·Clinical Research·

Magnetic resonance spectroscopy on hepatocellular carcinoma after transcatheter arterial chemoembolization

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[Abstract] **Background and Objective:** Proton magnetic resonance spectroscopy (MRS) of the liver in vivo is in experimental phase. MRS observation on liver cancer after transcatheter arterial chemoembolization (TACE) has seldom been reported. This study was to investigate the value of MRS in assessing the metabolic changes of hepatocellular carcinoma (HCC) after TACE. **Methods:** Twenty-five consecutive patients with pathologically-confirmed HCC received 1H MRS of all hepatic lesions using 1.5T whole body MR scanner before TACE and at 3–10 days after TACE. Choline-to-lipid (Cho/Lip), glucogen/glucose-to-lipid (Glu/Lip), and glutamine/glutamate-to-lipid (Glx/Lip) ratios were measured and analyzed statistically. **Results:** The Cho/Lip, Glu/Lip, and Glx/Lip ratios were 0.21 ± 0.08, 0.11 ± 0.05, and 0.28 ± 0.10 before TACE, respectively, and were 0.10 ± 0.08, 0.07 ± 0.07, 0.18 ± 0.12 after TACE, respectively, with significant differences ($P < 0.05$). **Conclusions:** Using MRS can evaluate the early metabolic responses of HCC to TACE.

Key words: liver neoplasm, proton magnetic resonance spectroscopy, transcatheter arterial chemoembolization, choline-to-lipid ratio, glucogen/glucose-to-lipid ratio, glutamine/glutamate-to-lipid ratio

Transcatheter arterial chemoembolization (TACE) is recognized as one of the most effective palliative treatments for hepatocellular carcinoma (HCC), which can lead to necrosis and apoptosis of HCC cells in varying degrees[1-3]. Functional magnetic resonances such as magnetic resonance spectroscopy (MRS), diffusion weighted imaging (DWI) and perfusion weighted imaging (PWI) can be performed to observe the changes of cell metabolism, energy and blood flow before necrosis and apoptosis, which are of great significance to the evaluation of the treatment efficacy and prognosis of HCC[4-5]. In recent years, the 1H proton MRS (1H MRS) on the liver in vivo has been investigated increasingly, but it is far below the study on the brain and prostate, and it is still in experimental stage[6-10]. Especially, there are few reports about changes of HCC after TACE by MRS observation[11-13]. We have observed the metabolic changes of 25 cases of HCC after TACE using MRS, and measured choline-to-lipid (Cho/Lip), glucogen/glucose-to-lipid (Glu/Lip) and glutamine/glutamate-to-lipid (Glx/Lip) ratios.

1 Materials and Methods

1.1 Clinical data

In 2007-2008, 25 patients with pathologically confirmed HCC were admitted to the intervention department of Xiangya Second Hospital of Central South University. Of the 25 patients, 17 were males, 8 were females, aged from 26 to 76 years old with a median age of 49 years. All patients underwent computed tomography (CT), magnetic resonance imaging (MRI), digital subtraction angiography (DSA) and alpha-fetoprotein (AFP) detection. Of the 25 cases of HCC, 18 were mainly located in the right lobe of the liver and 7 in the left lobe; all were bulky or nodular type. The largest lesion was about 14 cm × 9.5 cm × 14 cm and the smallest lesion was about 8 cm × 9 cm × 9.5 cm. All patients were TACE-naive. The regimen for TACE was 500–1 000 mg of 5-fluorouracil, 6–8 mg of mitomycin C, and 40-80 mg of cisplatin or epirubicin or pirarubicin, which were mixed in 15–40 mL of 38% lipiodol to embolize tumor blood vessels till flow interrupt.

