

ORIGINAL ARTICLE

Assessing Liver Function by T1 Maps on Gd-EOB-DTPA-Enhanced MRI for up to 50 Min in Rat Models of Liver Fibrosis: A Longer Hepatobiliary Time Period may Help

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Objectives To investigate whether a longer time period of gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA)-enhanced T1 mapping scanning, as well as dynamic contrast-enhanced (DCE) and multiple hepatobiliary phase magnetic resonance imaging (MRI) have the potential to provide information about liver function in rats with liver fibrosis.

Methods Forty rats were divided into the carbon tetrachloride-induced hepatic injury groups [carbon tetrachloride for four ($n=14$), eight ($n=8$), or twelve ($n=8$) weeks] and the control group ($n=10$). Gd-EOB-DTPA-enhanced MRI was performed including T1-mapping (delayed to 50 min), DCE, and multiple hepatobiliary phases. Indocyanine green retention rate at 15 min (ICG-R15) was determined. Parameters such as T1 reduction rate ($\Delta T1$), elimination half-life of $\Delta T1$ ($T_{\Delta T1\ 1/2}$), relative enhancement (RE), time to maximum RE (T_{max}), and perfusion parameters were calculated. *Pearson* correlation analysis was used for correlation analysis between ICG-R15 and each MRI indices.

Results $\Delta T1$ at 30, 40, and 50 min showed significant positive correlations with ICG-R15 ($r=0.784, 0.653, 0.757, P=0.007, 0.041, 0.030$). $T_{\Delta T1\ 1/2}$ showed a significant positive correlation with ICG-R15 ($r=0.685, P=0.029$). T_{max} showed a significant positive correlation with ICG-R15 ($r=0.532, P=0.019$).

Conclusions $\Delta T1$ in the late hepatobiliary phase and $T_{\Delta T1\ 1/2}$ exhibited moderate correlations with liver function. The longer time period of Gd-EOB-DTPA-enhanced T1 mapping scanning, as well as DCE and multiple hepatobiliary phases, may be of some value for estimating liver function in rats with liver fibrosis.

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LIVER function assessment plays an essential role in patients with chronic liver diseases and is an integral part of therapy decision making in patients who are candidates for surgical management, especially in those with diffuse liver diseases such as liver fibrosis.^[1] The liver performs a wide variety of critical biochemical, synthetic, and excretory functions. Most of the currently used methods for liver function measurement such as serum biochemical parameters are indirect methods, except for indocyanine green (ICG) clearance, which is considered as the gold standard for estimating the true global liver function.^[2, 3] Nevertheless, biochemical parameters are limited when the liver function is heterogeneously distributed in the organ.

Compared to serum parameters, image-based liver function tests have the advantages of offering liver spatial information.^[3] Gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA) is a liver-specific MR contrast agent that first distributes in the vessels and extracellular spaces during the arterial and portal phases, and then is selectively taken up and excreted by hepatic cell membrane transporters such as OATPB1/B3 and MRP2, in a mechanism similar to that of ICG.^[4] Therefore, Gd-EOB-DTPA has the potential to provide information on hepatic function with both dynamic contrast-enhanced (DCE) and hepatobiliary phase images.^[5]

Until now, various approaches have been used for the assessment of hepatic function using Gd-EOB-DTPA-enhanced liver MRI, including measurements of signal intensity (SI) at hepatobiliary phase,^[6-9] DCE MRI,^[10-13] and T1 mapping.^[14-19] Among these methods, T1 mapping has been considered to be less affected by MR parameters at the same magnetic field strength than SI measurements.^[14-19] Many previous studies used T1 mapping at 20 min for the evaluation of liver function and showed that dysfunctional liver has less uptake of Gd-EOB-DTPA.^[17, 20, 21] Nevertheless, limited knowledge is available regarding the results of a longer period of time. Since liver function involves not only uptake but also excretion, we hypothesized that a longer period of time might provide additional information about liver function. Thus, the assessment of the Gd-EOB-DTPA excretion might provide useful information for the quantification of liver function using MRI.

Therefore, the aim of this study was to use Gd-EOB-DTPA-enhanced MRI to explore whether longer time period T1 mapping scanning might yield addition-

al information for liver function measurements, as well as the potential of DCE perfusion parameters and multiple hepatobiliary phase parameters in liver function assessment.

