J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-008-0315-2
28 (3): 295-298, 2008

Application of Proton Magnetic Resonance Spectroscopy and Computerized Tomography in the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease

Nan WANG (王 南)[1], Hui DONG (董 慧)[2], Shichao WEI (魏世超)[3], Fuer LU (陆付耳)[2]
[1]Department of Radiology, [2]Institute of Integrated Traditional Chinese and Western Medicine, [3]Department of Pharmacy, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Summary: In order to investigate the application of proton magnetic resonance spectroscopy (^{1}H-MRS) and computerized tomography (CT) in the quantitative diagnosis of nonalcoholic fatty liver disease (NAFLD) and evaluation of therapeutic effects, 22 patients with NAFLD were selected according to the Chinese Medical Association's (CMA) standard of the NAFLD in comparison with 20 healthy volunteers (as control group). Blood samples for biochemistry were collected. The severity of hepatosteatosis was evaluated by ^{1}H-MRS scan and CT scan of liver. The intrahepatic content of lipid (IHCL) and CT value ratio of liver to spleen were calculated. The patients in NAFLD group were treated with Ganzhixiao Capsule for 8 weeks. The changes in IHCL and CT value ratio of liver to spleen were observed before and after treatment. In NAFLD group serum ALT, TG, IHCL calculated by ^{1}HMRS were increased and CT value ratio of liver to spleen decreased significantly as compared with control group. After treatment for 8 weeks serum ALT, TG, IHCL were decreased significantly, while CT value ratio of liver to spleen increased significantly in NAFLD group. It was suggested that IHCL could be measured precisely by ^{1}HMRS. NAFLD was treated effectively by Ganzhixiao capsule.
Key words: proton magnetic resonance spectroscopy; computerized tomography; nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized condition that may be aggravated to end-stage liver disease. The clinical implications of nonalcoholic fatty liver disease are mostly derived from its high prevalence in the population and its predisposition to aggravate to cirrhosis and liver failure. Ultrasound and computerized tomography (CT) examinations of liver are widely performed to make a diagnosis of NAFLD, but cannot make a quantitative diagnosis of hepatosteatosis. Recent study showed that liver proton magnetic resonance spectroscopy (^{1}H-MRS) was performed to be a new noninvasive method to evaluate the degree of NAFLD quantitatively. Meanwhile, Ganzhixiao capsule was known to be an effective Chinese medicine to treat NAFLD. The aim of present study was to apply ^{1}H-MRS of liver *in vivo* and evaluate the therapeutic effects of Ganzhifu capsule in treating NAFLD.

1 MATERIAL AND METHODS

1.1 Subjects and Diagnostic Criteria

Twenty-two NAFLD patients from Physical Examination Center of Tongji hospital were recruited in comparison with 20 healthy volunteers (as control group). The diagnosis of NAFLD was made according to the Chinese Medical Association's (CMA) standard of the NAFLD established in February, 2006. Among 22 patients with NAFLd there were 20 males and 2 females with the mean age of 39.4±15.6 years. In control group there were 18 males and 2 females with the mean age of 36.2±16.7 years. There was no significant difference in sex and age between the experimental group and the control group. The patients with following diseases were excluded from this study: diabetes mellitus, pancreatic disease, severe hepatic and renal dysfunction, severe cardiac disease, cancer, hypertension, viral hepatitis or autoimmune liver disease, drug-induced liver disease, pregnant and nursing women, and those who stopped the administration of medicines that had functions of antihyperglycemia, hepatocytoprotection, antihyperlipidermia and weigh reduction 2 weeks before treatment.

1.2 Experimental Protocol

Fasting blood samples were obtained for the measurement of biochemistry. The severity of hepatosteatosis was evaluated by ^{1}H-MRS scan and CT scan of liver. The NAFLD patients were orally administered with Ganzhixiao capsule three times a day with the dose of 3 or 4 capsules each time. Ganzhixiao capsule (composed of Artemisia capillaries Thunb, Rhizoma Polygoni Cuspidati, Radix Bupleuri Chinensis etc.) was provided by Department of Pharmacy, Tongji Hospital. Eight weeks later the above examinations were carried out again to evaluate the therapeutic effects of Ganzhixiao capsule in treating NAFLD.