1.2 MRS test

GE Signa Horizon LX, 1.5T dual-gradient superconducting magnetic resonance scanner was used, adopting Gpflex coil and

conventional axial Turbo Spin-Echo (TSE) sequences: T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), fat-saturation T1WI, and finally T1WI + FS + C. MRS was carried out before the first TACE treatment and at 3–10 days after TACE using the parameters in our previous report[7,8]. Multiple voxel point resolved surface coil spectroscopy (PRESS) sequences, TR 1000 ms, TE 30 ms, NEX 2, FOV 12 cm × 12 cm, multi-voxel phase matrix Freq10 × 10, voxel size 10 mm, thickness 10 mm, and A/P were adopted. Automatic center frequency was water, with automatic water suppression (WS) optimization. Saturated zone was set surrounding the region of interest (ROI). The time duration for one spectral acquisition was 204 s. Confining the abdomen with abdominal bandage can effectively avoid the impact of respiratory movement on the scan. The VOI positions of the preoperative and postoperative scans were the same. VOI included tumor lesion and peritumoral tissue, keeping away from large bile ducts and blood vessels, subcutaneous fat and intestine. Machine automatic pre-scan was performed for strict shimming and water suppression. The full width at half maximum (FWHM) and WS rates of water peak were observed. Spectral acquisition was collected only when pre-scan shimming achieved FWHM of ⩽ 10 Hz and WS of ⩾ 95%. For those did not meet the standards in the first scan, pre-scan was conducted again; if the results still did not reach the standards, requirements, the location of VOI was adjusted for automatic pre-scan. If necessary, higher-order shimming was conducted.

The MRS data were processed with the machine's supplementary analysis software FuncTool2.5.36. Four kinds of graphics, chemical shift image, spectrum (S), metabolic image (MI), and metabolic and anatomical overlay image (abbreviated as AI + MI image), were obtained simultaneously and observed by two radiologists jointly to evaluate the eligibility. The stability of spectrogram baseline, the recognition of significant peaks, and the impact of background noise on the major peaks were judged comprehensively. Severe deformation of the baseline, excessive overlap of major peaks (more than half of the peak), and wide,

deformed, or illegible fat peak were regarded as unqualified data and could not be used for further analysis.

Metabolic compounds were measured and analyzed by 1H MRS. Supplementary functional software Functool 2 was used to measure the metabolic image and overlay map, selecting the corresponding voxel of ROI. The metabolites of ROI and the corresponding ratios were automatically processed, and the wave peaks of NAA, Cho, Cr, LL metabolites as well as ratios of Cho/LL, Cr/LL and NAA/LL on the upper left corner of spectrums were obtained. The Glx/Lip ratios of the voxels within the VOI were measured, combined with T2WI anatomical positioning map, the mean of the Glx/Lip ratios of multiple voxels in VOI was took as the Glx/Lip ratio of this region. Cho/Lip and Glu/Lip measurement were the same as Glx/Lip measurement. On the illegible spectrums, a maximum of six major metabolite peaks could be seen (range, 0.49–4.30 ppm) (Figure 1). The chemical shifts were as follows: (1) lipid methyl peak (Lip1), 0.8–1.1 ppm; (2) lipid methylene peak (Lip2), 1.1–1.5 ppm; (3) lipid methylene peak with double-carbon bond (Lip3), 1.9–2.3 ppm; (4) glutamine and glutamate complex (Glx), 2.10–2.49 ppm; (5) choline compounds (Cho), 3.1-3.3 ppm; (6) glycogen and glucose complex (Glu), 3.35–3.90 ppm[6,14]. Among them, Lip2 was the highest and most obvious resonance peak on the spectrum (Figure 2). The ratios of integral area under curve of Glx, Cho and Glu to that of Lip were calculated using Functool2 software and expressed as Glx/Lip, Cho/Lip and Glu/Lip. Unqualified baselines were not included in statistical analysis. Twenty-one patients underwent a total of 42 successful MRS before and after TACE. Voxels in the VOI were measured. Two groups of mean values were obtained from each MRS, with a total of 84 groups of original data.