MATERIALS AND METHODS

Animal models

This study was approved by our Institutional Animal Care and Use Committee and was performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Forty healthy male Sprague-Dawley rats (180-220 g, 6-8 weeks old, obtained from Beijing Vital River Lab Animal Technology Co., Ltd.), were used in this study. The rats were maintained in the animal experiment center under standard conditions and controlled environment of 12-hour light/dark cycle at room temperature (23°C). All rats were allowed free access to standard laboratory food and water. After 1 week of adaptation, the rats were divided into the experimental ($n=30$) and control ($n=10$) groups. In the experimental group, a mixture of carbon tetrachloride (CCl₄; Sinopharm Chemical Reagent Co., Ltd.) and olive oil (Sinopharm Chemical Reagent Co., Ltd.) [2:3 (v/v)] was injected intraperitoneally twice a week at 0.3 ml/100 g body weight for 4 ($n=14$), 8 ($n=8$), or 12 ($n=8$) weeks to induce different stages of liver fibrosis. In the control group, normal saline was applied. At 4, 8, and 12 weeks, rats from both the control and experimental groups were randomly selected to undergo MRI. Liver fibrosis in CCl₄-treated rats was confirmed by histopathological analysis. Histopathological analysis was performed using hematoxylin and eosin (H&E) and Masson trichrome staining.

Biochemistry

Blood samples were drawn from each rat *via* the tail vein 1 day before MRI examination. The serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (Tbil), and γ -glutamyl transpeptidase (GGT) were measured using an automatic biochemical analyzer (Beckman Coulter, Brea, CA, USA).

MRI

MRI examinations were performed using a 3.0 T scanner (MAGNETOM Skyra, Siemens Healthcare, Erlangen, Germany) with a 16-channel wrist coil. The rats

received an intraperitoneal injection of 60 mg/kg of body weight of pentobarbital sodium (Sigma, St. Louis, MI, USA) for anesthesia before MRI examinations. The MRI protocol included a DCE sequence, multiple hepatobiliary phase acquisitions, and T1 mapping series. A volume interpolated breath-hold examination (VIBE) sequence was used for DCE imaging and multiple hepatobiliary phase acquisitions (TR=6.22 ms, TE=2.46 ms, FOV=90 mm×200 mm, slice thickness=2 mm). For DCE imaging, 60 consecutive measurements were obtained (including three pre-contrast ones), for a total scanning time of 3 min (time intervals of 3 s) after the injection of the contrast agent. Subsequently, 10 datasets were acquired at 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 min after injection. Each rat received a bolus injection of Gd-EOB-DTPA (Primovist, Bayer Schering Pharma AG, Berlin, Germany; 0.025 mmol Gd/kg body weight, concentration of 0.025 mmol/ml) *via* the tail vein within 3 s using the syringe manually. A dual-flip-angle T1 3D VIBE sequence^[22-24] was used for T1 mapping imaging (TR=6.22 ms, TE=2.46 ms, FOV=90 mm×200 mm, slice thickness=2 mm, flip angle 1=3°, flip angle 2=15°), obtaining six series including one acquired before and five obtained after the administration of contrast (every 10 min from 10 to 50 min).

ICG clearance test

The ICG clearance test as an index of liver function was performed in all rats after MRI examinations. A single dose of ICG (0.5 mg/kg; Dandong Yichuang pharmaceutical Co., Ltd., Liaoning, China) was freshly prepared in saline and injected into the tail vein. Blood samples (1.5 ml) were collected into tubes containing heparin sodium before and 15 min after ICG administration. The samples were centrifuged at 3000 *g* for 4 min and the plasma was isolated. The plasma ICG concentration (the ICG retention rate at 15 min after intravenous injection of ICG, ICG R15) was determined using a Varioskan Flash system (Thermo Fisher Scientific, Waltham, MA, USA) at 805 nm.

Image analysis

A MMWP post-processing workstation (Siemens Healthcare) was used for image analysis. All MR images were evaluated by one radiologist with 2 years of experience. In T1 mapping images, regions of interest (ROIs) were drawn manually to cover the whole liver at the level of the hepatic portal vein as large as possible (3-4 cm²), avoiding imaging artifacts, major vascular

branches, and gallbladder, to obtain T1 values for hepatic parenchyma. The reduction rate in T1 values at different time point (from 10 to 50 min) between pre- and post-Gd-EOB-DTPA enhancement was calculated as follows: reduction rate of T1 values ($\Delta T1$) = $(T1_{pre} - T1_{post})/T1_{pre} \times 100\%$, where $T1_{pre}$ is the T1 relaxation time before Gd-EOB-DTPA administration and $T1_{post}$ is the T1 relaxation time at different time points ($T1_{10min}$ to $T1_{50min}$) after administration. The elimination half-life of $\Delta T1$ ($T_{\Delta T1 1/2}$) was calculated: first, the data ($\Delta T1$ values at different time points from 10 to 50 min) were transformed to logarithm; then, the data (half of $\Delta T1$ at 10 min) was used to calculate $T_{\Delta T1 1/2}$.

The DCE-MR data (3 min) were transferred to the 4D Tissue software (Siemens Healthcare) to calculate the perfusion parameters. ROIs were drawn manually to cover the whole liver at the level of the hepatic portal vein, as large as possible in the perfusion maps, avoiding major branches of the portal and hepatic veins and gallbladder. Perfusion parameters including the extravascular volume transfer (K_{trans}), extravascular fluid volume (V_e), extravascular to plasma volume transfer (K_{ep}), and initial area under the enhancement curve (iAUC) were measured.

