MR diffusion-weighted imaging of rabbit liver

You-Hong Yuan, En-Hua Xiao, Zhong He, Jun Xiang, Ke-Li Tang, Rong-Hua Yan, Ke Jin, Zi-Wen Peng

AIM: To study the techniques of MR diffusion-weighted imaging (DWI) for normal rabbit liver.

METHODS: After 15 normal New Zealand white rabbits and one New Zealand white rabbit implanted with VX-2 tumor were anesthetized with 3% soluble pentobarbette, DWI was performed respectively for different b values, repetition times (TR) or thicknesses, when other parameters were the same and magnetic resonance imaging (MRI) (20 cm×15 cm) was best in our study, when other parameters were the same.

RESULTS: As b value increased, liver ADC, QI and SNR of DWI became smaller and simultaneously (F = 292.87, 156.1, 88.23, P <0.01). QI of DWI was high, when b value was 10, 50 or 100 respectively, but the distinction between them was insignificant; when b value was 10, 50 or 100 respectively, but the distinction between groups was analyzed by SPSS10.0 with (FOV) or coil when other parameters were the same. The distinction between groups was analyzed by SPSS10.0 with apparent diffusion coefficient (ADC), quality index (QI) or signal-noise ratio (SNR).

CONCLUSION: The scanning technique is very important in DWI of rabbit liver and the overall quality of DWI with b (100 s/mm²), thickness (2 mm), cranium coils and FOV (20 cm×15 cm) was best in our study, when other parameters were the same.

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Key words: Liver; Rabbits; Magnetic resonance imaging; Diffusion-weighted imaging; Technology


INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors[1]. Many researches on its computed tomography (CT)[2-6], ultrasonography (US)[7-9] and digital subtraction angiography (DSA)[7-9] have been performed in recent years and they have demonstrated their significant values in diagnosis or prognosis of HCC.

However, the application of MRI[10-14], especially diffusion-weighted imaging (DWI)[14-16], in diagnosing hepatic tumors and evaluating its progression is much less than that of CT and US. With the development of software and the scanning technology of MRI, common MRI, including T1WI, T2WI and digital subtraction angiography, has been used widely in diagnosing and evaluating progression of hepatic tumors in recent years.

Diffusion is caused by random water molecular motion. The amount of diffusion is determined by diffusion coefficient (DC) in vivo may be affected by many factors, such as temperature, blood perfusion, magnetic susceptibility in the tissue, or other kinds of motion, more of apparent diffusion coefficient (ADC) than DC is used clinically[17-19]. DWI was initially used to evaluate early ischemic stages of the brain and its value has been accepted in recent years all over the world. Meanwhile, several research groups have concluded that DWI has great potential for understanding normal and pathological brain function[20-23]. But only a few researches, including that of Inoha et al.[23,24], and Geschwind et al.[14,15], on the value of DWI in diagnosing or evaluating the progression of hepatic lesions, especially VX-2 tumor of rabbit, have been reported.

The rabbit VX-2 tumor, today, is seen as the valued animal modality of HCC in researching its imaging characteristics, because most experiments have demonstrated that the blood supply of VX-2 tumor is similar to that of HCC and VX-2 tumor is implanted easily and grows quickly so that
the diameter of the tumor can reach 2 cm or more, 3 wk after implantation and it is able to transfer or metastasize to the liver, the lump, mediastina, etc. in the early stage, and VX-2 tumor animal models have been applied increasingly in the study of HCC[10-13,24]. Thus, it is important to study the characteristics of rabbit liver MR DWI. Geschwind et al.[14], have performed researches on the value of DWI in assessing VX-2 tumor cell apoptosis and histologic analysis after transarterial arterial chemoembolization (TACE). However, the study on the characteristics of rabbit liver VX-2 tumor MR DWI has not been reported. The main reason is its poor quality of imaging which greatly limits its use in diagnosing or evaluating the progression of tumors[14-17].

The purpose of our experiment is to study the techniques of MR DWI of normal rabbit liver in order to improve its image quality and to prepare for the study of MR DWI of rabbit liver VX-2 tumor, which is an important part of the HCC study on MR DWI after TACE.

MATERIALS AND METHODS
Experimental animals
The animal studies were performed under the supervision of a veterinarian and were completely compliant with the National Institutes of Health (NIH) guidelines for the use of laboratory animals. All animals were provided by the Animal Department of the Second Xiangya Hospital and all protocols were approved by the Animal Use and Care Committee of the hospital.

