Enhanced Magnetic Resonance Imaging for Tissue Characterization of Liver Abnormalities with Hepatobiliary Contrast Agents: An Overview of Preclinical Animal Experiments

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Summary: Because of the demand for an extension of the range of diagnostic uses for currently available extracellular magnetic resonance imaging (MRI) contrast agents, a new generation of tissue-specific (i.e., hepatobiliary) contrast agents have been successfully developed to enhance the performance of hepatic MRI. These agents include mangafodipir trisodium (formerly manganese dipyridoxal diphosphate [Mn-DPDP]), gadoxetic acid (formerly gadolinium ethoxybenzyl diethylenetriaminopentaacetic acid [Gd-EOB-DTPA]), and gadolinium butylbenzyl diethylenetriaminepentaacetic acid (MS-264). In a series of experiments in animals, the potential of these agents for noninvasive characterization of liver lesions was evaluated with the use of a comprehensive methodology. The purpose of this report was to summarize the observed imaging behaviors of these agents toward different types of cholestasis and focal liver lesions, compare the similarities or dissimilarities of these agents in their possible mechanisms of action, and discuss the clinical implications of the results of the experiments. In summary, through intravenous administration of these agents, both global and local obstructive cholestases can be identified on the postcontrast image. With certain reliability, liver tumors of different origins and grades of cellular differentiation can be detected and classified according to their contrast enhancement patterns occurring at certain postcontrast phases. The invasiveness or degree of malignancy of the liver tumor can also be suggested by the presence or absence of a peritumoral rim sign. Such diagnostic information, otherwise only invasively achievable, may prove to be invaluable in clinical decision making. **Key Words:** MRI contrast medium—Liver neoplasm—Cholestasis—Hepatic dysfunction—Gadolinium—Manganese.

Imaging diagnosis plays a key role in detection, localization, staging, and selection of therapies for focal liver lesions. In contrast to advanced radiographic and echographic modalities such as computed tomography (CT) with hepatoarteriography or arterialportography (CTAP) (1,2) and intraoperative ultrasonography (3), which have greater precision at the price of invasiveness, magnetic resonance imaging (MRI) represents a relatively new

but ever-improving noninvasive technique for hepatic imaging.

Despite the greater inherent tissue contrast achieved with MRI compared with other imaging techniques, there remains substantial overlap in MRI signals between diseased and normal liver tissue. As a result, additional contrast agent (CA) injection is frequently preferred. Unfortunately, commercial MRI CAs such as gadopen-tetate dimeglumine (formerly gadolinium diethylene-triaminepentaacetic acid, or Gd-DTPA) and gadoterate meglumine (formerly gadolinium tetraazacyclododecanetetraacetic acid, or Gd-DOTA) distribute only nonspecifically in the extracellular fluid spaces of both

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normal and abnormal liver tissues, leading to a nonoptimal contrast enhancement. The need for more specific and effective CAs to enhance the performance of hepatic MRI has well been recognized. So far, great efforts have been made in designing liver-targeting MRI CAs, and several such new CAs have been successfully developed. Some of them have been investigated in advanced clinical trials and are waiting for or have received final approval for use in the clinical setting.

Other than particulate agents such as dextran-coated superparamagnetic iron oxide particles and liposomes that are taken up by intrahepatic mononuclear phagocyte system or Kupffer cells (4–6), a major category includes a variety of hepatocyte-targeting agents. These agents can be further divided into (1) receptor-directed agents such as arabinogalactan-coated or asialofetuin-conjugated iron oxide particles, which are specific for hepatocyte asialoglycoprotein receptors (7,8) and (2) hepatobiliary chelates such as manganese dipyridoxal diphosphate (mangafodipir trisodium) (9,10), gadolinium benzyloxypionictetraacetate (gadobenate dimeglumine, formerly Gd-BOPTA) (11), gadolinium ethoxybenzyl diethylenetriaminopentaacetic acid (gadoteric acid) (12,13), and gadolinium butylbenzyl diethylenetriaminopentaacetic acid (MS-264) (14).

Unlike T2 or T2* shortening agents such as superparamagnetic particles, which usually lead to a signal void of normal liver and therefore are called negative agents, the latter hepatobiliary chelates are positive agents and originally were designed to cause a preferential increase in liver intensity, leaving hepatic tumors as “negative defects” (9), and hence providing enhanced liver–tumor contrast. The basic efficacy of these agents was assessed in animal models of liver tumor implantation for improved lesion detection (10,11,13,14). However, little attention was initially paid to their potential use in noninvasive liver tissue characterization, probably due to a lack of well-adapted investigative methodology.

The present series of experiments in animals were intended to explore the hepatobiliary feature of these chelates for use in MRI liver lesion characterization. Comprehensive animal models of liver tumors, both implanted and primary, and obstructive cholestasis, both total and segmental, were used to examine contrast-enhancing effects of different agents on liver lesions of different origins and natures. The applied MRI-microangiographic or microcholangiographic-histologic and/or serologic matching techniques as well as the meticulous performance of these techniques proved to be indispensable for the accurate documentation of the experimental findings (15–20).