DCE MR images (3 min) and multiple hepatobiliary phase acquisitions (between 5 and 50 min) were transferred to the software to obtain the SI-time curve. ROIs were drawn manually to cover the whole liver at the level of the hepatic portal vein avoiding liver edge, large blood vessels, and the gallbladder to obtain the SI value. Relative enhancement (RE) at 3, 20, and 50 min was calculated from the SI measurements before (SI_{pre}) and after (SI_{post}) intravenous Gd-EOB-DTPA administration: $RE = (SI_{post} - SI_{pre})/SI_{pre} \times 100\%$. The maximum relative enhancement (RE_{max}) and the time to maximum RE (T_{max}) were obtained from the SI-time curve. The elimination half-life of RE ($T_{RE 1/2}$) was calculated as above. The MR scanning protocol and image analysis process are shown in **Fig. 1**.

For evaluation of inter-observer agreement, another radiologist drew the ROI in the same manner. After the MR examinations and ICG tests, each rat was euthanized by intravenous injection of pentobarbital sodium.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 (IBM, Armonk, New York, USA). Categorical variables were reported as counts. Continuous variables were

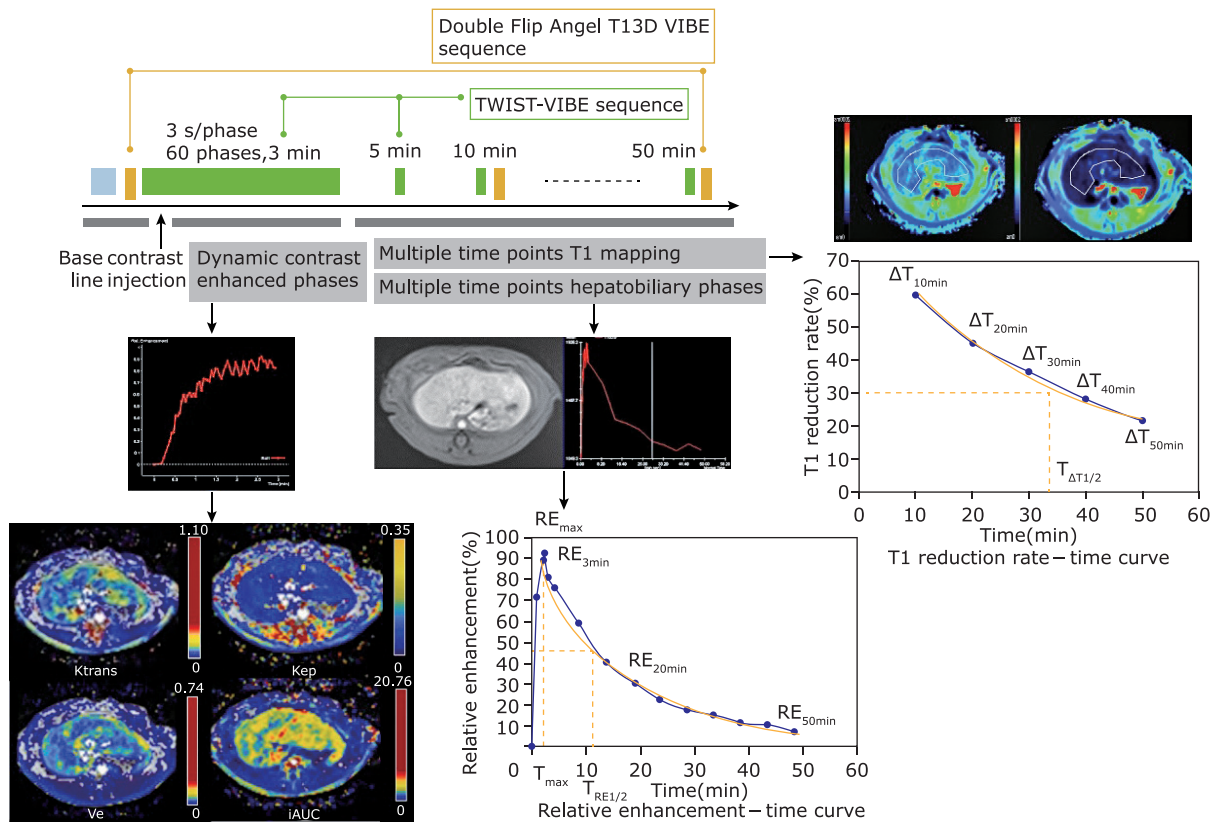


Figure 1. Magnetic resonance imaging (MRI) protocol and image analysis. The MRI protocol included dynamic contrast enhanced (DCE) sequence, multiple hepatobiliary phase acquisitions, and T1 mapping series. The regions of interest were drawn and the MRI parameters were measured and calculated from different datasets include: 1) T1 relaxation time, T1 reduction rate (ΔT_1) at different time points, and the elimination half-life of ΔT_1 ($T_{\Delta T_1 1/2}$); 2) Relative enhancement (RE) at 3, 20, and 50 min, $T_{RE1/2}$, T_{max} , and RE_{max} ; 3) extravascular volume transfer (Ktrans), extravascular fluid volume (Ve), extravascular to plasma volume transfer (Kep), and initial area under the enhancement curve (iAUC).