1.3 Statistical analysis

Cho/Lip, Glu/Lip and Glx/Lip ratios of each group were expressed as mean ± standard deviation, and analyzed by paired t test using SPSS12.0 software package. A P value of < 0.05 indicated significant difference.
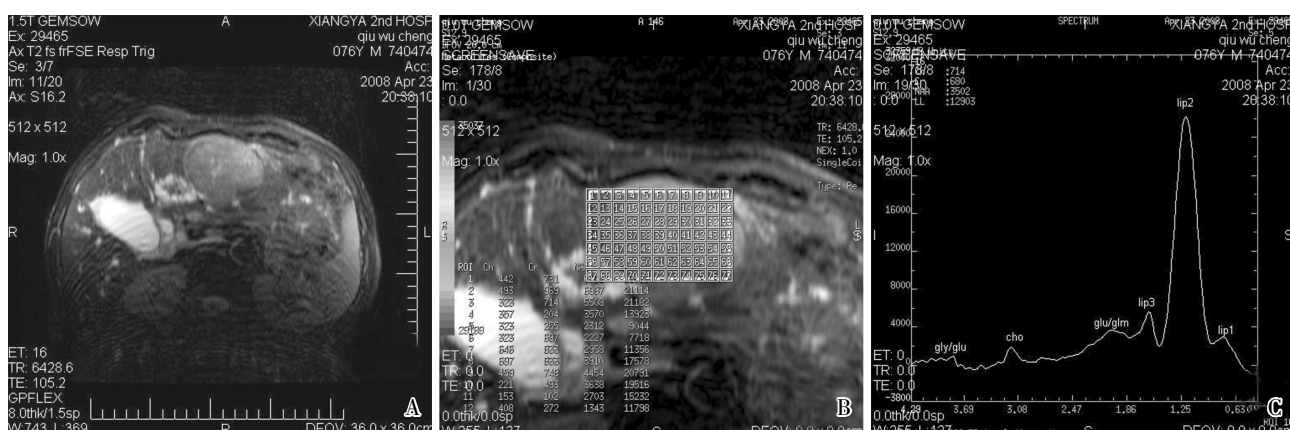

Figure 1 MRI and MRS images of a patient with hepatocellular carcinoma (HCC) in the left hepatic lobe