STUDIES OF LIVER TUMORS

Tumor models

Implanted liver tumors derived generally from undifferentiated or highly malignant cell lines are appropriate only for simulation of space-occupying liver metastasis in patients and useful mainly in lesion detection (10,11,13,14). Therefore, in addition to the use of models of tumor implantation such as VX2 carcinoma in rabbits and Novikoff hepatoma in rats, multifocal hepatocellular carcinomas (HCCs) were chemically induced with oral administration of diethylnitrosamine in rats to mimic primary liver cancer in humans (15–20). This model represented a wide morphologic heterogeneity. According to a classification similar to the Edmonson and Steiner typing of human hepatoma (21), a full spectrum of cellular differentiation of the HCC lesions ranging from highly or well-differentiated grade I to poorly differentiated or undifferentiated grade IV were identified. The tumors varied from being extremely hypervascular to hypovascular or avascular in their blood supply.

These characteristics plus other intratumoral features such as glandular or papillary formation and fatty degeneration, as well as coexisting benign cirrhotic lesions such as regeneration nodules, retention cysts, and cholangiofibrosis, rendered this tumor model highly relevant to the clinical setting and allowed an assessment of contributions of not only intravascular and extravascular compartments (22) but active cellular uptake in the final MRI contrast-enhancement pattern. The latter aspect proved to be particularly important in the study of tissue-specific CAs (15–20). On the other hand, the complexity in this model of the induced liver pathology with a large number of simultaneously occurring lesions may have made precise correlation between MRI and histology difficult, but with the introduced MRI-microangiography-histology matching approach, this technical problem was tackled (15).

Contrast agents

We studied three hepatobiliary chelates (mangafodipir trisodium, Teslascan[®], Byk-Gulden Lomberg Chemische, Constance, Germany), gadoteric acid (Eovist[®], Schering AG, Berlin, Germany), and MS 264 (Metasyn, Inc., Cambridge, MA, U.S.A.) at intravenous doses of 25, 30, and 50 $\mu\text{mol/kg}$, respectively, in separate studies (15–20). During the experiments, one nonspecific extracellular fluid agent (gadopentetate dimeglumine [Magnevist[®]], Schering AG, or gadoterate meglumine [Dotarem[®]], Guerbet, Aulnay-Sous-Bois, France) was always compared with one or two hepatobiliary agents, with an interval ≥ 24 h between two CA studies to allow

global elimination of the previous agent, in the same animal (intraindividual control) to assess both specific and nonspecific contributions of the specific chelates.

Imaging performance

Anesthetized animals were imaged with the use of a custom-made rat coil adapted to a 1.0-T imager (Magnetom SP, Siemens, Erlangen, Germany). Axial multislice T1-weighted (TR/TE, 600/15 ms) (T1W) spin-echo sequence was used for the precontrast and postcontrast measurements. Section thickness (without gap) was 2 mm in rats and 4 mm in rabbits; the field of view was 10 cm in rats and 15 cm in rabbits; the matrix was 256×256 ; and the number of excitations was four.

Imaging analysis and definition of tumor contrast enhancement

The signal intensities (SI) of tumors, liver parenchyma, and background noise were measured using a monitor-defined circular region of interest. The relative enhancement (RE) of the tumor and liver was calculated as $RE (\%) = (SI_{\text{post}} - SI_{\text{pre}}) / SI_{\text{pre}} \times 100$. The value of tumor–liver contrast-to-noise ratio (CNR) was obtained with the formula: $CNR = (SI_{\text{tumor}} - SI_{\text{liver}}) / \text{background noise}$. According to precontrast and postcontrast CNR values, tumor contrast enhancement (TCE) was defined by criteria of (1) $|CNR_{\text{post}}| < |CNR_{\text{pre}}|$ indicates no TCE and (2) $|CNR_{\text{post}}| > |CNR_{\text{pre}}|$ represents a TCE in which CNR_{post} of less than zero indicates a negative TCE , and CNR_{post} of more than zero indicates a positive TCE . Briefly, the definition given here of a negative or positive TCE is based on whether the signal intensity of the tumor is lower or higher than that of the liver, rather than whether a positive or negative agent has been used.

Data correlation

Serial MRI including precontrast, early, and delayed postcontrast imaging was performed and correlated with postmortem microangiographic and histologic findings. MRI enhanced with a nonspecific agent with microangiographic and histologic findings also facilitated assessment of the access of the hepatobiliary agent to individual lesions by depicting both the arterial blood supply and capillary vascular bed. All tumors were classified according to above-mentioned criteria as either negatively or positively contrast-enhanced tumors as they appeared most conspicuous at a certain postcontrast phase. The presence or absence of induced peritumoral rim enhancement also was recorded. These TCE patterns of different lesions were correlated to their histologic features, including the grade of tumoral differentiation and intratumoral and peritumoral topography.

Different hepatobiliary CAs were compared for the frequency of induced TCE patterns by using the χ^2 test to determine whether the differences were significant (Microsoft Excel Version 4.0).

