver preparations, we demonstrate how the drug-drug interactions through transporters determine cellular concentrations of the competing drugs BOPTA and RIF, and we show that the cellular concentrations by modulating transport through membranes regulate the rat Oatp-Mrp2 interplay. Moreover, drug interactions through transporters change greatly over time.

Introduction

Transporting membrane proteins in the liver are major determinants of drug disposition, so knowledge about the transport of commercialized compounds and drug candidates is crucial for drug safety and efficacy (Köck and Brouwer, 2012; Yoshida et al., 2013). The transport of drugs across hepatocytes is affected by changes in the expression and function of both sinusoidal and canalicular transporters induced by human diseases and genetic polymorphisms as well as by drug-drug interactions (Giacomini et al., 2010). For example, common variants of the SLCO1B1 gene encoding organic anion-transporting polypeptide B1 (OATP1B1) are strongly associated with an increased risk of statin-induced myopathy; the loss of uptake function through hepatic OATP1B1 impairs the clearance of statins and favors drug accumulation in muscles (Link et al., 2008). Thus, OATP1B1 and OATP1B3 are uptake transporters of considerable importance for drug disposition (Kallibokoski and Niemi, 2009; Ogasawara et al., 2010; Niemi et al., 2011; Shibara, 2011; Nakanishi and Tama, 2012).

Recently, Karlgren et al. (2012) extensively classified numerous drug competitions through these uptake sinusoidal proteins. Most drugs inhibit the transport of a well-defined substrate specific for each protein, but a few drugs promote their transport. Once inside hepatocytes, drugs (and metabolites) are excreted into bile through canalicular transporters. Drug-drug interactions and genetic polymorphisms also exist for canalicular proteins such as multiple resistance-associated protein 2 (MRP2) (Keppler, 2011; Oh et al., 2012; Simon et al., 2012).

For drugs with hepatobiliary transport across hepatocytes, the interplay between uptake and efflux transporters determines hepatic concentrations but the evolution over time of these concentrations is difficult to measure in humans, other than with magnetic resonance imaging (MRI) of contrast agents and tracers in the liver (Van Beers et al., 2012). Gadobenate dimeglumine (BOPTA, MultiHance; Bracco Imaging SpA, Milan, Italy) and gadoxetate dimeglumine (Gd-EOB-DTPA, Primovist; Bayer HealthCare Pharmaceuticals, Berlin, Germany) are two contrast agents used in liver MRI that enter into hepatocytes through OATP1B1 and OATP1B3 and exit unchanged into bile through MRP2. Concerns have emerged that liver imaging might be modified by the presence in sinusoidal blood of organic anions that compete with hepatobiliary contrast agents for entry into hepatocytes. Rifampicin (RIF) is an organic anion that enters into human hepatocytes through OATP1B1 and OATP1B3 and exits into bile through MRP2; consequently, this drug may compete with other organic anions for hepatic uptake (Lau et al., 2007; Zheng et al., 2009; Karlgren et al., 2012). When chronically administrated, RIF is also a potent inducer of metabolic enzymes and transporters.

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ABBREVIATIONS: BOPTA, gadobenate dimeglumine; DTPA, gadopentetate dimeglumine; EOB-DTPA, gadoxetate dimeglumine; Gd, gadolinium; IHUI, initial hepatocellular uptake index; KHB, Krebs-Henseleit-bicarbonate; MRI, magnetic resonance imaging; MRP2, human multiple resistance-associated protein 2; Mrp2, rat multiple resistance-associated protein 2; OATP, human organic anion transporting polypeptide; Oatp, rat organic anion transporting polypeptide; RIF, rifampicin.

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Drug-drug interactions through OATP1B1/OATP1B3 and MRP2 proteins are mainly determined by the vascular disappearance of competing drugs over time (Kalliokoski and Niemi, 2009; Kindla et al., 2009; Fahrmayr et al., 2010). Simultaneous drug-drug interactions through uptake and efflux transport systems in hepatocytes have never been investigated ex vivo. Our study elucidated the acute cellular interactions between BOPTA and RIF in perfused rat liver preparations. Only acute transport interactions of RIF were investigated to avoid the induction of transporters or metabolic enzymes.

