T_2-relaxation Time Measurement of ex vivo ^{1}H MR Metabolite Peaks for Evaluation of Human Stomach Cancer

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Abstract

In this study, transverse relaxation time (T2) measurement and the evaluation of the characteristics of the spectral peak related to stomach tissue metabolites were performed using ex vivo proton magnetic resonance spectroscopic imaging (MRSI) at 1.5-T MRI/S instruments. Thirty-two gastric tissues resected from 12 patients during gastric cancer surgery, of which 19 were normal tissue and 13 were cancerous tissue, were used to measure the T_2 of the magnetic resonance spectroscopy (MRS) peaks. The volume of interest data results from the MRSI measurements were extracted from the proper muscle (MUS) layer and the composite mucosa/submucosa (MC/SMC) layer and were statistically analyzed. MR spectra were acquired using the chemical shift imaging (CSI) point resolved spectroscopy (CSI-PRESS) technique with the parameters of pulse repetition time (TR) and echo times (TE) TR/(TE1,TE2)=1500 msec/(35 msec, 144 msec), matrix size=24×24, NA=1, and voxel size=2.2×2.2×4 mm^3. In conclusion, the measured T_2 of the metabolite peaks, such as choline (3.21ppm) and lipid (1.33ppm), were significantly decreased ($p<0.01$ and $p<0.05$, respectively) in the cancerous stomach tissue.

Key words : magnetic resonance spectroscopy (MRS), magnetic resonance spectroscopic imaging (MRSI, chemical shift imaging: CSI), stomach tissue, cancer, T_2-relaxation time

I. INTRODUCTION

It is well known that stomach cancer is one of the most common malignant tumors found throughout the Asian countries [1, 2]. The Korea National Cancer Center estimates that about 14,000 new cases of gastric cancer are diagnosed annually. Of those, about 7,500 patients die of this disease every year in South Korea [3]. The early detection, along with accurate preoperative staging and local resectability of gastric cancer, are most important to ensure the proper treatment and to improve the prognosis [3]. Usually endoscopic investigation, using gastrofiberscopic or fluoroscopic X-ray images, CT and endosonography (EUS) are used for the diagnosis of gastric cancer. However, these techniques have the following limitations: the layer of the gastric wall cannot be depicted clearly, and therefore the most important factors in the staging of the stomach, the degree of wall infiltration, cannot be evaluated reliably [4-6].

The peristaltic motion of the stomach wall, pulsations and respiration are main obstacles in studying the in vivo MR to diagnose gastric disease and causes the inherent artifacts. Besides, the thickness of the stomach tissue layer is too thin (< 2 mm) to allow the examination of the extent of the invasion of cancer cells into each layer. Therefore, there have been very few studies using MR imaging or spectroscopy of gastric carcinoma. Proton (^{1}H) MR imaging/spectroscopy is a sensitive technique that allows non-invasive access to metabolic information about organs within the human body [7-13]. To our knowledge there have been no previous reports concerning MR spectroscopy of the stomach tissue except our group [14-19]. Given these conditions, it is significant that the magnetic spectroscopy (MRS) has a great potential for the diagnosis and monitoring of cancer therapy. Spectral peak information of two composite layers (mucosa/submucosa: MC/SMC and proper muscle: MUS) of stomach tissue can be simultaneously acquired by MRSI technique.

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The purpose of this study is to compare and analysis the T_2-relaxation time of spectral peaks between normal and cancerous stomach tissue layers using clinical 1.5-T MRI system.

II. MATERIALS AND METHODS

A. Specimens of Stomach Tissues

Thirty-two stomach tissue specimens, resected during gastric cancer surgery at Pusan Paik Hospital Inje University and Gospel Hospital Kosin College, were obtained from twelve patients between March and September 2005. These patients consisted of ten male and two female between the ages of 49 to 75 years old (mean 62). Nineteen of them were diagnosed as cancerous tissue, while the others were non-cancerous specimens as confirmed by histopatho-logical examination (Table 1). The measured MRSI data of six speciemens were discarded due to sever low signal-to-noise ratio (SNR). These specimens were put in a refrigerator immediately after the gastrectomy and the MR data were obtained at the same day to prevent metabolic changes in the specimens.