expressed as means $\pm$ standard deviation. The *Pearson* correlation analysis was applied for correlation analysis between ICG R15 value and each of the MR indices. The interpretation of coefficients was as follows: weak, 0.2 to less than 0.5; moderate, 0.5 to less than 0.8; and strong, 0.8 or greater. Inter-observer agreement for the MRI parameters was analyzed by using intra-class correlation coefficient (ICC). ICC values were interpreted according to Kundel and Polansky:^[25] 0.81–1.00, excellent; 0.61–0.80, good; 0.41–0.60, moderate; 0.21–0.40, fair; 0.00–0.20, poor. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Animal model

Fig. 2 provides Masson trichrome slides of representative rats, showing the normal and liver fibrosis with CCl_4 exposure. Eleven rats died from the intraperi-

toneal injection of CCl_4 or anesthesia before MRI examinations. Three died during MR examination after contrast injection. Four rats failed to complete the ICG clearance test due to intravenous catheter blockage. Finally, 22 rats completed both the ICG test and DCE and multiple hepatobiliary phase imaging (6 in the control group and 13 in the experimental group), 10 rats completed both the ICG test and T1 mapping (4 in the control group and 6 in the experimental group). T1 mapping was not successfully performed in 12 rats due to the error of scanning sequence parameters which failed to generate adequate images.

The mean values of ALT, AST, ALP, Tbil and GGT in the control group were 39.7 ± 11.7 U/L, 80.2 ± 23.9 U/L, 178.7 ± 58.7 U/L, 0.57 ± 0.3 $\mu\text{mol/L}$ and 1.2 ± 0.4 U/L, respectively. The mean values of ALT, AST, ALP, Tbil, and GGT in the experimental group were 76.6 ± 47.5 U/L, 129.3 ± 75.9 U/L, 237.1 ± 68.8 U/L, 1.3 ± 1.9 $\mu\text{mol/L}$, and 2.3 ± 1.2 U/L, respectively. The mean ICG R15

values in the control and experimental groups were $6.2\% \pm 4.4\%$ and $13.4\% \pm 12.4\%$, respectively. ALT, AST, ALP, Tbil and GGT were significantly and positively correlated with ICG R15 ($r=0.745$, $P=0.001$; $r=0.636$, $P=0.011$; $r=0.699$, $P=0.004$; $r=0.787$, $P<0.001$ and $r=0.710$, $P=0.003$; respectively), indicating that the animal models were consistent with previous studies.^[8, 10]

T1 mapping parameters

The mean T1 relaxation time of all rats at pre-contrast and 10, 20, 30, 40, and 50 min ($T_{1_{10\text{min}}}$ to $T_{1_{50\text{min}}}$) were 886.3 ± 99.1 ms, 397.6 ± 87.8 ms, 545.9 ± 116.1 ms, 629.1 ± 123.1 ms, 723.1 ± 158.1 ms, and 764.2 ± 162.5 ms, respectively. The mean T1 reduction rate at 10, 20, 30, 40, and 50 min were $54.9\% \pm 10.2\%$, $39.3\% \pm 12.8\%$, $28.7\% \pm 13.1\%$, $17.7\% \pm 15.8\%$, and $17.6\% \pm 14.6\%$, respectively. The mean $T_{\Delta T1\ 1/2}$ was 36.7 ± 21.5 min. The inter-observer agreement of the T1 relaxation time was excellent (ICC: 0.973, 95%CI=0.955-0.984). Examples of the T1 maps and T1 reduction rate-time curve of rats with different ICG R15 are shown in **Fig. 3**.

T1 relaxation time at 50 min showed a significant negative correlation with ICG R15 ($r=-0.634$, $P=0.049$, **Fig. 4A**). No significant correlation was found between ICG R15 and $T_{1\text{pre}}$ ($r=-0.225$, $P=0.440$), ICG R15 and $T_{1_{10\text{min}}}$ to $T_{1_{40\text{min}}}$ ($r=-0.049$, $P=0.893$; $r=-0.193$, $P=0.592$; $r=-0.481$, $P=0.159$; and $r=-0.532$, $P=0.114$, respectively). $\Delta T_{1_{30\text{min}}}$, $\Delta T_{1_{40\text{min}}}$, $\Delta T_{1_{50\text{min}}}$ showed significant positive correlations with ICG R15 ($r=0.784$, $P=0.007$; $r=0.653$, $P=0.041$; and $r=0.757$, $P=0.030$, respectively, **Fig. 4B, 4C, 4D**). $T_{\Delta T1\ 1/2}$ showed a significant positive correlation with ICG R15 ($r=0.685$, $P=0.029$, **Fig. 4E**). No significant differenc-

es were found between ICG R15 and $\Delta T_{1_{10\text{min}}}$ ($r=0.422$, $P=0.225$) or ICG R15 and $\Delta T_{1_{20\text{min}}}$ ($r=0.527$, $P=0.117$).

