Magnetic resonance imaging with hepatospecific contrast agents in cirrhotic rat livers

PLANCHAMP MESSEILLER, Corinne, et al.

Abstract

During biliary cirrhosis in rats, organic anion-transporting peptides (Oatps) and ATP-dependent multidrug resistance-associated protein 2 (Mrp2) that are likely to transport the contrast agent Gd-BOPTA through hepatocytes are down-regulated. However, the consequences of such down-regulation on the signal intensity (SI) enhancement are unknown. Consequently, the aim of our study was to measure the hepatic SI enhancement during Gd-BOPTA perfusion as well as the Oatp and Mrp2 expression in normal and cirrhotic livers.

Reference


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Magnetic Resonance Imaging With Hepatospecific Contrast Agents in Cirrhotic Rat Livers

Corinne Planchamp, PhD,* Xavier Montet, MD,* Jean-Louis Frossard, MD,† Rafael Quadri,† Bruno Stieger, PhD,‡ Peter J. Meter, MD, PhD,‡ Marko K. Ivancevic, PhD,* Jean-Paul Vallée, MD, PhD,* François Terrier, MD,* and Catherine M. Pastor, MD, PhD*

Objective: During biliary cirrhosis in rats, organic anion-transporting peptides (Oatps) and ATP-dependent multidrug resistance-associated protein 2 (Mrp2) that are likely to transport the contrast agent Gd-BOPTA through hepatocytes are down-regulated. However, the consequences of such down-regulation on the signal intensity (SI) enhancement are unknown. Consequently, the aim of our study was to measure the hepatic SI enhancement during Gd-BOPTA perfusion as well as the Oatp and Mrp2 expression in normal and cirrhotic livers.

Materials and Methods: The hepatic SI enhancement during Gd-BOPTA perfusion was measured in livers isolated from normal rats and rats that had a bile duct ligation (BDL) 15, 30, and 60 days before the perfusion. Hepatic injury and transporter expression were measured in control and cirrhotic rats.

Results: BDL induced a severe hepatic injury that increased over time with a down-regulation of the transporter expression. The extracellular space (assessed by Gd-DTPA perfusion) increased with the severity of the disease. Gd-BOPTA-induced SI enhancement remained similar in BDL-15 and BDL-30 rats than in control rats but significantly decreased in severe cirrhosis (BDL-60 rats). In comparison, the Mn-DPDP-induced SI enhancement decreases proportionally to the severity of the disease.

Conclusion: During biliary cirrhosis, Gd-BOPTA-induced SI enhancement could not be related to the hepatic expression of transporters.

Key Words: magnetic resonance imaging, liver imaging, Gd-BOPTA, Mn-DPDP

In comparison with other imaging techniques, magnetic resonance imaging (MRI) exhibits a high contrast resolution that facilitates the distinction between normal and pathologic tissues. Differences in signal intensities (SI) among tissues result from the relative T1 and T2 relaxation times and relative proton density. Because some lesions induce minimal changes in T1 and T2 relaxation times, contrast agents are injected to accentuate the difference in signal intensity (SI) between healthy tissue and lesions. Such agents include gadopentetate dimeglumine (Gd-DTPA), which diffuses in the extracellular compartment and, in liver imaging, hepatocyte-specific contrast agents, such as gadobenate dimeglumine (Gd-BOPTA) and manganese dipyridoxal diphosphate (Mn-DPDP), which enter into hepatocytes after extracellular distribution.

Gd-BOPTA enhances the SI on T1-weighted images by entering into hepatocytes. In isolated perfused rat livers, we showed previously that the concomitant perfusion of bromosulphophthalein with Gd-BOPTA prevents the uptake of the contrast agent into hepatocytes. Consequently, Gd-BOPTA enters into hepatocytes through the same transporters as bromosulphophthalein, the organic anion transporting peptides (Oatp). Moreover, we also demonstrated that the transport of Gd-BOPTA into isolated rat hepatocytes is saturable with a $K_m = 0.3$ mM. In the literature, 3 transporters (Oatp1, Oatp2, and Oatp4) have been described on the sinusoidal (or plasmatic) membrane of rat hepatocytes, but the exact isoform responsible for Gd-BOPTA uptake is unknown. Following intracellular transport, Gd-BOPTA exits into bile through the ATP-dependent multidrug resistance-associated protein 2 (Mrp2, Abcc2). In biliary cirrhosis induced by a chronic bile duct ligation (BDL), rat Oatp1 and Mrp2 are down-regulated.
However, the consequences of such down-regulation on the SI enhancement induced by Gd-BOPTA perfusion are unknown. Consequently, the aim of our study was to measure the hepatic SI enhancement during Gd-BOPTA perfusion and the expression of the transporters in livers isolated from rats that had a biliary cirrhosis with various degree of severity. In comparison, we measured the SI enhancement by MRI during the perfusion of Mn-DPDP, another hepato-specific MRI contrast agent that acts by releasing Mn$^{2+}$ into hepatocytes.\(^9,10\) Consequently, Mn$^{2+}$ does not use membrane transporter of the Oatp family to enter into hepatocytes.