Experimental findings

On precontrast T1W spin-echo images, the primary and implanted liver tumors appeared hypointense, isointense, or slightly hyperintense (Figs. 1A, D, 2A). After injection of gadoterate meglumine or gadopentetate dimeglumine, these tumors were slightly to strongly enhanced (Fig. 2B), reflecting a variety of tumoral vascularization. In comparison with the lower liver enhancement ($RE = 25\text{--}37\%$) by a ~ 10 -fold higher dose (0.3 mmol/kg) of gadoterate meglumine or gadopentetate dimeglumine, the lower doses of hepatobiliary agents mangafodipir trisodium (0.025 mmol/kg), gadoxetic acid (0.03 mmol/kg) and MS-264 (0.05 mmol/kg) caused a much stronger liver enhancement ($RE = 50\text{--}60\%$). The patterns and degrees of the TCE varied with the specific hepatobiliary agent used, individual lesions observed, and certain postcontrast phases. Their typical appearances are illustrated as a schematic drawing in Fig. 3.

Soon after the injection of mangafodipir trisodium, 42 (50%) of 84 primary hepatomas that were hyperintense or isointense on precontrast images and slightly to moderately enhanced by gadoterate meglumine were simultaneously enhanced to a degree comparable to that in the liver parenchyma. During this early phase, the visibility of these HCC lesions was not significantly improved or even decreased (Fig. 1E). Only in the delayed phase (≥ 3 h) were these lesions shown as well-delineated bright spots, with the highest conspicuity occurring at 24 h (Fig. 1F). Such positive TCE lasted up to 48 h (16). On the other hand, all 26 (100%) implanted tumors and 42 (50%) of 84 primary liver cancers, which were hypointense on precontrast image but moderately to strongly enhanced with gadoterate meglumine remained hypointense but more conspicuous due to strongly enhanced surrounding liver parenchyma. After 24 h, when liver intensity had almost returned to normal, a hyperintense rim appeared around some of these lesions (17).

In comparison with mangafodipir trisodium, the other two agents (gadoxetic acid and MS-264) revealed a different pattern of liver tumor enhancement (18–20). After injection, all implanted and most primary liver tumors showed a prompt negative TCE due to maximal increase in liver intensity and negligible increase in tumor intensity (Figs. 1B, 2C, D). The highest conspicuity of these negatively enhanced lesions occurred at 15–20 min after

FIG. 1. T1-weighted spin-echo (TR/TE = 600/15 ms) MR images of a rat with chemically induced HCCs. **A**, Before contrast, a histologically proven moderately differentiated grade II HCC lesion (arrow) is almost invisible on this slice because it is isointense to the surrounding liver parenchyma. **B**, At 30 min after intravenous injection of gadoxetic acid at 0.03 mmol/kg, the lesion (arrow) is nicely delineated with a negative TCE due to a strong liver enhancement and negligible tumoral enhancement. Some hyperintensity shows up (arrowheads), presumably representing benign hepatocytic nodules, which take up but do not efficiently excrete the agent. **C**, At 3 h after gadoxetic acid injection, the lesion (arrow) tends to disappear when liver intensity is almost normalized. The enhanced small dots (arrowheads) are still faintly visible. **D**, At 24 h after gadoxetic acid but immediately before mangafodipir trisodium injection, the intensities of both the liver and tumor (arrow) have completely returned to normal, as shown in **A**. **E**, At 30 min after intravenous injection of mangafodipir trisodium at 0.025 mmol/kg, the lesion (arrow) shows a moderate positive TCE due to simultaneous contrast uptake in the liver and tumor, but impaired bile excretion only in the tumor. **F**, At 24 h after mangafodipir trisodium injection, the positive TCE of the tumor (arrow) becomes more pronounced as liver intensity largely decreases. There also are some positively enhanced small lesions (arrowheads), which were undetectable on both precontrast and early postcontrast images (**D** and **E**).

the injection. Surprisingly, 8 (5%) of 150 and 6 (9%) of 66 primary hepatomas showed a pronounced positive TCE with gadoxetic acid and MS-264, respectively (19,20). Such positive TCE lasted for 2 h, with the highest conspicuity at ~30 min (Figs. 2C, D), somewhat later than that of negative TCE (Figs. 1B, 2C, D). Similar positive TCE also was occasionally seen as scattered small dots in the cirrhotic liver (Figs. 1B and C). Furthermore, a faint peritumoral rim was observed in all implanted Novikoff hepatomas and some of the primary hepatomas. These rims remained visible for ~2 h (19,20).

At microangiography, most primary hepatomas showed one or more hypertrophic and tortuous feeding arteries and a varying amount of intratumoral vessels. A clearly recognizable vascular network was seen in

some hepatomas, whereas others were characterized by intense but amorphous staining (16–20). All implanted hepatomas showed a moderate degree of hypervascularization and central inhomogeneity (18,19). This vascular aspect was particularly informative for interpretation of the TCE at both perfusion and hepatobiliary phases (16–20).