DCE and multiple hepatobiliary phase parameters

The mean RE_{max} and RE at 3, 20, and 50 min were $83.7\% \pm 21.3\%$, $82.1\% \pm 18.6\%$, $37.5\% \pm 20.6\%$, and $11.6\% \pm 9.4\%$ respectively. The mean T_{max} was 2.9 ± 0.7 min. The mean $T_{RE1/2}$ was 19.5 ± 12.7 min. The inter-observer agreement of the SI was excellent (ICC: 0.980, 95%CI=0.966-0.988). T_{max} showed a significant positive correlation with ICG R15 ($r=0.532$, $P=0.019$, **Fig. 4G**). $T_{RE1/2}$ showed a significant positive correlation with ICG R15 ($r=0.535$, $P=0.018$, **Fig. 4F**). RE_{max} showed no significant correlation with ICG R15 ($r=-0.416$, $P=0.077$). RE at 3, 20, and 50 min showed no significant correlation with ICG R15 ($r=-0.058$, $P=0.813$; $r=0.321$, $P=0.193$; and $r=0.233$, $P=0.338$, respectively).

The mean Ktrans, Kep, Ve, and iAUC were $0.44 \pm 0.12\ \text{min}^{-1}$, $1.43 \pm 0.26\ \text{min}^{-1}$, 0.34 ± 0.08 , and 11.49 ± 3.45 , respectively. The inter-observer agreement of the parameters was excellent (ICC: 0.996, 95%CI=0.994-0.998). No significant correlations were found between these perfusion parameters and ICG R15 ($r=-0.328$, $P=0.252$; $r=-0.461$, $P=0.097$; $r=-0.157$, $P=0.592$; and $r=-0.161$, $P=0.583$, respectively).

DISCUSSION

The present study showed that $\Delta T1$ at 30, 40, and 50 min was increased and $T_{\Delta T1\ 1/2}$ as well as $T_{RE1/2}$ and T_{max} were prolonged in rats with impaired liver function. This study added information to the present knowledge by focusing on the excretion of Gd-EOB-

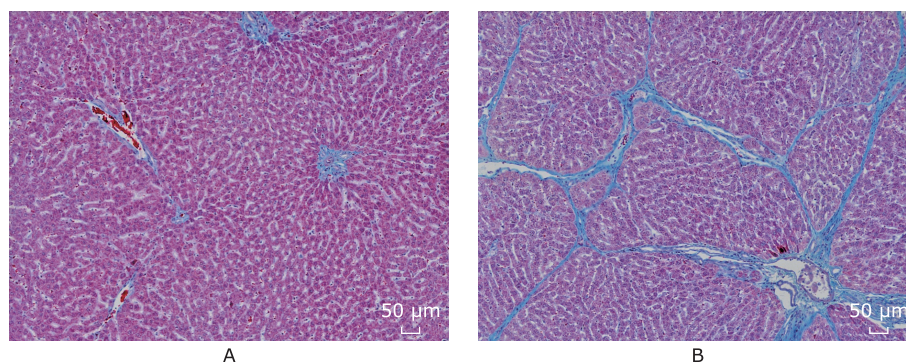


Figure 2. Pathological micrographs of rats in the control group and experimental group.

Histopathological analysis of liver sections stained with Masson trichrome ($\times 100$) showing normal (A) and liver fibrosis with CCl_4 exposure (B).