Nan WANG, male, born in 1974, Doctor in Charge
E-mail: southern1@sohu.com

1.3 ^{1}H-MRS Scan and CT Scan of Liver

^{1}H-MRS of liver was acquired on a 1.5T MRI scanner (GE company, USA) using a standard body coil and breath hold mode. MRS for water and fat quantification was accomplished by using a Point Resolved Spectroscopy Sequence (PRESS). Sagittal, coronal, and axial slices of the liver were acquired, a spectroscopic volume of interest in right posterior hepatic lobes was positioned, avoiding major blood vessels, intrahepatic bile ducts, and the lateral margin of the liver. The voxel size and position were optimized to prevent contamination of signal from liver by signal from abdominal adipose fat. The volume of voxel was not limited and a relatively large voxel was used to collect good quality data in a short time. Saturated zone was placed around the voxel. The imaging parameters for PRESS sequence were as follows: TR 1500 ms, TE 35 ms, Echo=1, NEX=8, Research mode, without water saturation. CT scan of liver was acquired on a 16 slices spiral CT scanner (GE Light-speed Ultra, USA).

1.4 Measurements of Intrahepatic Lipid Content (IHCL) and CT Value Ratio (Liver/Spleen)

Spectroscopic data were processed using the SAGE software. The peak area of the water (*S*w) at 4.77 ppm and fat resonance (*S*f) at 1.4 ppm were measured. IHCL was calculated by dividing 100 times *S*f by the sum of *S*f and *S*w. CT values of liver and spleen were determined and the CT value ratio (liver /spleen) were calculated.

1.5 Statistical Analysis

SPSS software was used to perform data analysis. The results of measurement data were expressed as $\bar{x}\pm s$. Data between two groups was tested with *t*-test. Significance test before and after the treatment was tested with paired *t*-test. $P<0.05$ was considered to be significant.

2 RESULTS

2.1 General Conditions of the Patients with NAFLD

After 8 weeks 3 patients lack of compliance were dropped out of the trial. Nineteen cases completed the 8-week treatment. Through the clinical trial some patients complained symptoms of diarrhea and relieved after 2 weeks.

2.2 Difference of Clinical Features between NAFLD before Treatment and Control Group

Serum ALT activity and serum concentration of triglycerides (TG) were all elevated significantly in NAFLD group. Meanwhile, in NAFLD group the CT values ration of liver to spleen were decreased significantly and IHCL calculated through ^{1}HMRS were increased.

Table 1 Difference of clinical features between NAFLD before treatment and control group ($\bar{x}\pm s$)

Parameters	Control (*n*=20)	NAFLD (*n*=22)
Serum ALT (U/L)	20.4±10.1	71.5±24.8$^{\Delta\Delta}$
Serum TG (mmol/L)	1.05±0.40	2.48±1.46$^{\Delta\Delta}$
IHCL (100%)	1.34±0.79	27.49±12.27$^{\Delta\Delta}$
CT value ratio	1.21±0.14	0.69±0.24$^{\Delta\Delta}$

$^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$ vs control group

2.3 Difference of Clinical Features before and after Treatment in NAFLD Group

After treatment for 8 weeks, serum ALT activity, and concentrations of TG and IHCL were decreased significantly, while CT value ratio increased significantly.

Table 2 Difference of clinical features before and after treatment in NAFLD group ($\bar{x}\pm s$)

Parameters	Before treatment (*n*=19)	After treatment (*n*=19)
Serum ALT (U/L)	73.2±25.2	54.6±19.9**
Serum TG (mmol/L)	2.44±1.52	2.14±1.38*
IHCL (100%)	27.8±13.2	19.7±12.7**
CT value ratio	0.67±0.24	0.84±0.21**

$^{*}P<0.05$, $^{**}P<0.01$ vs before treatment

2.4 ^{1}H-MRS Imaging in NAFLD and Control Groups

^{1}H-MRS imaging in NAFLD and control groups were obtained successfully. Resonances from water and fat could be clearly identified. The peak area of the water (*S*w) was at 4.77 ppm and fat resonance (*S*f) at 1.4 ppm respectively. Typical proton magnetic resonance liver spectra from healthy volunteers only showed water peak without fat peak (fig. 1). Liver spectra from 3 patients with NAFLD showed progressive degrees of fatty infiltration (fig. 2－4) and increasingly higher fat peak. After treatment for 8 weeks hepatosteatosis was improved with reduced fat peak and the peak area of fat resonance (fig. 5－6).