At histology, primary hepatomas of grades I to III were highly to poorly differentiated HCCs with a solid macroscopic appearance, whereas grade IV lesions were undifferentiated HCCs generally with an angioma-like appearance. All implanted VX2 carcinomas in rabbits and Novikoff hepatomas in rats were solid but grade IV undifferentiated tumors. Peritumoral infiltration of malignant cells between hepatocytes was a common finding

FIG. 2. A–F, MRI, macroscopic and histologic findings in a rat with multiple HCCs. A–D, T1-weighted spin-echo (TR/TE = 600/15 ms) images containing two small tumors (~3 mm in diameter each) adjacent to branches of hepatic vein. A, Before contrast, the tumors appear slightly hyperintense (long arrow) and slightly hypointense (short arrow). B, At 10 min after gadolinium diethylenetriaminepentaacetic acid (0.3 mmol/kg) injection, which was done 24 h earlier, together with a moderate enhancement in the liver, one tumor (long arrow) is slightly enhanced, and the other (short arrow) is surrounded by an enhanced rim due to perfusion and diffusion of this nonspecific agent. C, At 10 min after intravenous injection of gadoxetic acid at 0.03 mmol/kg, these two tumors display typical negative (short arrow) and positive (long arrow) TCE with a strong liver enhancement. D, At 30 min after gadoxetic acid injection, when the intensity of the liver has largely decreased, leading to a less conspicuous negative TCE (short arrow), the tumor with positive TCE (long arrow) becomes more prominent in both signal intensity and marginal delineation. E, Macroscopic view of the two tumors with gadoxetic acid-induced positive TCE (long arrow) and negative TCE (short arrow). Notice the adjacent branch of hepatic vein as a landmark. Square indicates the location of the microscopic picture. F, Light microscopic view reveals a highly differentiated grade I HCC (bottom left), which showed gadoxetic acid-induced positive TCE, and a moderately differentiated grade II HCC (bottom right), which showed gadoxetic acid-induced negative TCE. V represents the adjacent branch of hepatic vein. (Hematoxylin and eosin stain; original magnification, 40 $\times$.)

among grade IV primary HCC and implanted Novikoff hepatoma, whereas peritumoral liver parenchyma compression was more generally seen with other types of tumors (16–20).

The correlation between patterns of TCE induced by different hepatobiliary agents and grades of cellular differentiation of liver tumors are summarized in Table 1. Among separate studies with similar tumor models, the frequency of the positive TCE with mangafodipir trisodium (50%) is significantly higher ($p < .001$) than that with gadoxetic acid (5%) or MS-264 (9%).

Mangafodipir trisodium induced persistent (2 days) positive TCE in all differentiated HCCs, including those of grades I to III, with negative TCE for grade IV undifferentiated primary and secondary liver tumors. However, gadoxetic acid and MS-264 caused a prolonged (2 h) positive TCE only in grade I highly differentiated HCCs and benign hepatic nodules and a prompt negative

TCE in the remainder of the tumors. As exceptions, two and one grade I HCCs in mangafodipir trisodium and MS-264 studies, respectively, did not show positive CE. They were neither enhanced by gadoterate meglumine and gadopentetate dimeglumine nor opacified at microangiography, suggesting poor accessibility of the lesions by the agents.

The scattered areas of positive TCE seen with gadoxetic acid and mangafodipir trisodium (Figs. 1C and 1F) were too small (<1 mm) to make a one-to-one match between MRI and histology, but they most likely correspond to precancerous benign lesions, such as regenerative and hyperplastic nodules, which may coexist in this cirrhotic rat model (19).

Comments and discussions

The persistent (2 days) pronounced rim enhancement with mangafodipir trisodium and prolonged (2 h) faint

FIG. 3. Schematic of liver-tumor contrast-enhancement patterns induced by different hepatobiliary MRI contrast agents. Mn-DPDP, mangafodipir trisodium; Gd-EOB-DTPA, gadoxetic acid. (Refer to the text and Table 1 for detailed explanations.)

rim enhancement with gadoxetic acid or MS-264 presumably were caused by retention of the CAs within peritumoral hepatocytes as a result of impaired local bile drainage due to malignant infiltration, or compression, or both (18–20). In comparison with those induced with gadoxetic acid or MS-264, the rims induced with mangafodipir trisodium were more prominent and therefore probably more valuable in the clinical assessment of liver malignancies (17). Although unexpected on the basis of other preclinical studies with implanted tumor models (10,11,13,14), positive TCE was clearly documented in the present series of studies for all three hepatobiliary chelates (15–20). This TCE pattern can be reasonably attributed to both active uptake of the agent by those hepatomas with a residual hepatocytic function and an impaired biliary excretion within the intratumoral region (16–20). However, the important distinctions in terms of frequency, intensity, and duration of positive TCE must be related to different pharmacokinetics of individual agents and their interactions with the individual lesions.