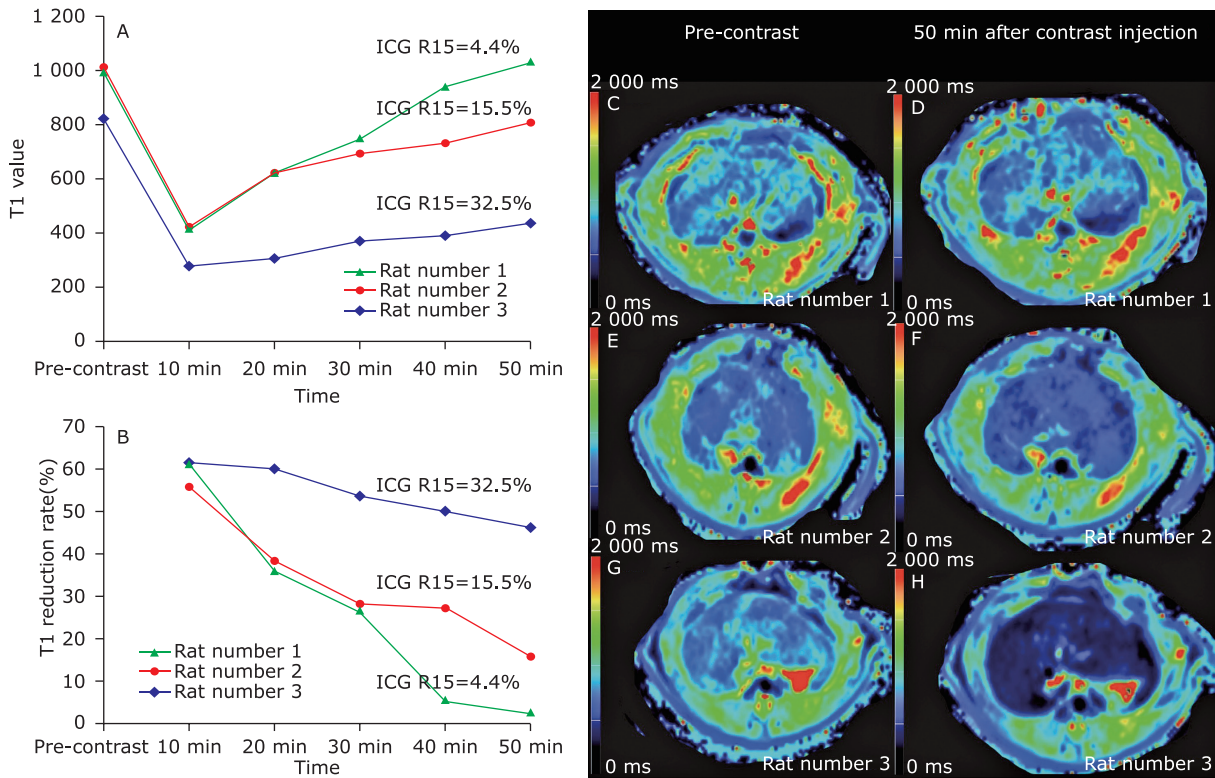


Figure 3. The T1 value-time curve (A), T1 reduction rate-time curve (B), and T1 maps obtained before (C, E, G) and 50 min after Gd-EOB-DPTA administration (D, F, H) of three rat models with varying liver function.

The indocyanine green retention rate at 15 min (ICG R15) values of rat number 1, rat number 2, and rat number 3 was 4.4%, 15.5%, and 32.5%, respectively. The worse the liver function was, the smaller T1 value and larger T1 reduction rate were found in the late hepatobiliary phase.

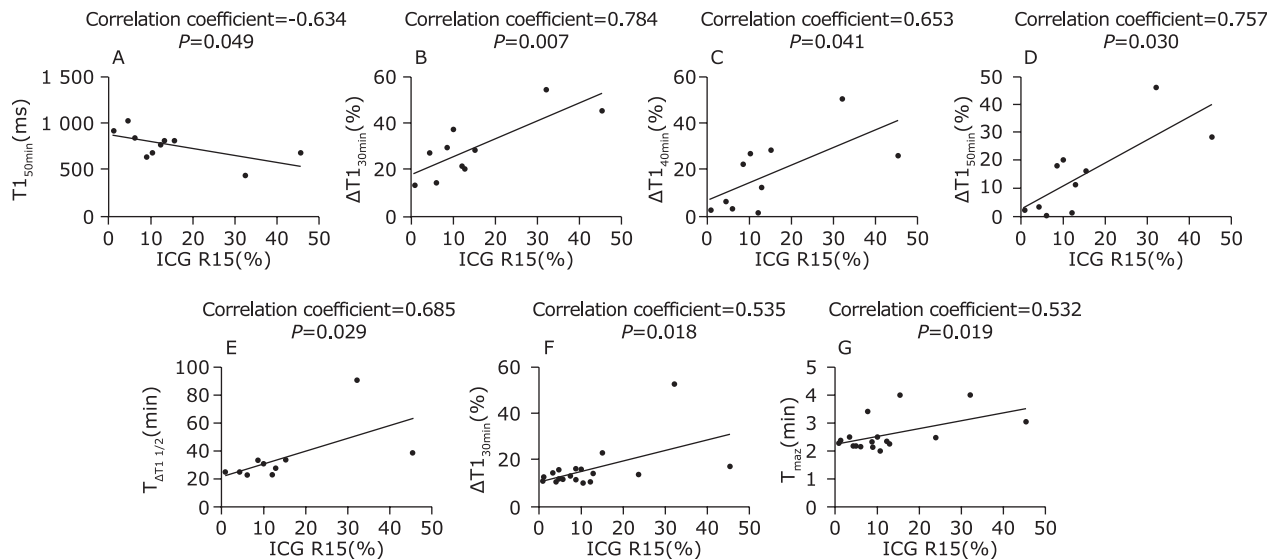


Figure 4. Scattergrams showing the relationships between the T1 mapping parameters, relative enhancement parameters, and indocyanine green retention rate at 15 minutes (ICG R15).