Materials and Methods

Animals

Normal Sprague-Dawley male rats (Charles River, Saint-Germain-sur-l’Arbeles, France) and male rats lacking the canalicular transporter Mrp2 (TR– rats; Division of Clinical Pharmacology and Toxicology, University Hospital of Zurich, Zurich, Switzerland) were anesthetized with pentobarbital (50 mg · kg$^{-1}$ i.p.). The protocol was approved by the veterinary office in Geneva and followed the guidelines for the care and use of laboratory animals.

Rat Liver Perfusion

Livers were perfused in situ as previously described elsewhere (Pastor et al., 1996). Briefly, the abdominal cavity was opened, and the portal vein was cannulated and secured. The abdominal vena cava was transected, and the Krebs-Henseleit-bicarbonate (KHB) solution (118 mM NaCl, 1.2 mM MgSO$_4$, 1.2 mM KH$_2$PO$_4$, 4.7 mM KCl, 26 mM NaHCO$_3$, 2.5 mM CaCl$_2$) was immediately pumped into the portal vein. The flow rate was slowly increased over 1 minute up to 30 ml/min. In a second step, the chest was opened, and a second cannula was inserted through the right atrium into the thoracic inferior vena cava and secured. Finally, the ligature around the abdominal inferior vena cava was tightened. The KHB solution was perfused without recirculation to the liver through the portal catheter and was eliminated by the catheter placed in the thoracic inferior vena cava and secured. In each experiment, the common bile duct was cannulated with a PE10 catheter.

The entire perfusion system included a reservoir, a pump, a device regulating the perfusate temperature to 37°C, a bubble trap, a filter, and an oxygenator. The perfusate was equilibrated with a mixture of 95% O$_2$–5% CO$_2$. The livers were perfused with a KHB buffer ± contrast agents and RIF, and the concentrations of drugs entering the liver were steadily maintained.

Quantification of Hepatic Concentrations of Contrast Agents

BOPTA was labeled by adding $^{153}$GdCl$_3$ (1 MBq/ml) to a 0.5 M BOPTA solution, which contained a slight excess of ligand (Planchamp et al., 2005a,b). The contrast agent was diluted in the KHB solution to obtain two concentrations (200 or 1600 µM). To assess the exact intracellular concentrations of BOPTA, Gd-DTPA (labeled gadopentetate dimeglumine; Bayer HealthCare Pharmaceuticals) was previously perfused at the same concentrations (200 and 1600 µM). DTPA has the same extracellular distribution as BOPTA but does not enter into hepatocytes. To quantify hepatic DTPA and BOPTA, a gamma scintillation probe that measures radioactivity every 20 seconds was placed 1 cm above the liver (Fig. 3). To transform radioactivity counts into contrast agent concentrations, we used the exact concentrations related to the last count measured by the probe. Samples were also collected every 5 minutes from the perfusate and bile to measure the concentrations of the labeled drugs.

Experimental Protocols

Concentrations of Contrast Agents in the Extracellular Space and Hepatocytes. To study the hepatic concentrations of the extracellular contrast agent DTPA and of BOPTA, which also enters into hepatocytes, we perfused the livers isolated from normal rats with KHB solution (45 minutes, recovery period). The perfusate samples were injected directly into the high-performance liquid chromatography (HPLC) system; the bile samples were diluted in water (1:5) before the analysis. Separation was performed on a Zorbax SB-aa C18 column (100 mm $\times$ 2.1 mm i.d.; particle size 3.5 µm; Agilent Technologies, Santa Clara, CA) coupled with a guard column with the same stationary phase (20 mm $\times$ 2.1 mm i.d.; particle size 3.5 µm). The mobile phase, consisting of a mixture of ammonium formate 20 mM/formic acid 0.1% (A) and acetonitrile (B) (70/30), was delivered at 0.3 ml/min. Separation was performed under gradient conditions (from 0 to 4 minutes 70% of B; from 4 to 5 minutes 50% of B; and at 5.5 minutes a return to initial conditions). We performed at least three measurements from each experiment.

Statistics

The parameters are mean ± S.D. We used the Mann–Whitney test or Kruskal-Wallis test to compare the means between different experimental groups.