Mangafodipir trisodium, a chelate of pyridoxal-5-phosphate, which is a catalytically active form of vitamin B₆,

was designed to be recognized specifically by the membrane transport system, taken up by normal hepatocytes, and excreted into the bile (9,10). By coincidence, experiments have shown that a supply of vitamin B₆ is essential to the growth of highly to poorly differentiated hepatomas (23). These arguments constituted the main rationale to explain the preferential uptake of mangafodipir trisodium by both normal hepatocytes and hepatoma cells of grades I to III (16–19). However, in a recent comparative study (Y. Ni, unpublished data, 1996), mangafodipir trisodium and MnCl₂ induced similar positive or negative TCE patterns on the same HCCs in the same animals, suggesting that it is mainly Mn²⁺ rather than mangafodipir trisodium chelate as a vitamin B₆ analog that is responsible for the intracellular uptake. This finding is in agreement with other basic research, which disproves the previous assumption (24–26). After intravenous injection, mangafodipir trisodium is gradually metabolized mainly in the plasma through steps of dephosphorylation and transmetallation with endogenous zinc, a process that accounts for the decreased toxicity of ionic manganese when this agent is used. The released Mn²⁺ binds to plasma protein and is rapidly cleared from the blood, pre-

TABLE 1. Comparison of tumor contrast-enhancement patterns among hepatobiliary MRI contrast agents

	TCE patterns		
	Negative TCE	Positive TCE	Peritumoral rim ^a
Duration			
Mangafodipir trisodium	>3 h	48 h	48 h
Gadoxetic acid	<1 h	2 h	2 h
MS 264	<1 h	2 h	2 h
Optimal phase			
Mangafodipir trisodium	30 min	24 h	24 h
Gadoxetic acid	15–20 min	30 min	30 min
MS 264	15–20 min	30 min	30 min
Frequency			
Mangafodipir trisodium	HCC, 50%; IT, 100%	HCC, 50%; IT, 0%	HCC, 33%; IT, 70%
Gadoxetic acid	HCC, 95%; IT, 100%	HCC, 5%; IT, 0%	HCC, 10%; IT, 100%
MS 264	HCC, 91%	HCC, 9%	HCC, 9%
Histology			
Mangafodipir trisodium	Grade IV HCC, IT	Grades I–III HCC	Grade IV HCC, IT
Gadoxetic acid	Grades II–IV HCC, IT	Grade I HCC	Grade IV HCC, IT
MS 264	Grades II–IV HCC	Grade I HCC	Grades II–IV HCC

TCE, tumor contrast enhancement; HCC, hepatocellular carcinoma; and IT, implanted tumor.

^a Rims are generally more pronounced and last longer with mangafodipir trisodium than with the other two agents.

dominantly by hepatic uptake. In contrast, the slight observed hepatic uptake of DPDP ligand indicates that the ligand does not play a major role in facilitating intracellular uptake of Mn^{2+} (24). Because manganese is an essential trace element and liver is the key organ for maintenance of body homeostasis of manganese, the mechanisms of mangafodipir trisodium-induced positive and negative TCE of liver tumors therefore can be attributed to hepatic function-determined local Mn^{2+} kinetics. The uptake of Mn^{2+} appears to be a fundamental or primitive function that is present not only in normal hepatocytes but also in a large number of HCC cells. Furthermore, local cholestasis is a common feature of nodular hepatic lesions as a result of dysplasia of biliary channels. Thus, a high incidence of positive TCE with mangafodipir trisodium should not be surprising because the capability of Mn^{2+} uptake and incapability of biliary excretion coexist in the majority of HCCs as well as in some benign hepatomas. Only when intracellular uptake of Mn^{2+} is virtually deficient, as seen with grade IV HCC and implanted liver tumors, can a negative TCE be expected.

On the other hand, both gadoxetic acid and MS-264 are similar agents derived from the hydrophilic extracellular fluid agent gadopentetate dimeglumine. The addition of lipophilic ligands ethoxybenzyl and butylbenzyl to the metal chelator DTPA renders the intracellular character or hepatobiliary specificity to the new derivatives (12–14). Studies have suggested that the pathway for mangafodipir trisodium and its metabolites is distinct from that used by bilirubin and its pharmacological competitors such as gadoxetic acid and MS-

264 (27,28). The uptake and elimination of these two agents are believed to require a higher level of hepatic function that involves the organic anion transport system and biliary excretion system. It has been demonstrated that they compete at least in part with bilirubin for the same organic anion excretory mechanisms (27,29–31). It seems that only the most differentiated hepatomas and benign nodules can possibly preserve this "normal" uptake function. However, with the absence of a normal biliary drainage network, these tumors retain gadoxetic acid or MS-264 for a longer period of time than does normal liver, leading to a prolonged positive TCE. Ultimately, the agent may reenter to the blood circulation and is excreted by the kidneys, normal liver, or both. The majority of the hepatomas, as well as implanted tumors, lack this uptake function and therefore show only a negative TCE. The scarcity of this negative TCE pattern has been acknowledged on the basis of both experiments in animals (18,32–34) and clinical experiences (35,36). Indeed, the eventual role of this pattern in grading hepatocyte-derived tumors must be defined by an enlarged sample size of histologically proved HCC cases during further clinical trials.

The present results emphasize that early and delayed MRI after the injection of mangafodipir trisodium can be equally important because the latter provides better delineation of differentiated HCCs and easier recognition of the rims around highly malignant liver tumors (16–19). This has been continuously supported by the results of clinical trials (37,38). Although such an overnight delayed scan with mangafodipir trisodium is

not suitable for use with gadoxetic acid and MS-264, a slightly delayed postcontrast imaging at 20–40 min (away from perfusion phase) can be helpful to gain greater conspicuity with both negative TCE in hypervascular lesions and positive TCE in highly differentiated hepatocellular tumors (19).