T1 relaxation time at 50 min showed a significant negative correlation with ICG R15 (A, $r = -0.634$, $P = 0.049$). $\Delta T1_{30min}$, $\Delta T1_{40min}$, $\Delta T1_{50min}$ showed significant positive correlations with ICG R15 (B, $r = 0.784$, $P = 0.007$; C, $r = 0.653$, $P = 0.041$; and D, $r = 0.757$, $P = 0.030$, respectively). $T_{\Delta T1 \ 1/2}$ showed a significant positive correlation with ICG R15 (E, $r = 0.685$, $P = 0.029$). $T_{RE1/2}$ and T_{max} showed significant positive correlation with ICG R15 (F, $r = 0.535$, $P = 0.018$; G, $r = 0.532$, $P = 0.019$).

DTPA by multiple time points from early to late hepatobiliary phases to explore the relationship between T1 mapping with a longer period of time and liver function.

The potential of T1 mapping using Gd-EOB-DTPA in evaluating liver function has been examined in many studies, especially the relationship between T1 relaxation time/ $\Delta T1$ of the liver parenchyma 20 min after contrast injection and Child-Pugh classification, ICG clearance, or the model for end-stage liver disease (MELD) score.^[14-16, 20, 26] Since Gd-EOB-DTPA has a T1 shortening effect on the liver parenchyma and can be specifically taken up by hepatic cells, and a higher uptake of Gd-EOB-DTPA by hepatic cells leads to shorter T1 relaxation time and larger $\Delta T1$. Liver dysfunction may lead to changes in transporter number or activity, resulting in lower uptake and smaller $\Delta T1$ compared with healthy controls. Katsube *et al.*^[14] compared the T1 relaxation time and $\Delta T1$ at 3, 8, 13, and 18 min after Gd-EOB-DTPA administration between four groups of patients (normal liver function, chronic hepatitis, liver cirrhosis with Child-Pugh A, and liver cirrhosis with Child-Pugh B) and their results showed that post-contrast T1 relaxation times were significantly longer for the Child-Pugh B group than for the other groups at all time points. Similarly, previous studies also showed that T1 relaxation time/ $\Delta T1$ of the liver at 20 min after injection has a strong correlation with the extent of liver dysfunction expressed by the MELD score and ICG.^[16, 27] In this study, T1 in the late hepatobiliary phase and $T_{\Delta T1\ 1/2}$ exhibited moderate correlations with liver function, indicating the potential of T1 mapping in the late hepatobiliary phase for the evaluation of liver function. Importantly, the parameters mentioned above were also consistent with the biochemistry results evaluating liver function by routine methods.

Different from the previous studies that showed a decreased T1 reduction rate at 20 min in dysfunctional liver,^[16, 18, 19, 27-30] the present study used a longer scanning time and the results showed that in rat models with impaired liver function the T1 reduction rate at 30, 40, and 50 min increased. The possible explanation may be that liver dysfunction not only influences the uptake of the contrast agent, as shown by previous studies,^[16, 18, 19, 27-30] but may also be accompanied by diminished excretion.^[31] Therefore, a slower excretion of Gd-EOB-DTPA will lead to more accumulation of the contrast agent in the hepatocytes in the late hepato-

biliary phase, resulting in a larger T1 reduction rate at late hepatobiliary phase and a longer half elimination time in a functionally impaired liver compared with a normal functioning liver. A previous study done in Mrp2-deficient rats using Gd-EOB-DTPA enhanced MRI showed that the relative enhancement values at 10 to 40 min after contrast injection were significantly higher in the Mrp2-deficient group than those in the control group,^[32] supporting the present study. Nevertheless, a study carried out in patients with primary sclerosing cholangitis showed significant negative correlations of $\Delta T1$ at 16 and 132 min after contrast injection with MELD score.^[33] One possible explanation may be that the circulation rate in the liver is different between rats and humans. Besides, contrary to previous studies, we did not observe a correlation between T1 relaxation time/ $\Delta T1$ at 10 or 20 min and liver function; this may also be due to a faster circulation rate in rats than in humans. The liver parenchyma may reach the hepatobiliary phase at a much earlier time point and the $\Delta T1$ may be influenced by both the uptake and the excretion of Gd-EOB-DTPA, with counter effects at this period of time.

We also compared the relative enhancement and liver function. Similar to $T_{\Delta T1\ 1/2}$, the $T_{RE1/2}$ was also prolonged in the dysfunctional liver. This is consistent with previous studies that showed that prolongation of the elimination half-life of RE on Gd-EOB-DTPA-enhanced MRI correlated with the severity of fibrosis in nutrient-deficient dietary NASH models.^[31, 34] Nevertheless, different from what we found in T1 maps, we found no significant correlation between ICG R15 and RE at 20 min or later time points. SI measurement is influenced by many technical parameters such as potency of the radiofrequency amplifier, the receiver coil, and the pulse sequence, while T1 relaxation time is not affected by those limitations.^[14] A study reported that the T1 reduction rate of the liver at 20 min showed a higher correlation coefficient with ICG clearance than RE.^[17] Correlation analysis between each variable and pathological variables, especially Mrp2 immunostaining, has been conducted and the data are consistent with the present study.