3 DISCUSSION

NAFLD comprises a spectrum of conditions extending from simple hepatic steatosis to end-stage liver disease. It represents a significant problem among patients referred for evaluation of abnormally elevated circulating levels of liver enzymes. Despite its clinical importance, the prevalence of NAFLD in the general population remains poorly defined due to limitations of the available noninvasive techniques used to diagnose hepatic steatosis.

The imaging modalities to assess hepatic fat content are usually CT or ultrasound. Ultrasound is the preferred imaging modality for the qualitative assessment of fatty infiltration, but the results are correlated with the objective evaluation of operator and vary over a wide range due to variations in techniques selected for analysis. CT scan of liver is also semiquantitative. From the results of our study, it was found in CT imagines of liver in healthy volunteers hepatic density was always higher than spleen and CT value ratio of liver to spleen was larger than 1.0. However, liver density becomes lower and lower as well as hepatic fatty infiltration developing, and CT value ratio of liver to spleen is lower than 1.0. In addition, liver biopsy is regarded as the golden diagnostic criteria but unaccepted because of its invasive method[1].

In the present study, we applied a new method–^{1}H-MRS to detect the IHCL and evaluate the therapeutic effects of medicine. Unlike CT scanning and ultrasound, which rely on nonspecific attenuation of X-ray and Doppler signals, ^{1}H-MRS provides a quantitative rather

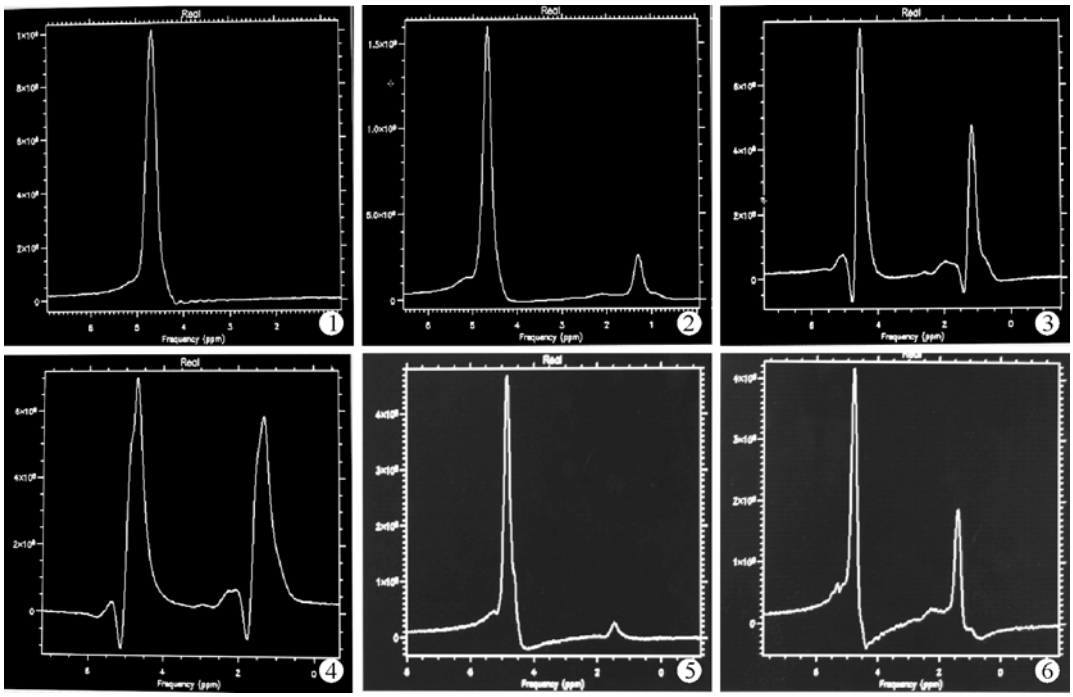

Fig. 1 ^{1}H-MRS of showing water peak without fat peak
Fig. 2 ^{1}H-MRS of liver in one patient with mild NAFLD
Fig. 3 ^{1}H-MRS of liver in patients with moderate NAFLD
Fig. 4 ^{1}H-MRS of liver in patients with severe NAFLD
Fig. 5 ^{1}H-MRS of liver in one patient with mild NAFLD (fig. 2) after treatment
Fig. 6 ^{1}H-MRS of liver in one patient with moderate NAFLD (fig. 3) after treatment