**METHODS**

**Animals**

Before liver perfusion, Sprague–Dawley rats (300–450 g) were anesthetized with pentobarbital (50 mg·kg\(^{-1}\) ip). The protocol was approved by the animal welfare committee of the University of Geneva and the veterinary office and followed the guidelines for the care and use of laboratory animals.

**Induction of Biliary Cirrhosis**

Biliary cirrhosis was induced by ligating the bile duct. After laparotomy (2–3% isoflurane anesthesia), rats had a double ligation of the common bile duct with section between the 2 ligatures. Rats recovered from surgery and were perfused 15 (BDL-15), 30 (BDL-30), and 60 (BDL-60) days later. An additional group of rats had BDL the day of perfusion (BDL-1). Moreover, to assess that laparotomy by itself did not modify hepatic histology, additional rats had laparotomy without BDL (sham rats).

**Liver Perfusion**

Livers were perfused in situ as previously described.\(^11\) Briefly, the abdominal cavity was opened and the portal vein was cannulated and secured. A 16-G catheter (outer diameter, 1.8 mm) was introduced into the portal vein up to 2–3 mm from the liver. A ligature was placed around the inferior vena cava above the left renal vein. After the cannulation of the portal vein, the abdominal vena cava was transected and the Krebs-Henseleit-bicarbonate (KHB) solution was pumped without delay into the portal vein. The flow rate was slowly increased over one minute up to 35 mL/min. In a second step, the chest was opened and a second cannula (14-G) inserted through the right atrium into the thoracic inferior vena cava and secured with a ligature. Finally, the ligature around the abdominal inferior vena cava was tightened. The KHB solution was perfused to the liver through the portal catheter and eliminated by the catheter placed in the thoracic inferior vena cava. With this technique, rats die as soon as the catheter is introduced into the portal vein. Then, it is possible to explant the livers or to leave them in the carcass as done in our study.

In the MRI room, livers in the carcass were inserted into a wrist coil and placed in a plastic box maintained at a steady temperature (37°C). The entire perfusion system consisted of reservoir, pump (Ismatec, CH8152 Glattbrugg-Zürick, Switzerland), bubble trap, filter, and oxygenator. The livers were perfused with a KHB buffer (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\(_2\)) during the entire protocol with a nonrecirculating perfusion. The perfusate was equilibrated with a mixture of 95% O\(_2\)–5% CO\(_2\) during the protocol. Then, the plastic box was placed inside the magnet. All devices that were MRI incompatible were installed in an adjacent room.

**MRI**

MRI was performed on a 1.5-T Eclipse MR system (Philips Medical System, Cleveland, OH). An axial image was obtained using a fast-gradient echo T\(_1\)-weighted MR sequence (FAST) preceded by a 90° saturation pulse with the following parameters: inversion time (29 milliseconds); repetition time (6.8 milliseconds); echo time (3 milliseconds); flip angle (90°); matrix 256 × 256; 1 image/8 seconds; field of view 14 cm; slice thickness 7 mm. To record the SI over time and visualize the hepatic kinetics of the contrast agents, the mean SI were measured in a region of interest drawn on the short axis view of the liver, excluding all large vessels such as portal and hepatic veins from the region. SI was normalized to muscle SI and baseline value to allow comparison between experiments. For each liver, the region of interest remained constant during the entire experiment, but the region varied between livers.