In addition, we found that the time to maximum RE showed a positive correlation with ICG R15, indicating a slower liver enhancement in rats with impaired liver function. Tsuda *et al.*^[31] reported a significantly prolonged time to maximum RE in NASH 10-week rats compared to control and NASH 7-week rat models,

which may support our result to some extent. Yamada *et al.*^[34] also reported a correlation between time to maximum RE with steatosis score and the severity of fibrosis. Since Gd-EOB-DTPA may be distributed in both the extravascular extracellular space and in hepatocytes during this period of time (which is around 3 min), this delay of peak enhancement may be either due to a diminished hepatic microcirculation or a slower uptake of the contrast medium by hepatocytes or both. We also compared the perfusion parameters Ktrans, Kep, Ve, and iAUC with ICG R15, and no significant correlations were found. This may imply that the impaired liver function may be reflected by a slower uptake by hepatocytes more than the change in hepatic microcirculation. Zhang *et al.*^[35] found that the Ktrans, Kep, Ve, and iAUC were significantly different between different fibrosis stages in rat models, but we did not find significant relationships between these parameters and liver function, probably because liver fibrosis does not always correlate with liver dysfunction.

The present study has some limitations. First, the number of rats was small and the number of rats with ICG R15 >20% was limited, which may lead to sample error. Secondly, this study was done in rat models and what we discovered in rats may be different in humans. Thirdly, the model we used to process DCE-MR images was the Tofts model rather than a dual-input two-compartment model, the latter is considered more accurate when investigating the hepatic blood supply.^[36] However, previous research had used Tofts model to evaluate liver fibrosis in rat models,^[35] which is similar in our study. Fourthly, the expressions of Oatp1 and Mrp2 were not obtained and further evaluation of Oatp1 and Mrp2 would be helpful in explaining the results of this study. Fifthly, the exact longer scanning time remains to be investigated in different animal models and human subjects, which means that the time points found in rat model with significant correlation of imaging parameters and ICG-R15 cannot directly correspond to the same time points in humans, but the results of the present study indicate the potential of T1 mapping in the late hepatobiliary phase for the evaluation of liver function. Whether the longer time period of T1 mapping time points have practical application value and operability in human remains to be further confirmed.

In conclusion, ΔT_1 reduction rate in the late hepatobiliary phase and $T_{\Delta T_1 \ 1/2}$ exhibited significant

correlations with liver function, indicating the potential of late hepatobiliary phase for providing information about liver function. The longer time period of Gd-EOB-DTPA-enhanced T1 mapping scanning, DCE, and multiple hepatobiliary phases may be of some value for estimating liver function in rats with liver fibrosis.

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Conflicts of Interest Statement

The authors have no competing interest to declare.

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中文摘要

探索应用延长至 50 分钟的钆赛酸二钠增强磁共振 T1 Maps 评价大鼠肝纤维化模型肝功能的价值： 延长的肝胆期可能提供帮助

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摘要

目的 探索延长成像时间的钆赛酸二钠 (Gd-EOB-DTPA) 增强磁共振 T1 mapping 成像及快速动态增强扫描序列 (DCE)、多期肝胆期磁共振成像对于大鼠肝纤维模型肝功能的评价是否存在潜在价值。

方法 40 只雄性 SD 大鼠，分为采用四氯化碳溶液腹腔注射的肝功能损伤组 [四氯化碳溶液分别注射 4 周 ($n=14$)、8 周 ($n=8$)、12 周 ($n=8$)] 以及对照组 ($n=10$)。钆赛酸二钠磁共振增强扫描序列包括 T1-mapping 成像 (延至注射对比剂后 50 分钟)、DCE、以及多期肝胆期成像。测量获得吲哚菁绿 15 分钟滞留率 (ICG R15)。图像分析计算获得影像学参数如 T1 弛豫时间减低率 ($\Delta T1$)、 $\Delta T1$ 减半时间 ($T_{\Delta T1\ 1/2}$)、相对强化率 (RE)、RE 达峰时间 (T_{max})、灌注参数等。应用 *Pearson* 相关性分析比较影像学参数与 ICG R15 的关系。

结果 30、40、50 分钟的 $\Delta T1$ 和 ICG R15 具有显著正相关性 ($r=0.784, 0.653, 0.757, P=0.007, 0.041, 0.030$)。 $T_{\Delta T1\ 1/2}$ 与 ICG R15 具有显著正相关性 ($r=0.685, P=0.029$)。 T_{max} 与 ICG R15 具有显著正相关性 ($r=0.532, P=0.019$)。

结论 晚期肝胆期的 $\Delta T1$ 及 $T_{\Delta T1\ 1/2}$ 与肝功能具有中度相关性。延长成像时间的钆赛酸二钠增强磁共振 T1 mapping 成像及 DCE、多期肝胆期磁共振成像对于大鼠肝纤维模型肝功能的评价具有一定价值。

关键词： 肝功能；T1 mapping；钆赛酸二钠；磁共振成像