The experimental findings as summarized in Fig. 3 and Table 1 display a general trend of the behaviors of three hepatobiliary agents in the tumor models used and imaging protocol applied. Despite a high degree of consistency between these findings and clinical outcomes (35–41), a few potential pitfalls should be recognized before translating them into clinical situations. First, the frequency of TCE patterns for each CA was based on a specific group of animals with probably biased proportions of tumor types and do not necessarily reflect the frequency in the clinical setting. For example, the undifferentiated HCCs could be less commonly seen in patients than in this rat model; therefore, a higher percentage of mangafodipir trisodium-induced positive TCE in patients with an HCC can be expected. On the other hand, the implanted tumor models may not cover all possibilities for human metastases. For example, positive TCE with mangafodipir trisodium has occasionally been noticed in liver metastases of endocrine tumors (E. Rummeny, personal communication, 1997) due to a preferential uptake by these tissues (42–44).

Second, the potential of hepatobiliary CAs for use in liver tumor characterization could have been underestimated by the present studies. Because the fast MRI acquisition was not available when the studies were performed, only conventional T1W spin-echo sequences were applied, which required ~10 min for one measurement. Therefore, the biphasic contrast-enhancing property of the CAs failed to be fully used, and the described TCE patterns were relevant mainly to the second hepatobiliary phase. It is now known that the dramatic changes of TCE at the first distribution or perfusion phase occurring within a couple of minutes are also crucial for distinguishing focal liver lesions such as metastases, HCC, focal nodular hyperplasia, and hemangioma (35,36). To determine whether a positive TCE is caused by real intracellular uptake or by extracellular perfusion combined with a delayed washout could indeed be problematic, especially when “short-life” agents such as gadoxetic acid are being used (33,36).

Third, the capability to depict lesions as small as 2–3 mm has been well demonstrated in the present study, as exemplified in Fig. 2; it is due to the high MRI spatial resolution produced by the combined use of a dedicated rat coil, small field of view of 10 cm, and thin-slice

thickness of 2 mm. These obviously are not realistic from a clinical point of view.

Last, the doses of the CAs used in animal experiments usually are larger than those used in a clinic setting. Therefore, it should not be surprising to see a less-pronounced TCE pattern in patients with use of the same CA but a lower dose. On the other hand, because the MRI sequences used in animal experiments were not optimized to maximize the contrast-enhancing capacity, if a recently developed acquisition technique is manipulated, a comparable or even better TCE may be obtained despite a lower clinical dose. This is a well known advantage of MRI over other imaging modalities.

For more-specific tumor detection and characterization, many efforts have been made to develop antibody- and porphyrin-based magnetopharmaceuticals (45–47). These agents are unlikely to be able to reach clinical stage due to practical limitations for antibody agents (45,46) and questionable tumor targetability for porphyrin agents (48–50), whereas most of the hepatobiliary agents have displayed promising clinical profiles of both safety and efficacy. Although they are not tumor specific, the different spectra of positive and negative TCE induced by these agents may enable not only determination of tumors of hepatocytic origin but also assessment of the grade of cellular differentiation and degree of invasiveness with certain reliability. The demand for accurate noninvasive tissue characterization can be highlighted by a recently reported unexpectedly high incidence of benign liver tumors in the adult population (51) and by an increased tendency to use aggressive therapies for patients with hepatic tumors (52). In this context, the ability to discriminate differentiated HCCs from others or to recognize benign hepatocytic nodules and highly differentiated HCCs with mangafodipir trisodium- or gadoxetic acid-enhanced MRI could be meaningful.

Histologic examination at autopsy and needle or surgical biopsy has been considered the only reliable way to accurately characterize malignancies. However, surgeons seem to be increasingly reluctant to operate on patients who have had percutaneous liver biopsy and are at risk of a postoperative recurrence due to a prior tumor seeding along the needle tract (53). On the other hand, morphologic dysplasia of neoplasm is always accompanied by functional and metabolic alterations. The latter aspect can also be used for imaging diagnosis. For example, the positive TCE as a result of uptake and retention of hepatobiliary agents may serve as an *in vivo* “functional marker” for certain hepatic tumors, and the peritumoral rim could be regarded as an *in vivo* sign for the invasiveness of the tumor. Efforts in this

direction may lead to a single-step approach with the use of noninvasive contrast-enhanced MRI for both detection and characterization of liver tumors.

STUDIES OF BILIARY OBSTRUCTION

Animal models

Models of acute and chronic, total (TBO) and segmental (SBO) bile duct obstruction were surgically induced in rats and rabbits during studies with mangafodipir trisodium and gadoxetic acid (29,54–57). Removable total biliary obstruction (RTBO) also was induced in rats to investigate the potential side effect of the secondary intrahepatic cholestasis associated with the use of mangafodipir trisodium in patients with jaundice (56).