**SI Enhancement During the Perfusion of Contrast Agents**

To evidence the extracellular diffusion space, we perfused each liver with KHB solution + Gd-DTPA during 20 minutes (extracellular distribution of Gd-DTPA) and KHB solution during 10 minutes (hepatic elimination of Gd-DTPA). This 10-minute delay was sufficient to ensure a SI return to baseline. To study the hepatocyte entry of Gd-BOPTA, we perfused the same liver with KHB solution + Gd-BOPTA during 30 minutes (extracellular distribution and hepatocyte entry of Gd-BOPTA) and KHB solution during 30 minutes (hepatic elimination of Gd-BOPTA). In each liver, we perfused Gd-DTPA before Gd-BOPTA. Five groups were studied: control livers (no BDL, n = 4), BDL-1 (BDL at the time of hepatic perfusion, n = 4), BDL-15 (BDL 15 days before the hepatic perfusion, n = 4), BDL-30 (BDL 30 days before the hepatic perfusion, n = 4), and BDL-60 (BDL 60 days before the hepatic perfusion, n = 4). The concentration of both contrast agents in the perfusion solution was 0.5 mM.

In other experimental groups we perfused Mn-DPDP after Gd-DTPA. Three groups were studied: livers from control rats (n = 3), livers from BDL-30 rats (n = 3), and
livers from BDL-60 rats (n = 3). The concentration of Mn-DPDP in the perfusion solution was 0.5 mM.

**Hepatic Test**

Before liver perfusion, blood was collected to measure the degree of cholestasis (serum bilirubin concentration), hepatic injury (serum aspartate aminotransferase and alanine aminotransferase concentrations), as well as the synthetic capacity of the liver (serum albumin concentration).

**Histologic Examinations**

To quantify the severity of cirrhosis, hepatic tissues were collected in 15 rats (n = 3 in each group). For each liver, 3-μm paraffin sections from the right and left lobes were stained with hematoxylin eosin. Fibrosis was detected according to the Masson technique.

**Protein Extraction**

**Oatp and Mrp2 Transporters**

Liver tissues were snap-frozen in liquid nitrogen. They were homogenized with ice-cold Tris buffer (100 mM Tris-HCl, pH 7.6) containing protease inhibitors, AEBSF, aprotinin, leupeptin, bestatin, pepstatin A and E-64 (Sigma, Buchs, Switzerland). Homogenates were centrifuged at 10,000g for 10 minutes at 4°C. This first supernatant was used for the detection of Oatp1 and Oatp4 because no Oatp1 and Oatp4 were present in the pellet. Because no Mrp2 was present in this first supernatant, the pellet was homogenized with the same buffer containing 1% Triton X-100. After centrifugation at 10,000g for 10 minutes at 4°C, this second supernatant was used for the detection of Mrp2. To better extract Oatp2 a second Tris buffer was used (50 mM Tris-HCl, 0.1 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid [EGTA], 0.1 mM ethylenediamine tetraacetic acid [EDTA], 0.1% sodium dodecyl sulfate [SDS], 0.1% deoxycholic acid, 1% Triton X-100, pH 7.5). Protein concentration was determined according to Bradford (Bio-Rad, Glattbrugg, Switzerland). The supernatants were stored at –70°C until use.

**Albumin and Cytokeratin 19**

To assess the modification of the number of hepatocytes and cholangiocytes induced by cirrhosis, we also mea-

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**TABLE 1.** Hepatic Tests (Median [Minimum–Maximum]) in Sham-Operated Rats and in Rats With Bile Duct Ligation (BDL-days)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BDL-15</th>
<th>BDL-30</th>
<th>BDL-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASAT (IU/L)</td>
<td>50 [40–113]</td>
<td>170 [160–252]</td>
<td>800 [357–998]</td>
<td>252 [171–374]</td>
</tr>
<tr>
<td>Total bilirubin (μmol/L)</td>
<td>4 [0–12]</td>
<td>152 [146–164]</td>
<td>119 [99–144]</td>
<td>194 [142–204]</td>
</tr>
<tr>
<td>Direct-reacting bilirubin (μmol/L)</td>
<td>3 [0–5]</td>
<td>90 [88–95]</td>
<td>66 [62–71]</td>
<td>116 [90–124]</td>
</tr>
</tbody>
</table>

n = 4 in each group.

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.
sured albumin (for hepatocytes) and cytokeratin 19 (for cholangiocytes). Tissues were homogenized in ice-cold Tris buffer (50 mM Tris-HCl, 0.1 mM EGTA, 0.1 mM EDTA, 0.1% SDS, 0.1% deoxycholic acid, 1% Triton X-100, pH 7.5) containing protease inhibitors, AEBSF, aprotinin, leupeptin, bestatin, pepstatin A, and E-64. Homogenates were centrifuged at 10,000 g for 10 minutes at 4°C. Albumin was detected in the supernatant whereas cytokeratin 19 was found in the pellet resuspended in the same buffer.