Results

RIF Hepatobiliary Transport: Effect of BOPTA. During the perfusion of RIF (100 µM over 30 minutes from time points 45 to 75 minutes), RIF enters into hepatocytes and the vascular clearance (determined from the difference in concentrations between inflow and outflow perfusates) was maximal at time point 50 minutes (Fig. 1A).
and decreased thereafter until time point 75 minutes. Because 3000 nmol/min were perfused (100 μM with a 30 ml/min perfusate flow rate), the extraction ratio was 67% at time point 50 minutes. During this period, the RIF bile excretion was 2 nmol/min (Fig. 1B). The hepatic concentrations steadily increased until the end of the RIF perfusion (668 ± 93 nmol/g or μM) because the RIF uptake was higher than the cell excretion (Fig. 1C).

During the rinse period, the RIF was excreted from the hepatocytes. Because the extracellular space was completely cleared of RIF within 2 minutes, we could assess the efflux rates from the hepatocytes back to the sinusoids by measuring the RIF concentrations in the outflow perfusate. During this period, the hepatic concentrations gradually decreased until residual concentrations remained (197 ± 64 nmol/g or μM; Fig. 1C). The efflux of RIF from the hepatocytes back to the sinusoids was mainly responsible for this decrease, and the bile excretion rates remained low (Fig. 1B).

RIF perfusion greatly decreased the bile flow; a full recovery occurred when the RIF perfusion was stopped and switched to a KHB solution (Fig. 1D). Thus, the presence of RIF in cells decreased the canalicular transport of intracellular compounds, including its own transport, but both bile flow (Fig. 1D) and RIF bile excretion (Fig. 1B) recovered when the RIF hepatic concentrations decreased.

The coperfusion of 200 μM BOPTA with 100 μM RIF had no effect on the vascular clearance (Fig. 1A), bile excretion rates (Fig. 1B), intracellular concentrations of RIF (Fig. 1C), or efflux back to the sinusoids (Fig. 1B) because RIF blocked BOPTA uptake and little BOPTA was present in the hepatocytes. Mrp2 mediated the canalicular transport of RIF, and the hepatocytes lacking Mrp2 could not excrete it while the hepatic concentrations were higher in these groups of rats (Table 1). However, the perfusate efflux rates were not significantly different in the livers with or without Mrp2.

**BOPTA Hepatobiliary Transport: Effect of RIF.** As observed with RIF, the BOPTA uptake clearances were maximal at the time point 50 minutes and decreased thereafter (Fig. 2A). With the perfusion of a 200 μM concentration (or 6000 nmol/min), the liver cleared 22% of the contrast agent in a single pass at time point 50 minutes. Besides this classic parameter, an uptake index (IHUI, nmol/min/g) is available (Fig. 3). We used a gamma probe to measure the hepatic concentrations every 20 seconds (315 measurements over 105 minutes; Fig. 3A) to yield the uptake index. Moreover, by subtracting the concentrations induced by the perfusion of the extracellular contrast agent DTPA, we can quantify the exact concentrations of BOPTA in hepatocytes (Fig. 3, B and D). All radioactivity of DTPA was cleared from the liver by the KHB solution within 2 minutes (Fig. 3A) to yield the uptake index. Moreover, by subtracting the concentrations induced by the perfusion of the extracellular contrast agent DTPA, we can quantify the exact concentrations of BOPTA in hepatocytes (Fig. 3, B and D). All radioactivity of DTPA was cleared from the liver by the KHB solution within 2 minutes (Fig. 3A). An IHUI (nmol/min/g) was calculated between time points 46 and 48 minutes (46 ± 6 nmol/min/g; Fig. 3D). During this 2-minute interval, a limited amount of BOPTA was excreted into bile, and the slope of the relation between concentrations and time accurately reflects the hepatic uptake rate of the contrast agent.

After the time-point 48 minutes, the hepatic concentrations did not measure the hepatocellular uptake but rather the combination of drug uptake into hepatocytes and exiting from the cells. The concentrations...
Drug-Drug Interactions within Hepatocytes

in hepatocytes constantly increase until the end of BOPTA perfusion, with drug uptake higher than drug elimination (Fig. 2D). During this period, the bile excretion rates (Fig. 2B) and the bile flow (not shown) were high. During the following rinse period, BOPTA exited from the hepatocytes mainly through the canalicular membrane (Fig. 2B), and the efflux rates to the sinusoids were much lower (Fig. 2C).