A, Transverse fast spin-echo fat-saturated T2-weighted MR image shows a hyper–intense tumor in the left hepatic lobe. B, Localized MR image shows location of the voxel of interest in the tumor. C, Six peaks are shown in high resolution spectroscopy at amplitude (0. 49–4. 30 ppm). Lipid 1, 0.8–1.1 ppm; Lipid 2, 1.1–1.5 ppm; Lipid 3, 1.9–2.3 ppm; glycogen/glucose (Gly/Glu), 3.35–3.90 ppm; choline (Cho), 3.1–3.3 ppm; glutamine/glutamate (Glu/Gln), 3.35–3.90 ppm.
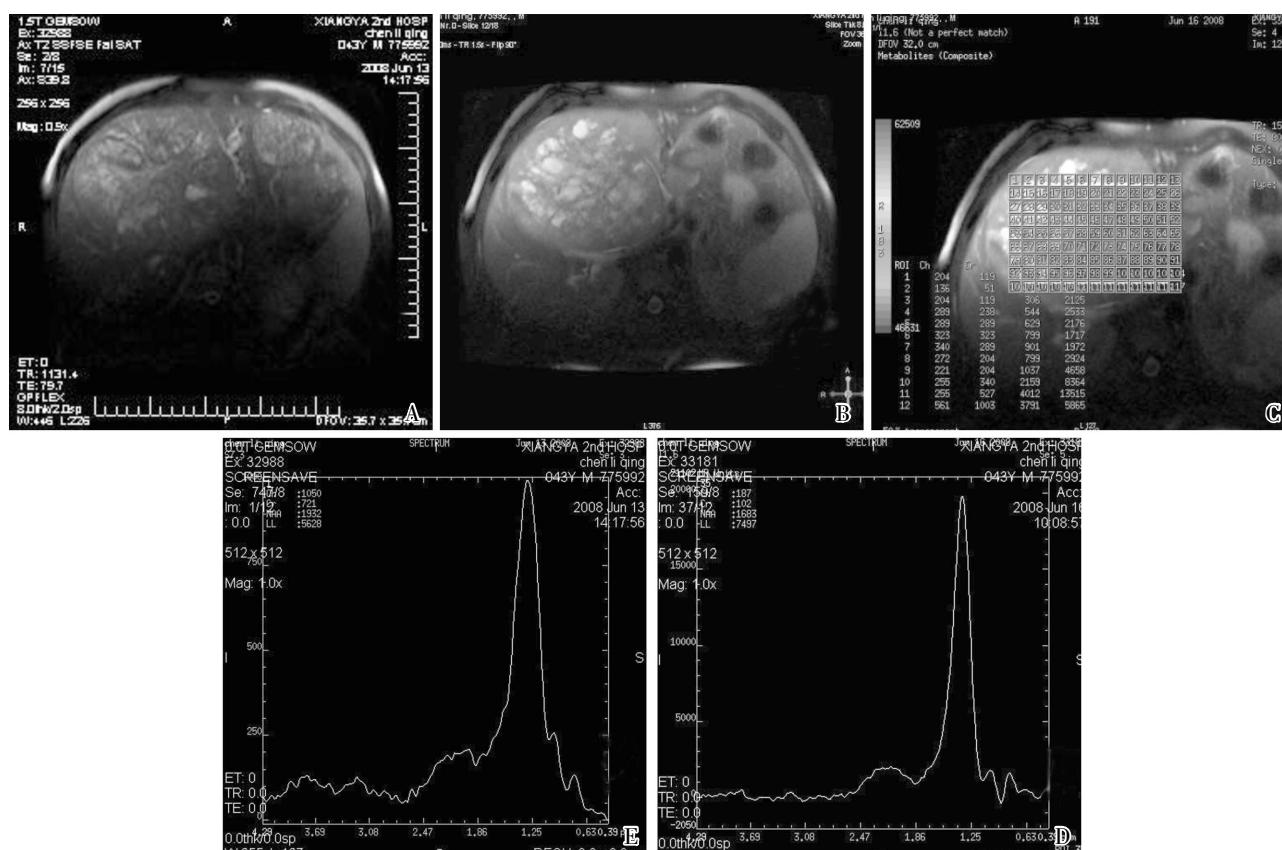
Figure 2 MRI and MRS images of a patient with HCC in the right hepatic lobe before and after transcatheter arterial chemoembolization (TACE)

A, Transverse fast spin-echo fat-saturated T2-weighted MR image shows a hyper-intense tumor in the right hepatic lobe. B, After TACE, T2-weighted MR image reveals more hyper–intense areas in the tumor. C, Localized MR image shows location of the voxel of interest in the tumor. Comparing with the data before TACE (D), choline-containing compounds, Gly/Glu peak and Glu/Gln peak descended after TACE (E).

2 Results

1H MRS was performed on 21 of the 25 cases successfully; the spectrums of 4 cases could not be used for diagnosis. The success rate was 84%.

Compared with preoperative data, the peak values of Cho/Lip, Glu/Lip, and Glx/Lip ratios were significantly reduced after TACE (Table 1, Figure 2)

Table 1 Comparative of the mean of Cho /Lip，Glu/Lip，and Glx/Lip before and after TACE

Item	Before TACE	after TACE	t	P
Cho/Lip	0.20 ± 0.08	0.10 ± 0.08	9.218	< 0.05
Glu/Lip	0.11 ± 0.06	0.07 ± 0.07	4.078	< 0.05
Glx/Lip	0.28 ± 0.10	0.18 ± 0.12	6.526	< 0.05

Cho/Lip，Choline Lipied；Glu/Lip，glutamin/glutmate to lipid；GLx/Lip，glutemine/ glutamate to lipia；TACE，transcatheter arterial chemoemblization.