Western Blotting Analysis

Rabbit polyclonal antibodies against rat-oatp1 (Slc21a1),6,12 rat-Oatp2 (Slc21a5),13 rat-Oatp4 (Slc21a10),14 and rat-Mrp2 (Abcc2)15 were used. SDS polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed according to standard procedures. One hundred micrograms of protein extracts were separated on a 7.5% polyacrylamide gel. After the gel had been transferred to a polyvinylidene difluoride membrane (Millipore, Volketswil, Switzerland), the membrane was blocked with 5% nonfat dry milk in phosphate-buffered saline (PBS; 1 hour at room temperature) and incubated with the specific antibodies. We assessed, through staining with Ponceau Red before blocking, that an equal quantity of proteins was loaded on each line. The membrane was then incubated overnight at 4°C with anti-Oatp1 (1:1,000), anti-Oatp2 (1:5,000), anti-Oatp4 (1:2,000), anti-Mrp2 (1:2,000), rat polyclonal antialbumin (1:400, Anawa, Zürich, Switzerland), or mouse monoclonal anticytokeratin-19 (1:1000, Progen, Heidelberg, Germany) antibodies containing 5% nonfat dry milk in PBS. For albumin detection, 10 µg proteins were charged and the membrane was blocked with 3% gelatin. The membrane was washed 4 times with PBS–0.2% Tween-20 and incubated for 1 hour with an alkaline phosphatase-conjugated goat antirabbit IgG antibody at 1:5,000 (Stressgen, Victoria, Canada). Then, the membrane was washed 4 times with PBS–Tween buffer. Development was performed using an Immune-Star chemiluminescent protein-detection system (Bio-Rad) according to the manufacturer’s instructions. Molecular weight markers, normal controls, and positive controls were included during each experiment.

Statistical Analysis

Data are given as median [minimum − maximum], and differences for hepatic tests and SI at the end of each contrast agent perfusion were compared between the experimental groups (control livers and livers from BDL rats) with a Kruskal–Wallis analysis for nonparametric variables and Dunn posthoc tests. P < 0.05 was considered significant.

**Drugs**

Gadopentetate dimeglumine, Gd-DTPA, Magnevist (Schering, Berlin, Germany); mangafodipir, Mn-DPDP, Teslascan (Nycomed, Oslo, Norway); and gadobenate dimeglumine, Gd-BOPTA, MultiHance (Bracco, Milano, Italy) were used.

**RESULTS**

**Bilirubin, Albumin, and Hepatic Enzymes in Serum After BDL**

In BDL-15 rats, total bilirubin concentrations in serum significantly increased (Table 1, P < 0.02). Fifty percent of elevated serum bilirubin was direct-reacting bilirubin. The serum concentration of albumin decreased with the length of in vivo BDL (P < 0.02) whereas ASAT concentrations slightly increased over time (P < 0.02).
Hepatic Injury Induced by BDL

After BDL, cholangiocytes proliferated and formed an organized network of well-defined tubular structures (Fig. 1). Ductular hyperplasia predominated in the portal areas in BDL-15 rats and extended to the entire hepatic parenchyma in BDL-30 and BDL-60 rats. Fibrosis also extended with the duration of BDL: fibrous connective tissue septa bridged portal areas and the normal lobular pattern was disorganized. The decrease in albumin expression and the increase in cytokeratin 19 expression paralleled the decreased number of hepatocytes with a concomitant increase in cholangiocytes (Fig. 2).

Expression of Hepatic Transporters After BDL

Oatp1 expression decreased in BDL-15 rats, and the low expression was maintained in BDL-30 and BDL-60 rats (Fig. 3). Oatp2 expression slightly decreased over time whereas Oatp4 expression transiently increased in BDL-15 and BDL-30 rats. The Mrp2 expression was low in BDL-15 rats and further decreased in BDL-30 and BDL-60 rats.

MRI

Livers were isolated from control or rats with various severity of biliary cirrhosis and imaged during contrast agent perfusion. In control livers, Gd-DTPA perfusion slightly