Of 15 New Zealand normal white rabbits, seven were male rabbits and eight were female, their weights ranging from 1.5 kg to 2.0 kg and their ages ranging from 5 mo to 6 mo. One New Zealand white rabbit was transplanted with VX-2 tumor for 21 d, and four rabbits were for pre-experiment. All New Zealand white rabbits were healthy.

Magnetic resonance imaging protocol
After animals were anesthetized, by injecting 3% soluble pentobarbitone into auricular vein at a dose of 1 mL/kg or different dose based on different animal status in order to make sure that the breath of animals was slowest, other than for the dead ones, and stable, T1WI, T2WI or DWI was performed on a 1.5-Tesla Signa Twinspeed MR scanner (General Electron Medical Systems, USA). Our experiment was divided into seven steps.

Firstly, to clearly observe the effect of animal breathing on MRI quality and choose the most suitable dose of anesthesia, T1WI and T2WI were obtained repeatedly in pre-experiment after four rabbits (excluding 15 rabbits in formal experiment) were anesthetized with different dosage, from low to high, of 3% soluble pentobarbitone injected into auricular vein. Then we compared the quality of images with that of the images after the rabbits died from an overdose of anesthesia. From this, we concluded that the quality of the images was the best, when breathing of animals was slow and stable and this dose was the most suitable anesthetic dosage. Secondly, T1WI and T2WI of 15 rabbits were obtained respectively with the cranium coil and the knee joint coil, and the other similar parameters included fast reverse fast spin echo (FRFSE) series, T1WI [TR 400 ms/echo time (TE) 12.3 ms], T2WI (TR3000/TE80 ms), FOV 20 cm×15 cm, NEX 4, thickness of the layer 5 mm, spacing 0 mm, matrix 256×192 (T1WI) and 320×256 (T2WI). Thirdly, different FOVs included 24 cm×24 cm, 20 cm×15 cm, 16 cm×12 cm, while the other parameters were similar to those in step two; T1WI and T2WI of 15 rabbits were carried out respectively. Fourthly, DWIs of 15 rabbits were acquired respectively for different b values including 10, 50, 100, 300, 800 s/mm2 while other parameters remained the same (Table 1). Fifthly, DWIs of 15 rabbits were performed respectively with different TR values including 4 000, 6 000, and 8 000, while the other parameters remained the same (Table 1). Sixthly, DWIs of 15 rabbits were obtained respectively for different layer thicknesses including 2 and 5 mm, while other parameters remained the same (Table 1). Finally, DWI of VX-2 tumor rabbit was performed for a b value of 100 or 300 s/mm2, TR 6 000, and a 2-mm thick cranium coil.

Data analysis
Based on the ADC value of the region of interest (ROI), QI and SNR, the distinction between different scanning parameter groups was respectively evaluated quantitatively. Of them, the ADC and SNR values were obtained through GE working station.

ADC values of 15 normal rabbit liver parenchyma were obtained by getting the ADC average value from five different ROIs at the central layer of the liver, of which each area was 50 mm2, which was measured five times, with the help of the relative functional software in GE working station. QI value was an image marker, which was obtained by combining SNR, the artifact and the liver anatomy manifestation of the image with the same weight after they were quantified respectively with five levels, which could reflect the image quality.