When RIF was coperfused with BOPTA (200 μM), the IHUI significantly decreased with the increasing concentrations of perfused RIF (Fig. 3D). RIF (10 and 100 μM) nearly blocked BOPTA entry into the hepatocytes (Fig. 2D). With a lower concentration of RIF (1 μM), the cellular concentrations of BOPTA increased, reached a plateau by time point 54 minutes, then slightly decreased until time point 75 minutes (Fig. 2D). This decrease is explained by the excretion rates being higher than the uptake rates. During the rinse period, hepatic concentrations of BOPTA decreased to the residual concentrations (47 ± 7 nmol/g).

Can 1600 μM BOPTA Overcome the Uptake Inhibition Induced by 1 μM RIF? With interactions being potentially concentration-dependent, a concentration of 1600 μM BOPTA was tested. The IHUI was not significantly affected (Fig. 3D). However, when RIF was perfused from 45 to 75 minutes, the IHUI decreased with increasing concentrations of perfused RIF (Fig. 3D). With 100 μM RIF, the cellular concentrations of BOPTA increased, reached a plateau by time point 54 minutes, then slightly decreased until time point 75 minutes (Fig. 2D). This decrease is explained by the excretion rates being higher than the uptake rates. During the rinse period, hepatic concentrations of BOPTA decreased to the residual concentrations (47 ± 7 nmol/g).

### Table 1

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Time</th>
<th>Hepatic Concentrations</th>
<th>Bile Efflux Rates</th>
<th>Perfusate Efflux Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μM RIF in normal livers</td>
<td>90</td>
<td>380 ± 110</td>
<td>3.3 ± 1.3</td>
<td>216 ± 39</td>
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<tr>
<td></td>
<td>105</td>
<td>197 ± 64</td>
<td>2.3 ± 0.8</td>
<td>71 ± 31</td>
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<tr>
<td>100 μM RIF in liver lacking Mrp2</td>
<td>90</td>
<td>538 ± 215</td>
<td>0</td>
<td>269 ± 32</td>
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<tr>
<td></td>
<td>105</td>
<td>270 ± 100</td>
<td>0</td>
<td>85 ± 7</td>
</tr>
<tr>
<td>100 μM RIF and 200 μM BOPTA</td>
<td>90</td>
<td>348 ± 194</td>
<td>3.1 ± 0.7</td>
<td>244 ± 61</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>151 ± 44</td>
<td>2.3 ± 0.7</td>
<td>53 ± 34</td>
</tr>
<tr>
<td>10 μM RIF and 200 μM BOPTA</td>
<td>90</td>
<td>103 ± 40</td>
<td>0.9 ± 0.2</td>
<td>58 ± 9</td>
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<tr>
<td></td>
<td>105</td>
<td>71 ± 19</td>
<td>0.5 ± 0.1</td>
<td>26 ± 9</td>
</tr>
<tr>
<td>1 μM RIF and 200 μM BOPTA</td>
<td>90</td>
<td>46 ± 24</td>
<td>0.2 ± 0.1</td>
<td>15 ± 11</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>22 ± 23</td>
<td>0.1 ± 0.1</td>
<td>7 ± 6</td>
</tr>
</tbody>
</table>

*P = 0.05 vs. 100 μM RIF in normal livers (with Mrp2).

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![Fig. 2](image_url)

**A** Vascular clearances [nmol/min]  
**B** BOPTA bile excretion rates [nmol/min]  
**C** BOPTA efflux back to sinusoids [nmol/min]  
**D** BOPTA Concentrations in hepatocytes [nmol/g]