FIGURE 4. MRI signal intensities in livers isolated from control rat, livers isolated from rats that had a bile duct ligation (BDL) at the time of hepatic perfusion (BDL-1), livers isolated from BDL-15, BDL-30, and BDL-60 rats. Each liver was perfused with a Krebs–Henseleit bicarbonate (KHB), KHB + gadopentetate dimeglumine (Gd-DTPA, 20 minutes), and KHB + gadobenate dimeglumine (Gd-BOPTA, 30 minutes) solutions. Gd-DTPA and Gd-BOPTA concentrations in KHB were 0.5 mM.
increased the SI whereas the SI was much higher when the livers were perfused with Gd-BOPTA (Fig. 4 control and Fig. 5). During the KHB perfusion after Gd-DTPA perfusion, the SI rapidly returned to baseline value whereas during the KHB perfusion after Gd-BOPTA perfusion, the SI remained high. In the portal and hepatic tributary veins, the SI increased and decreased sharply after the start and the end of the contrast agent perfusions and remained steady during the perfusion of both contrast agents (corresponding to 0.5 mM concentration, data not shown). In livers isolated from BDL-1, BDL-15, and BDL-30 rats, similar patterns were observed (Fig. 4, BDL-1, BDL-15, and BDL-30), the increase in SI during Gd-BOPTA perfusion being higher than that observed during Gd-DTPA perfusion. In contrast, in BDL-60 rat, the increase in SI during Gd-BOPTA perfusion was similar to that observed during Gd-DTPA perfusion.

At the end of Gd-DTPA perfusion, the SI significantly increased in BDL-60 rats (Table 2, $P = 0.024$). In BDL-15 and BDL-30 rats, the SI at the end of Gd-BOPTA perfusion was similar to control rats, whereas in BDL-60 the SI was markedly decreased (Table 2. $P = 0.04$). Interestingly, the SI at the end of Mn-DPDP perfusion decreased proportionally with the severity of biliary cirrhosis (Table 3, $P = 0.027$).

### DISCUSSION

Our study shows that biliary cirrhosis induces a severe hepatic injury over time associated with a down-regulation of Oatp1, Oatp2, and Mrp2. The extracellular distribution space increases in severe biliary cirrhosis. Gd-BOPTA-induced SI enhancement is preserved in mild cirrhosis but impaired when cirrhosis is severe. In contrast, the Mn-DPDP-induced SI enhancement decreases proportionally to the severity of the disease.

**BDL-Induced Biliary Cirrhosis**

After BDL, serum concentration of bilirubin increases and cholestasis is associated with hepatic injury and a decreased albumin synthesis. Cholangiocyte proliferation remains confined to portal tracts in the early phase and spread over the entire lobules over time. Although these hyperplastic cells resemble normal cholangiocytes, their origin and the mechanism responsible for their proliferation remain obscure. The increase in cholangiocytes is evidenced by an increase in cytokeratin 19 expression, which is specifically expressed by these cells, as already published. The decrease in hepatic expression of albumin with the severity of cirrhosis (Fig. 3) associated with a low serum albumin concentration in serum (Table 1) paralleled the decreased number of hepatocytes in tissues (Fig. 1).

**Gd-BOPTA Entry into Hepatocytes During Cirrhosis**

In cirrhotic patients, the modifications of SI in hepatic parenchyma are not fully understood, and conflicting results have been published. One hour after the injection of Gd-BOPTA (0.1 mmol/kg), the parenchymal SI is either altered (the decreased SI parallels the alteration of hepatic tests and

| TABLE 2. Median [Minimum–Maximum] Signal Intensity Measured at the End of the KHB + 0.5 mM Gd-DTPA and KHB + 0.5 mM Gd-BOPTA Perfusions in Control Livers and Livers Isolated From Rats With Bile Duct Ligation (BDL-Days) |
|-----------------|----------------|----------------|
|                 | Gd-DTPA        | Gd-BOPTA       | Gd-BOPTA / Gd-DTPA |
| Control         | 0.58 [0.37–0.84] | 2.58 [1.73–3.28] | 4.19 [3.03–6.64] |
| BDL-1           | 0.51 [0.43–0.62] | 2.99 [2.44–3.73] | 5.40 [3.03–6.62] |
| BDL-15          | 0.51 [0.43–0.62] | 2.99 [2.45–3.73] | 5.85 [5.40–6.62] |
| BDL-30          | 0.51 [0.42–0.52] | 2.90 [1.84–3.52] | 6.59 [3.59–6.97] |
| BDL-60          | 0.78 [0.71–0.85] | 1.32 [1.34–3.28] | 1.70 [1.57–1.85] |

n = 4 in each group.

*P < 0.05 versus control rats.