Table 1 The parameters of normal rabbit MR DWI

<table>
<thead>
<tr>
<th>Series</th>
<th>b (s/mm2)</th>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>FOV (mm)</th>
<th>NEX</th>
<th>Thickness (mm)</th>
<th>Spacing (mm)</th>
<th>Matrix</th>
<th>Coils</th>
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<tr>
<td>SE-EPI</td>
<td>10</td>
<td>6 000</td>
<td>45</td>
<td>20×15</td>
<td>8</td>
<td>2</td>
<td>0.5</td>
<td>128×128 Cranium</td>
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<tr>
<td>SE-EPI</td>
<td>50</td>
<td>6 000</td>
<td>45</td>
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<td>SE-EPI</td>
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<tr>
<td>SE-EPI</td>
<td>100</td>
<td>4 000</td>
<td>45</td>
<td>20×15</td>
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<td>2</td>
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SE-EPI: spin echo echo planar imaging.
quality collegiately. SNR is the contrast of SH to SD. SH was the average signal of the hepatic parenchyma from the average value of five different ROIs, each area of which was 50 mm$^2$ excluding biliary ducts and blood vessels. SD is standard deviation of background signal intensity in the direction of the phase coding. It was obtained by calculating the average value of five ROIs which were not only in front of the abdominal wall but in the field of the scanning as well. The values of “SNR<5”, “5<SNR<10”, “10<SNR<20”, “20<SNR<30”, “30<SNR<50” and “SNR>50” are thought of as 0, 1, 2, 3, 4 and 5 scores respectively. As far as the artifact of the images is concerned, when the artifact was the highest and the images could not be used for diagnosis, we considered it as 1 score and when the artifact was very high and it affected diagnosis seriously, we considered it as 2 scores. When the artifact of the images was general and it had some effects on manifestation and diagnosis of the lesions, we regarded it as 3 scores and when the artifact was not high and it did not affect diagnosis seriously, we regarded it as 4 scores. Then, when the artifact was little or none, we regarded it as 5 scores. As for the liver anatomy manifestation of the images, 1 score represented the poorest quality of the images which could not display, at all, the border of the organ or the intrahepatic vessels and 3 scores indicated the average quality of the images which could display the border of most organs, comparatively display the major intrahepatic vessels and vaguely display the border of stomach and bowel. When the quality of the images was between 1 and 3 scores, we regarded it as 2 scores and, when the quality of the images was between that of 3 scores and 5 scores, we regarded it as 4 scores.

All MR images were retrospectively analyzed by three experienced radiologists and evaluation of the images artifact and liver anatomy manifestations were obtained by average value statistical analysis, based on the above criteria.

The statistical significance was calculated using SPSS10.0 with analysis of variance (ANOVA) of the randomized block design or paired $t$-test.

RESULTS

Considering only the increase in $b$ value and no other parameter, the mean ADC value of hepatic parenchyma was decreased and, at the same time, QI also decreased (Table 2). The distinction of ADC among $b$ values of 100 and that of 50, 300 or 800 was significant, respectively (Tukey HSD, $P<0.01$). The distinction of QI among groups of $b$ values 10, 50 and 100 s/mm$^2$ was insignificant, and those among $b$ values 100, 300 or 800 was significant (Tukey HSD, $P<0.01$), and was significant between groups of $b$ values of 800 and 300 (Tukey HSD, $P<0.01$). With $b$ value increase, SNR became smaller and, when $b$ value was 800, SNR was no more than 10 (Table 2, Figures 1 and 2). The distinction of SNR between $b$ values 100, 300 or 800 was significant (Tukey HSD, $P<0.01$) and that between $b$ values 100 and 50 was not significant.

<table>
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<th>Table 2 DWI of normal rabbit liver with different $b$ values (s/mm$^2$)</th>
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<td>$b$ = 10</td>
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<td>ADC$^1$</td>
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<tr>
<td>QI</td>
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<td>SNR</td>
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$^1$(mean±SD)$\times10^3$mm$^2$/s.
were 2.48±0.52, 2.58±0.43 and 2.70±0.37 (F = 5.6, P<0.01)
respectively, QI were 11.11, 11.80 and 10.83 (F = 3.15,
P>0.05) respectively, and SNR were 26.61, 28.65 and 29.21
(F = 0.94, P>0.05) respectively, which indicates that QI
and SNR did not change significantly. The distinction of
ADC between TR 4 000 and 8 000 was significant (Tukey
HSD, P<0.05) and that between TR 6 000 and 4 000 or
8 000 was not significant.

When the thickness was 2 or 5 mm and other parameters
were the same, ADC values of hepatic parenchyma were
8 000 was not significant.

If rabbit liver was scanned with the cranium coil and the
knee joint coil respectively 10, 100, 300 and 800 s/mm
MR DWI were also different. The distinction between the
VX-2 tumor and surrounding hepatic parenchyma was
significant and the border of the VX-2 tumor was clear (Figure 4).

**Figure 3** When b value was 100 and the thickness was 2 mm, SNR and the
liver anatomy manifestation of the images were all good, while the artifact was
low and the quality was high.

**Figure 4** The VX-2 tumor manifests low signal on MR T1WI in image (A), while
it displayed high signal on T2WI in image (B). The VX-2 tumor manifested
significantly high signal while surrounding hepatic parenchyma displayed low
signal on DWI with a b value of 100, TR of 6 000 and a thickness
of 2 mm. The distinction between VX-2 tumor and surrounding
hepatic parenchyma was significant and the border of VX-2
tumor was clear (Figure 4).