Fig. 2. BOPTA transport through hepatocytes. (A) Vascular clearances (nmol/min) are quantified from the difference of concentrations between inflow and outflow perfusates (nmol/ml) times perfusate flow rates (30 ml/min). BOPTA concentrations in inflow perfusate were 200 μM or 6000 nmol/min. Livers isolated from normal rats were perfused with a KHB solution (from 0 to 45 minutes), 200 μM BOPTA ± RIF (from 45 to 75 minutes), and KHB solution (from 75 to 105 minutes): 200 μM BOPTA alone (white squares) and 100 μM RIF (heavy gray), 10 μM RIF (light gray), or 1 μM RIF (black squares). (B) BOPTA bile excretion rates (nmol/min). (C) BOPTA efflux rates from hepatocytes back to sinusoids (nmol/min). (D) BOPTA concentrations in hepatocytes (nmol/g). In D, BOPTA concentrations in extracellular space were subtracted from liver concentrations to quantify BOPTA in hepatocytes (see Fig. 3). Vertical lines limit the period of drug perfusion.
dependent, we perfused livers with 1600 μM BOPTA to overcome the effect of RIF 1 μM on the transport of 200 μM BOPTA. Concentrations within hepatocytes were slightly higher during the perfusion of 1600 μM BOPTA and 1 μM RIF than during the perfusion of 200 μM alone but were much lower than they were during the perfusion of 1600 μM BOPTA alone (Fig. 4B). The IHUI was not statistically significantly different when comparing 1600 μM BOPTA (151 ± 22 nmol/min/g) with 1600 μM BOPTA plus 1 μM RIF (110 ± 33 nmol/min/g, P = 0.20), but it was higher than during the perfusion of 200 μM BOPTA (46 ± 6 nmol/min/g).

BOPTA entry into the hepatocytes was similar at the beginning of the perfusion (Fig. 4B) in the two groups and BOPTA concentrations increased until time point 54 minutes, when concentrations reached a plateau in the group perfused with 1 μM. In this group, from time point 54 minutes to the end of the perfusion, the uptake rates became similar to the excretion rates. Because from time points 54 to 75 minutes the bile excretion rates decreased (Fig. 4A), the uptake rates should have similarly evolved. During the rinse period, BOPTA bile excretion rates decreased with the cellular concentrations while BOPTA efflux back to sinusoids was higher in the presence of RIF (Fig. 4C).

Drug-Drug Interactions through Hepatocyte Transporters. No effect of BOPTA perfused at 200 μM was found for 100 μM RIF transport because little of the contrast agent entered into the hepatocytes (Fig. 1). RIF competition with BOPTA transport was more informative. The IHUI (Fig. 3D) and vascular clearances (Fig. 2A) clearly showed that RIF impeded BOPTA entry into the hepatocytes. RIF had priority over BOPTA for uptake through rat Oatp. When 1600 μM BOPTA was perfused with 1 μM RIF, the initial uptake index was unchanged, but impaired uptake appeared by time point 54 minutes.

The drug-drug interactions through exit transporters are more difficult to measure because cell excretion depends on concentrations in hepatocytes. We then plotted the excretion rate in relation to BOPTA cellular concentrations in all experimental groups according to RIF presence or absence (Fig. 5). Bile excretion rates increased with the cellular concentrations of BOPTA until a limit of 1000–1200 μM; thereafter, the bile excretion rates remained constant. The presence of RIF in the hepatocytes did not interfere with this relationship (Fig. 5, A and B).

In contrast, the perfusate efflux rates were statistically significantly higher in the presence of RIF. At similar concentrations of BOPTA,
the efflux rates back to perfusate were higher in the presence of RIF (Fig. 5C). The high efflux of RIF back to the sinusoids at various cellular concentrations (Table 1) probably promoted the transport of BOPTA.

**Discussion**

For years, information on the hepatobiliary transport of drugs and drug-drug interactions was mainly obtained by measuring vascular clearance or drug disappearance from sinusoidal blood. However, we hypothesized that measuring hepatic concentrations over time would bring more information for various reasons. 1) The hepatic concentrations of MRI contrast agents correlate with images and recent liver imaging with hepatobiliary contrast agents was used to assess OATP1B1 and OATP1B3 polymorphisms (Nassif et al., 2012). 2) The importance of drug concentrations acting within hepatocytes such as statins...
is obvious (Rodrigues, 2010). 3) The metabolism of drugs depends on hepatic concentrations (metabolizing enzymes-transport interplay) (Benet, 2009; Zhang et al., 2009).

Nevertheless, apart from liver imaging with contrast agents and tracers, cellular pharmacokinetics is difficult to assess in humans. The novelty of our study was then to present drug-drug interactions through uptake and efflux transport systems between two drugs with different hepatobiliary behavior. The interactions were studied in perfused rat livers because it is easy to control hepatic perfusate flow (set to 30 ml/min). The composition of perfused solutions is well controlled and interference with extrahepatic organs is avoided by liver isolation.