Experimental protocols

Groups of animals with different forms of obstructed cholestasis and normal and sham-treated animals were imaged with serial T1W MRI before and after the injection of mangafodipir trisodium at 25 $\mu\text{mol/kg}$ (54,57) or gadoxetic acid at 30 $\mu\text{mol/kg}$ (or with a repeated dose as designed) (29,55). Imaging was quantified as either absolute enhancement signal-to-noise ratio or relative

enhancement and compared between the different treatment groups. The imaging findings also were correlated with postmortem microcholangiography, histomorphology, and serum bilirubin levels.

Results and discussions

There is concern over both the fate of hepatobiliary agents and imaging outcomes when the agents are used in the presence of biliary obstruction and abnormal biliary excretion (29–31,54–60). In the present series of studies, different forms of obstructed cholestasis were virtually undetectable with unenhanced MRI, whereas with injection of mangafodipir trisodium and gadoxetic acid, both total and segmental obstructive cholestasis could be identified with enhanced MRI (Figs. 4 and 5). However, the cholestatic liver was characterized differently on MRI with different hepatobiliary CAs depending on their pharmacodynamic properties (Table 2).

After the injection of mangafodipir trisodium, the liver of both TBO and SBO rats was strongly enhanced at a level comparable to that of normal rats. The enhanced liver intensities were normalized within 24 h in unobstructed lobes of SBO rats as well as in normal rats but decreased slowly for a couple of days in the obstructed lobes of SBO rats, leading to a prolonged

FIG. 4. Schematic of hepatobiliary MRI contrast agent-induced contrast-enhancement patterns in different status of bile flow. (Refer to the text and Table 2 for detailed explanations.)

FIG. 5. T1-weighted spin-echo (TR/TE = 600/15 ms) MR images before (A) and 10 min (B) and 40 min (C) after intravenous injection of gadoxetic acid at 0.03 mmol/kg display an inability of unenhanced MRI to show segmental obstructive cholestasis (A), almost equal enhancement in both obstructed and unobstructed lobes soon after contrast administration (B), and a distinct lobar contrast between obstructed and unobstructed lobes at a slightly delayed phase (C).

interlobar contrast. The liver enhancement persisted in TBO rats as long as the common bile duct was obstructed (54,56,57). The enhancement of extrahepatic tissues such as renal cortex and spleen paralleled to some extent the prolonged enhancement of the obstructed lobes in SBO rats and the persistent liver enhancement in TBO rats, suggesting a certain degree of Mn^{2+} retention (54).

Results were largely different with the use of gadoxetic acid. In contrast to normal and SBO rats, this CA did not cause substantial liver enhancement in TBO rats as long as the obstruction was effective as demonstrated by a high bilirubin level (29,55). However, segmental cholestasis was equally visible but started at an earlier postcontrast phase (Fig. 5) (29,55) as opposed to that shown with mangafodipir trisodium on more-delayed images (54). The phenomenon of interlobar contrast enhancement with both mangafodipir trisodium and gadoxetic acid lasting a couple of days can be attributed to the fraction of the CAs that have been excreted to and temporarily retained in the obstructed part of the biliary tree (from the ligated hepatic duct to its distal bile canaliculi between hepatocytes). Such biliary excretion across the hepatocyte membrane on the bile canalicular side is known to be an active process against concentration gradient and therefore is unidirectional. Although the other proportion of the CAs being taken up but not yet excreted by the hepatocytes may reenter the blood across the membrane on the sinusoidal side and be eliminated by unobstructed liver lobes, kidneys, or both, this proportion responsible for the prolonged enhancement can be drained only through a new bile collateral network, which in rats takes a couple of days to form (29,55). The prolonged lobar enhancement as a result of normal contrast uptake but impaired bile drainage in the SBO

rats mimicks the phenomena in tumor studies and therefore supports the proposed mechanisms for the observed positive TCE in differentiated HCCs (16–20), the peritumoral rim enhancement in highly malignant tumors (17,19,20), and the wedge-type enhancement in patients with liver tumors (38).

In bile duct obstruction, unlike mangafodipir trisodium, which is still effective in enhancing the liver even in obstructive hyperbilirubinemia (54), whether gadoxetic acid can enhance the liver depends on the amount of accumulated endogenous bilirubin, which is known to be a metabolic competitor of this agent (27,29–31).

With time, both TBO and SBO models became invalid in rats due to biliary collateral formation that bypassed the obstruction, as demonstrated with serum bilirubin testing in correlation with microcholangiography (29,55). Because such internal evolution in biliary excretion system can be nicely detected with hepatobiliary CA-enhanced MRI (29,54–57), noninvasive mapping of both global and regional obstructive cholestases of varying causes can be implicated as a novel clinical application for these agents, although the imaging must be carefully interpreted regarding the specific agent used.