**DWI manifestation of rabbit liver**

With b value, thickness, TR value, scanning coil and NEX
were different, ADC value, the ability of liver anatomy
manifestation or differentiation and SNR of rabbit liver on
MR DWI were also different. In Figure 1, b values were
respectively 10, 100, 300 and 800 s/mm of the images
(1), (2), (3) and (4). ADC value, SNR and the liver anatomy
manifestation of the images became lower and lower with
increasing b value. The distinction between b values 10 and
100 was insignificant and the diagnostic value of images at
b value 800 was low. In Figure 2, b value, the thickness and
the NEX of image (1) were 300, 2 mm and 8 respectively
while those of image (2) were 100, 2 mm and 1 respectively.
The quality and SNR of the images were poorer than that
of Figure 3. In Figure 3, when b value was 100 and the thickness
was 2 mm, SNR and the liver anatomy manifestation of
the images were all good while the artifact was low and
the quality was high. In Figure 4, the VX-2 tumor manifests
low signal on MR T1WI in image (1), while it displayed high
signal on T2WI in image (2). The VX-2 tumor manifested
significantly high signal while surrounding hepatic parenchyma displayed low signal on DWI in image (3) with $b$ value 100, TR 6 000 and thickness 2 mm, the distinction between the VX-2 tumor and surrounding hepatic parenchyma was significant and the border of the VX-2 tumor was clear. The image (4) is the ADC map from VX-2 tumor DWI of the image (3).

**DISCUSSION**

Diffusion is caused by water molecular random motion, so-called “Brownian motion”, and it is able to change the intensity of local magnetic field around hydrogen proton so that its phase position in the magnetic field is changed. When we added a powerful polar and quick switching gradient radiofrequency (RF) pulse besides routine RF pulse, we were able to amplify these phase changes so that we could detect water molecular diffusion motion, the so-called DWI. DWI was initially used to evaluate early ischemic stages of the brain and its value has been recognized in recent years. However, because of its poor image quality, DWI is rarely used in diagnosing and evaluating the lesions of the liver\[24-28\], $b$ value is the sensitivity of diffusion and it is the most important parameter of DWI scanning because changing it will directly lead to a change of the complemented gradient magnetic field and affect the ability of detecting water molecular diffusion. Ichikawa et al.\[15,27,28\], reported that high $b$ value or $b$ value deviation has an important influence on the quality of DWI and ADC value or DC value. When $b$ value or its deviation is higher, ADC value would be stabler and could get closer to the DC value, which could better reflect water molecular diffusion motion, little affected by the microcirculation. When $b$ value or its deviation is lower, ADC value will, to some degree, reflect the perfusion of the microcirculation while the ability of reflecting water molecular diffusion motion is worse than that of the former and the ADC value is also unstable. However, with increasing $b$ value, gradient RF pulse will prolong, thus enlarging TE value, and the quality of images will be reduced\[25,27,28\]. Our results showed that ADC value, QI and SNR of the images would reduce with $b$ value increase but, when $b$ value was 100, QI of the images was basically the same as that when $b$ value was 10 or 50 and SNR of the images was also better. Moreover, QI and SNR of the images were still high when $b$ value was 300. Thus, we consider that at a $b$ value of 100, QI and SNR of the images are good, together with a $b$ value of 300, at which lesions are more sensitively detected as a good choice on DWI (Figures 1 and 3).

TR enlarging will raise the quality of the images because it prolongs the signal collecting time. But it leads to prolongation of the scanning time or reduction of the scanning coverage which prolongs the signal collecting time. But it leads to prolongation of the scanning time or reduction of the scanning coverage which is not beneficial to control breathing. Our results indicated that the major reason for the artifact was the gas in the stomach and it was effective to use some precluding method, thickness, coil, FOV, respiratory movement and control of stomach gas, are very important in DWI of rabbit liver and the overall quality of DWI with a $b$ value (100 s/mm$^2$), thickness (2 mm), cranium coils and FOV (20 cm×15 cm) was considered the best in our study.

In conclusion, the scanning techniques, including appropriate $b$ value, thickness, coil, FOV, respiratory movement and control of stomach gas, are very important in DWI of rabbit liver and the overall quality of DWI with a $b$ value (100 s/mm$^2$), thickness (2 mm), cranium coils and FOV (20 cm×15 cm) was considered the best in our study.

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