During the perfusion of drugs, only bile excretion rates were assessed because drug concentrations measured in outflow perfusate do not distinguish molecules coming from hepatocytes from those not taken up. In contrast, after a 5-minute perfusion of KHB (long enough to rinse the entire extracellular space), all molecules measured in the outflow perfusate originated from hepatocytes. We could then compare how RIF and BOPTA exited from the hepatocytes and determine whether any interactions occurred through transporters.

The cellular transport of RIF, which enters into human hepatocytes through OATP1B1 and OATP1B3, is incompletely known (Vavricka et al., 2002, 2004; Tirona et al., 2003). We show that RIF is mainly excreted via efflux back to the systemic circulation. Little RIF is excreted into bile through rat Mrp2. During RIF perfusion, a steady increase in the cellular concentrations shows that uptake rates are higher than the excretion rates. The extraction ratio is 67% during a single pass 5 minutes after the start of perfusion, but it decreases thereafter.

RIF accumulation in hepatocytes decreases the bile flow below the baseline value, emphasizing the cholestatic effect of the drug at a 100 μM concentration (Stieger et al., 2000). Recovery of the impaired bile flow is rapid when the hepatic RIF concentrations decrease. This finding may explain the early hepatocellular dysfunctions that were associated with RIF treatment in patients (Tostmann et al., 2008).

The cellular mechanisms of cholestasis associate interactions with drug uptake in mice (van de Steeg et al., 2010; Neyt et al., 2013) as well as decrease bile excretion through Mrp2 in mice (Neyt et al., 2013) and human bile salt export pump in transfected cells (Mita et al., 2006). However, RIF has also beneficial effects in cholestatic liver diseases; chronic treatment with RIF enhances bile acid detoxification, bilirubin conjugation, and excretion in association with an increased hepatic expression of CYP3A4, UGT1A1, and MRp2 (Marschall et al., 2005).

During the rinse period, we show that RIF mainly exits from hepatocytes through the sinusoidal membrane. However, the mechanism of this efflux into the sinusoids is unknown. RIF (as well as BOPTA) may use Oatp that are bidirectional or Mrp transporters of the sinusoidal membrane (Li et al., 2000).

We know that BOPTA distributes to the extracellular space and enters into rat hepatocytes through the sinusoidal transporters Oatp1a1, Oatp1a4, and Oatp1b2 (Planchamp et al., 2007). The V_max of maximal BOPTA accumulation was 2133 nmol/g/30 minutes in normal livers and 2945 nmol/g/30 minutes in livers lacking Mrp2 (Millet et al., 2011). BOPTA is excreted unchanged into bile through Mrp2, and rats lacking Mrp2 do not excrete BOPTA (de Haën et al., 1999; Millet et al., 2011). The time needed for BOPTA to reach the bile is ≤5 minutes, as observed with the bile salt taurocholate (Crawford et al., 1988). BOPTA bile excretion increases bile flow because the transport of BOPTA through the hepatocytes drives water across the membranes (Tietz et al., 2005; Mottino et al., 2006; Lehmann et al., 2008). Similarly to RIF, BOPTA effluxed back to the sinusoids, but the contrast agent was mainly excreted from the hepatocytes via bile excretion. Interestingly, bile excretion rates rely on hepatic concentrations until a threshold value of 1000–1200 nmol/g is reached. Perfuse excretion rates are also related to cellular concentrations (Millet et al., 2011).

The cellular drug-drug interactions of BOPTA and RIF are dose and time-dependent. BOPTA (200 μM) is not taken up and does not interfere with the hepatobiliary transport of RIF until the concentrations of RIF in sinusoids decrease to 1 μM. Moreover, RIF (1 μM) does not modify the IHUI of 1600 μM BOPTA until RIF finds the way through transporters and decreases the BOPTA uptake (time point 54 minutes). Then, the concentrations within hepatocytes stop increasing and instead remain steady, with uptake and excretion rates similarly decreased. In contrast, with a perfusion of 200 μM BOPTA, RIF decreases the IHUI as early as 2 minutes after the start of perfusion. RIF likely inhibits BOPTA entry through rat Oat1a4 rather than through Oatp1a1 (Fattinger et al., 2000).