The next sensitive issue is whether the use of these CAs in cholestatic patients can cause detrimental effects due to retention by the body of contrast materials as a result of impaired biliary excretion. In contrast to mangafodipir trisodium, which possesses only a rigid hepatobiliary pathway for dissociated Mn^{2+} , gadoxetic acid has flexible dual excretion routes, (through the bile or urine (59,60)) and therefore can be efficiently eliminated even in long-term cholestasis without the risk of CA retention. This may make gadoxetic acid more suit-

TABLE 2. Hepatobiliary contrast agents in normal and impaired biliary excretion

Hepatobiliary contrast agents	Bile flow					
	Mangafodipir trisodium			Gadoxetic acid		
	Normal	SBO	TBO	Normal	SBO	TBO
Contrast enhancement pattern	Strong enhancement	Interlobar contrast	Strong enhancement	Strong enhancement	Interlobar contrast	Minimal enhancement
Duration	<24 h	3–48 h	>48 h	<2 h	20 min to 48 h	
Optimal phase	30 min	24 h	30 min–48 h	15–30 min	30 min to 24 h	30 min
Mechanism	Normal uptake and biliary excretion	Normal uptake; impaired biliary elimination in obstructed lobes	Normal uptake; impaired biliary elimination	Normal uptake and biliary excretion	Normal uptake; impaired biliary elimination in obstructed lobes	Impaired uptake and biliary elimination

SBO, selective or segmental biliary obstruction; and TBO, total biliary obstruction.

able than mangafodipir trisodium for monitoring of cholestasis with MRI. In fact, this CA has been advocated to be used clinically in MRI cholangiography (61). However, with complete biliary obstruction, gadoxetic acid cannot produce significant liver enhancement because its uptake by hepatocytes is impaired by the competition with increased bilirubin (29). Therefore, for effective liver lesion detection and characterization in patients with obstructive jaundice, in theory, gadoxetic acid with bile drainage before imaging to ensure sufficient liver enhancement or mangafodipir trisodium with bile drainage before or after imaging to reduce the potential risk of Mn^{2+} retention may be tried.

Increased attention has been paid to the safety of mangafodipir trisodium (30,56,62,63). The combined administration of free Mn^{2+} and bilirubin can induce intrahepatic cholestasis in an animal model (64–70). Free Mn^{2+} is released after mangafodipir trisodium injection (71), and persistent liver enhancement after mangafodipir trisodium injection in cholestatic subjects suggests the coexistence of free Mn^{2+} and high levels of endogenous bilirubin (54). These observations have triggered understandable concern that in jaundice commonly associated with hepatic and pancreatic tumors, the use of mangafodipir trisodium might cause a secondary intrahepatic cholestasis in addition to the primary cholestatic disease due to an interaction between the dissociated Mn^{2+} and increased endogenous bilirubin (30,54,62). To investigate this possible side effect, an experiment in animals was conducted (56). Removable total biliary obstruction was surgically induced in rats to build up a high bilirubin level and to mimic the situation of a cholestatic patient before and after biliary drainage. After the intravenous injection of mangafodipir trisodium at 25 $\mu\text{mol/kg}$ (the highest dose used in humans (72) but five times the rec-

ommended clinical dose (73)), a period of interaction between free Mn^{2+} and bilirubin in the liver was ensured for 12 h before removal of the obstruction. Sequential blood sampling and T1W spin-echo MRI were conducted over 4 days. Another group of rats with removable total biliary obstruction underwent the same experimental regimen except for mangafodipir trisodium injection and served as blank controls. The evolution of the bilirubin level and liver intensity on MRI were evaluated. In both mangafodipir trisodium-injected and control rats, the bilirubin levels rapidly decreased within 4 h after restoration of bile flow and almost normalized within 24 h. In the mangafodipir trisodium group, the progressive reduction of liver intensity paralleled the decreasing bilirubin levels. Neither persisting liver enhancement nor prolonged high bilirubin levels were observed. This study suggests mangafodipir trisodium is unlikely to cause secondary intrahepatic cholestasis in animals with biliary obstruction and retained free Mn^{2+} was readily eliminated by restoration of bile flow.

There are differences between the clinical setting of a patient with obstructive jaundice who is administered mangafodipir trisodium and the experimental condition of the Mn^{2+} bilirubin-induced animal model of intrahepatic cholestasis. First, only exogenous unconjugated bilirubin was effective in producing cholestasis (64–66), whereas acute obstructive cholestasis presents mainly conjugated bilirubin. Second, only when free Mn^{2+} was injected before bilirubin could the cholestatic model be established (70), whereas in clinical or experimental jaundice, mangafodipir trisodium would be used in subjects who already have high bilirubin levels.

Finally, the highest clinical dose of mangafodipir trisodium is still much lower than the minimum amount of free Mn^{2+} that was effective in the cholestatic model

(65,72,73). Therefore, based on the results of this dedicated experiment as well as these arguments, the potential side effect of mangafodipir trisodium in inducing intrahepatic cholestasis virtually can be ruled out. Indeed, because Mn^{2+} is an essential nutritional trace element with a regular daily turnover, the proportion of dissociated Mn^{2+} from a single clinical dose of mangafodipir trisodium (5 μ mol/kg), even if it is retained in the body, would be very unlikely to cause an important side effect.

CONCLUSIONS

Preclinical studies in comprehensive animal models have revealed extremely promising potentials for a new generation of tissue-specific MRI CAs (i.e., hepatobiliary agents) in the noninvasive diagnosis of liver disease. The extensive clinical application of these CAs will undoubtedly prove and even expand their capabilities in both lesion detection and tissue characterization of diffuse and focal hepatic abnormalities, including malignant and benign liver tumors and global and local cholestases.

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