than qualitative or semiquantitative assessment of hepatic triglyceride by directly measuring the protons in the fatty acids of the triglycerides. In addition, ^{1}H-MRS is highly reproducible in contrast to CT scanning, where there is significant intraindividual variability in the measurements[2, 3]. IHCL was calculated by dividing 100 times *S*f by the sum of *S*f and *S*w. Previous study has shown IHCL obtained by spectroscopy closely coincided with biopsy or autopsy derived triglyceride concentrations and IHCL of fatty liver was larger than 5.5%. If IHCL was just calculated by dividing 100 times *S*f by *S*w, fatty liver was larger than 20%[4, 5]. ^{1}H-MRS provides a noninvasive method for the quantitative determination of hepatic steatosis.

Currently less medical therapy is available for all patients with NAFLD. Treatments targeting insulin resistance reverse the associated accumulation of hepatic triglyceride. The following NASH targeting medications including metformin[6] and the thiazolidinedione[7] would be inspiring, but they need to be further evaluated in carefully controlled clinical trials that display sufficient statistical power and good clinically relevant end points. Chinese medicine Ganzhixiao capsule is effective to treat NAFLD. Previous study on Ganzhixiao decoction and its effective component−emodin demonstrated good therapeutic effects experimentally[8−11]. The mechanisms was possibly associated with up-regulation of the expression of PPAR-gamma mRNA in liver tissue. From the above clinical data, it was suggested that after treatment with Ganzhixiao capsule for 8 weeks serum ALT activity, and concentrations of TG and IHCL were decreased significantly, while CT value ratio of liver to spleen increased significantly. It was concluded that NAFLD could be effectively treated by Ganzhixiao capsule.

Localized ^{1}H-MRS is an alternative, reproducible and noninvasive method to measure IHCL. The IHCL can be measured precisely by ^{1}H-MRS. But its clinical application is limited because of expensive cost and prolonged examination time.

FERERENCES

1 Jiangao F. Effect of liver biopsy in the diagnosis of fatty liver. Chin J Hepatol (Chinese), 2000,8(2):116
2 Du X K, Feng Y L. Medical magnetic resonance spectrum is one of physiological examinations to body functional metabolic disease. Chin J Radiol (Chinese), 2000,34(11):725-726
3 Bai L C, Guo S L, Zhou H Q *et al*. The pilot study of ^{1}H magnetic-resonance spectroscopy to quantitatively evaluate the fatty liver. Chin J Med Imaging Technol (Chinese), 2006,22(4):529-531
4 Ricci P E, Pitt A, Keller P J *et al*. Effect of voxel position on single-voxel MR spectroscopy findings. AJNR, 2000, 21(2):367-374
5 Lee J K, Dixon W T, Ling D *et al*. Fatty infiltration of the liver: Demonstration by proton spectroscopy imaging. Radiology, 1984,153(2):195-201
6 Nair S, Diehl A M, Wiseman M *et al*. Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. Aliment Pharmacol Ther, 2004,20(1):23-28
7 Neuschwander-Tetri B A, Brunt E M, Wehmeier K R *et al*. Improved nonalcoholic steatohepatitis after 48 weeks

of treatment with the PPAR-γ ligand rosiglitazone. Hepatology, 2003,38(4):1008-1017
8 Fuer L, Hui D, Zhiqiang G *et al*. Effect of Chinese medicine Ganzhifu decoction on muring fatty liver induced by alcohol and high fat feeding. World Chin J Digestology, 2004,12(7):1596-1599
9 Gao Z Q, Lu F E, Dong H *et al*. Effect of Ganzhifu decoction on experimental fatty liver rats induced by high fat feeding. Chin J Integ Tradi Western Med Diges (Chinese), 2004,12(5):263-266
10 Dong H, Lu F E, Gao Z Q *et al*. Effects of emodin on murine fatty liver induced by feeding with alcohol and high fat laboratory chow. Chin J Hospital Pharmacy, 2005,25(3):11-13
11 Dong H, Lu F E, Gao Z Q *et al*. Effects of emodin on treating murine nonalcoholic fatty liver induced by high caloric laboratory chaw. World J Gastroenterol, 2005,11(9):1339-1344

(Received Feb. 20, 2008)