The competition for BOPTA and RIF uptake transport may rely on the physicochemical structures of the drugs (such as lipophilicity and polar surface area), as recently noted by Karlgren et al. (2012). As shown in our study, drug concentrations are also important determinants of drug-drug interactions. Over time, the increase of drug concentrations in hepatocytes might modulate uptake through Oatps, the concentration gradient across membrane decreasing with constant concentrations in inflow perfusate and increasing concentrations inside cells. With perfused rat livers examined via MRI, we also showed that bromosulfophthalein blocks the uptake of BOPTA into hepatocytes (Pastor et al., 2003).

To evidence whether drug-drug interactions occur through efflux transport systems, we plotted the efflux rates against cellular concentrations. With BOPTA, efflux rates and hepatic concentrations are available every 5 minutes. For RIF only two intracellular concentrations (90 and 105 minutes) are available. Interestingly, RIF did not interfere with the transport of BOPTA through Mrp2 and the low bile excretion rates of RIF may explain the absence of interaction with BOPTA through Mrp2. In contrast, RIF increases the sinusoidal efflux rates of BOPTA for a given concentration, a finding in line with the high efflux back of RIF to sinusoids. Karlgren et al. (2012) also show that a few compounds may stimulate transport of a test drug through OATP1B1 and OATP1B3. Similar results were observed through MRp2 where interacting compounds may serve as both stimulators and inhibitors depending on concentrations used (Zelcer et al., 2003).

Extrapolating our data to the clinical situation, it is likely that if a patient treated with RIF needed a liver MRI, RIF uptake into hepatocytes would be favored until blood RIF concentrations decrease to 1 μM. In patients, the therapeutic concentrations of RIF in serum are in the range of 5 to 10 μM, with unbound concentrations of 1–2 μM for sinusoidal uptake (Acocella, 1983). Thus, no toxicity with RIF is anticipated because RIF has priority over BOPTA.

Extrahepatic toxicity of BOPTA is unlikely, but delayed hepatobiliary clearance of the contrast agent will compromise liver imaging. When Kato et al. (2002) injected Gd-EOB-DTPA (6.25 μmol i.v.) 30 minutes after RIF (40 mg/kg or 12 μmol i.v.), the contrast agent did not enter into hepatocytes, rendering the examination useless. Other acute drug-drug interactions have been published. Concomitant intravenous administration of RIF (600 mg/kg) increases the plasma concentrations of atorvastatin (Lau et al., 2007). Atorvastatin clears from blood within 24 hours, but delayed hepatobiliary clearance may induce myopathy.

Drug-drug interactions in pharmacologic studies are mainly determined by the kinetics of drug plasma disappearance. Such clearance
methods are useful in clinical practice but do not convey comprehensive information, including the hepatic concentrations. Consequently, physiologically based pharmacokinetic models and mathematical simulations were used to show how alterations in the transport through hepatic transporters can modify the concentrations of drugs (Shitara and Sugiyama, 2006; Kusuhara and Sugiyama, 2009; Watanabe et al., 2009, 2010). However, in these studies, pharmacokinetic modeling and simulations estimate the hepatocellular concentrations. In contrast, our model measures all parameters. Besides liver MRI, positron emission tomography (PET) imaging with [11C]temsiran of the liver can also evidence the accumulation of the tracer in rat livers. PET imaging with (15R)-11C-TIC-TMC assesses hepatobiliary transport in humans (Takashima et al., 2011, 2012). We recently showed the pharmacokinetics of BOPTA in cholestatic fatty rat livers with compartmental analysis and simulations in relation to the transport function across membrane transporters (Pastor et al., 2013).

In conclusion, our study demonstrates how the drug-drug interactions through transporters determine cellular concentrations of competing drugs and shows that cellular concentrations by modulating transport through membranes regulate the rat Oatp-Mrp2 interplay. Moreover, drug interactions through transporters greatly change over time.

Acknowledgments

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Authorship Contributions

Participated in research design: Daali, Millet, Dayer, Pastor. Conducted experiments: Daali, Millet, Dayer, Pastor. Contributed new reagents or analytic tools: Daali. Performed data analysis: Daali, Millet, Dayer, Pastor. Wrote or contributed to the writing of the manuscript: Daali, Millet, Dayer, Pastor.

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