KHB, Krebs-Henseleit bicarbonate; Gd-DTPA, gadopentetate dimeglumine; Gd-BOPTA, gadobenate dimeglumine.
Increased enhancement in regenerating nodules, possibly due to impaired bile excretion, explains why enhancement of impaired liver parenchyma is higher in patients with hepatocarcinomas than the enhancement of normal liver parenchyma in patients with focal nodular hyperplasia.²⁷

**Gd-BOPTA Secretion into Bile**

Mrp2 is a phosphoglycoprotein that transports a wide range of products, including conjugated bilirubin and Gd-BOPTA, out of cells by an ATP-dependent mechanism. Mrp2 is mainly expressed on the canalicular membrane of hepatocytes. The hepatocyte expression of Mrp2 is down-regulated in rats subjected to BDL.⁷,⁸,²⁸ –³¹ The down-regulation may be caused by endocytosis of transporters followed by an increased lysosomal breakdown.²⁹ Other members of the multiple drug resistance-associated transporters, such as Mrp3, have been recently identified on the sinusoidal membrane of hepatocytes, where they mediate the efflux of organic anions, bile salts, anticancer drugs, methotrexate, and bilirubin monoglucuronide. Mrp3 is normally expressed at low levels, but down-regulation of Mrp2 is compensated by Mrp3 up-regulation.⁸,²⁸,³⁰ Thus, Gd-BOPTA might return to sinusoids through Mrp3 or through Oatp that are bidirectional.²² In human liver with primary biliary cirrhosis, Mrp2 expression does not change at the beginning of the disease³³ but decreases with the severity of the disease.³⁴ In conclusion, during biliary cirrhosis, Gd-BOPTA-induced SI enhancement could not be related to the hepatic expression of transporters.

**ACKNOWLEDGMENTS**

The authors thank Martha Jordan for excellent technical assistance.

**REFERENCES**


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**TABLE 3.** Median [Minimum–Maximum] Signal Intensity Measured at the End of the KHB + 0.5 mM Gd-DTPA and KHB + 0.5 mM Mn-DPDP Perfusions in Control Livers and Livers Isolated From Rats With Bile Duct Ligation (BDL-Days)

<table>
<thead>
<tr>
<th></th>
<th>Gd-DTPA</th>
<th>Mn-DPDP</th>
<th>Mn-DPDP / Gd-DTPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.29 [0.18–0.46]</td>
<td>8.98 [6.94–9.60]</td>
<td>33.79 [19.47–38.53]</td>
</tr>
<tr>
<td>30-d BDL</td>
<td>0.28 [0.25–0.29]</td>
<td>4.39 [3.60–4.75]*</td>
<td>15.28 [12.74–19.31]*</td>
</tr>
<tr>
<td>60-d BDL</td>
<td>0.47 [0.44–0.50]</td>
<td>3.23 [1.36–3.24]*</td>
<td>6.42 [3.13–6.97]*</td>
</tr>
</tbody>
</table>

n = 3 in each group.

*P < 0.05 versus control rats.

KHB, Krebs-Henseleit bicarbonate; Gd-DTPA, gadopentetate dimeglumine; Mn-DPDP, manganese dipyridoxal diphosphate.

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the extent of fatty metaplasia²⁰ or higher than the SI obtained in normal patients.²¹ In this last study, the increased enhancement in cirrhotic livers have been attributed to a preserved transport in regenerating nodules and a high extracellular distribution. An increased extracellular distribution was confirmed in our experimental model because the maximal SI of Gd-DTPA is significantly increased in BDL-60 rats. The entry of hepato-specific contrast agents into hepatocytes also differentiates benign hyperplastic nodules from malignant hepatocarcinomas.²²

We previously showed that bromosulphophthalein was able to abolish the hepatic signal enhancement induced by Gd-BOPTA in normal perfused rats.² Because bromosulphophthalein enters into hepatocytes through Oatp transporters, Gd-BOPTA is likely to enter by the same transporters. However, direct evidence has not yet been published. Thus, protein expression of these hepatic transporters is important to investigate to better understand liver MRI with hepato-specific contrast agents, such as Gd-BOPTA. In our study, the decrease in hepatic transporter expression induced by mild cirrhosis was not associated with change in MR SI that remained similar in BDL-15 and BDL-30 livers to control livers. We can hypothesize that less Gd-BOPTA that remained similar in BDL-15 and BDL-30 livers to control livers. We can hypothesize that less Gd-BOPTA entry of hepato-specific contrast agents into hepatocytes also could be caused by endocytosis of transporters followed by an increased lysosomal breakdown. Gd-BOPTA Secretion into Bile

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In human liver with primary biliary cirrhosis, Mrp2 expression does not change at the beginning of the disease but decreases with the severity of the disease. In conclusion, during biliary cirrhosis, Gd-BOPTA-induced SI enhancement could not be related to the hepatic expression of transporters.

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