3 Discussion

At present, MRS is the only non-invasive method for investigation on the metabolic and biochemical changes of the liver and compounds quantitative analysis. On MRS, the area ratios of different resonance peaks can represent the relative amount of various types of nuclei. It is in direct proportion to the concentration of the materials which create peaks, and is also related with the structure of compounds. This is the theoretical basis of adopting MRS to do structure deduction and quantitative analysis of the corresponding compounds. 1H MRS may show metabolic and biochemical changes of liver disease in a non-invasive manner, present different number and shape of metabolite peaks of normal liver tissue, cirrhosis and liver cancer tissues, and provide a theoretical basis for the study on liver cancer occurrence and development[6,10-14]. However, the spectroscopic imaging requires high magnetic field uniformity, while the liver is affected by respiratory motion, so it is difficult to collect the data of lesions at the same level. In our study, abdominal bandage was used to confine the abdomen of the

patients, and MRS was performed successfully on 21 patients with a success rate of 84%.

The peak of Cho was 3.2 ppm, which included glycerophosphoryl choline, phosphoric acid choline and phosphatidyl choline. Cho is a component of cell membrane phospholipid metabolism and is involved in cell membrane synthesis and degradation. It plays a primary role in evaluating tumor cell metabolism. Cho is significantly increased in malignant tumors. In the preliminary 3.0 TMR 1H MRS study on HCC, Wu et al.[9] found that the Cho peak of HCC was elevated.

The peaks of Lip were at 0.8, 1.2, 1.5 and 6.0 ppm, which was composed of methyl, methylene and the vinyl of unsaturated fatty acid. Usually, the peak of Lip is significantly higher in most malignant tumors than in normal tissues, and it is related to tumor cell necrosis and lipid release. In the liver cell-derived tumors, the lipid content is increased; in the non-liver cell-derived tumors, the lipid content is generally not increased. Lip is a composition of cell membrane, rather than free molecules. In the MRS, Lip peaks producing resonance come from free lipid inside the cell vesicles or extracellular free fat granules, which are released due to structural damage or collapse of cell membrane caused by various reasons, such as hypoxia, necrosis, apoptosis and inflammation[6,14].

The peak of creatine (Cr) was at 3.03 ppm, which included creatine and phosphocreatine (PCr). The role of PCr may be through storing high-energy phosphate bonds, adenosine triphosphate and adenosine diphosphate in the cell to act as buffer to maintain the energy-dependent system[14].

Because the chemical shifts of glutamine and glutamate complex (Glx) are located close to each other, and also overlap partly with Lip3, covering a wide range. Within the same resonance frequency, there may exist other amino acids such as γ-aminobutyric acid, alanine, lysine, or some other metabolites such as acetate, which will cause the Glx peaks become extremely complex.

In our study, preoperative Cho/Lip ratio was 0.21 ± 0.08 and postoperative ratio was 0.10 ± 0.08. TACE decreased the Cho/Lip ratio of HCC cells significantly. Wu et al.[11] performed 1.5T MRS on HCC, and found that TACE preoperative Cho/Lip ratio was 0.205 ± 0.060, postoperative ratio was 0.070 ± 0.020. Using 3.0T MRS, Kuo et al.[13] found that TACE preoperative Cho/Lip ratio was 0.23 ± 0.11, postoperative ratio was 0.01 ± 0.00. In this study, we first observed the changes of Glu/Lip and Glx/Lip ratios of HCC cells before and after TACE, and found that these two ratios were significantly decreased after TACE, indicating that the membrane metabolism of HCC cells was significantly reduced after TACE. Our previous studies have also proved that necrosis and apoptosis of HCC cells reached to the peak at 7–10 days after TACE, while cell proliferation was inhibited to the maximal extent[15-17].

Although 1H MRS, as a non-invasive technique, can detect the change of liver metabolites before and after TACE, there are still a lot of problems: ① the detection is time-consuming and is vulnerable to be interfered by respiration; ② data processing is complex; ③ study sample is few, technical parameters are not unified, and the experimental findings need further clinical validation and improvement.

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