Table 1. Number of specimens extracted from 12 gastric cancer patients used for each modality

Modality / Tissue type	Number of ROIs at MC/SMC and MUS layers	Number of specimens
Non-cancerous	9	13
Cancerous	17	19
Total	26[a]	32

a The measured CSI data of six specimens were discarded due to severe low SNR

B. 64-MHz (1.5-T) ^{1}H MRI and CSI

Magnetic resonance scans were performed with a 64-MHz (1.5-T) MRI system (Echo Speed, General Electronics Co., Milwaukee, WI, USA) at the Pusan Paik Hospital using the same custom-made volume coil as the previous work [17-19]. The subjects were 19 cancerous- and 13 normal-gastric tissues. Each specimen was dissected into sections measuring 25×50 mm^2 and placed into polyethylene tubes (inner diameter, 30 mm) filled with normal saline solution within four hours after they were resected at surgery. Spin echo T1 weighted images (repetition time: TR = 300 msec and echo time: TE = 15 msec) in the oblique plane were ob- tained using 70 mm field of view (FOV), matrix size of 256×256, slice thickness of 3 mm, slice gap of 0.1 mm, and number of excitations (NEX) of 4. These images were used mainly for localizer images of CSI. The CSI data were obtained by CSI point-resolved spectroscopy (CSI-PRESS) sequence, repetition time (TR) = 1500 msec, matrix size = 24×24, NA = 1, and voxel size of 2.2 ×2.2×4mm^3. Two sequential measurements using echo times (TE) of 35 and 144 msec were performed in order to calculate the T2-relaxation time in each voxel. ROI was placed to cover both the MC/SMC and MUS layers of the specimens. Auto-gradient shimming was performed before all data acquisitions. The cases with bad shimming were initially discarded from this study. All calculations were performed on a Pentium IV PC (3 GHz and 2 Gbytes RAM), running Windows XP using a Visual C++6.0 (Microsoft, U.S.A.) and Matlab (MathWorks, Massachusetts, U.S.A.) based custom designed software and commercial SPSS software package (version 11.0, ISP Inc., Chicago, IL, USA).

The T_2-relaxation times were measured from spectral peaks of each metabolite using CSI processing software developed by the author. The measurements of T_2-relaxation time, in two selected layers of the gastric tissue, were performed in three metabolite peaks such as lipid, NANA and Choline. The selected two layers were the proper muscle (MUS) layer and the composite of mucosa/submucosa (MC/SMC) layer because the thickness of the MC and SMC layer (less than 2 mm) is usually thinner than the voxel size.

After analysis of MR data, all specimens were prepared for histological examination. Histopathological sections corresponding to the areas examined by MR study were selected for comparison and for determination of cancer invasion. A pathologist, blinded to the findings of the MR study, diagnosed the cancer invasion into each layer of the human stomach tissue.

C. Data Analysis

Acquired data were analyzed using MRSI proce- ssing S/W developed by the authors based on Visual C++ 6.0 (Microsoft, U.S.A.). From reconstructed spectroscopic imaging, spectra were extracted from the selected ROIs such as MC/SMC and MUS layers. Most data processing was performed in automatic mode to prevent operator-dependent error in the same manner of the previous studies [15-18].

The T_2-relaxation time of the three metabolite peaks in two selected layers of cancerous gastric tissue was calculated using the above MRSI processing S/W. In order to compare with the normal tissue and statistically analyzed using the SPSS software package (version 11.0, SPSS, Inc., Chicago, IL, USA), the statistical evaluation was performed using the one-way ANOVA test.

III. RESULTS

A. Histopathological Examination

The histopathological analysis of H-E stained sections of twelve human stomach cancer tissue specimens indicated that

eleven specimens were tubular adenocarcinomas. The twelfth tissue was a signet ring cell carcinoma including tubular and papillary adenocarcinoma. This histological examination results were used as a reference to categorize the T2 values of MRS data according to the infiltration of the tumor cells in stomach tissue.

B. T_2-relaxation Time of ^{1}H MR Spectrum

Non-cancerous specimens: The statistical T_2-relaxation time at three resonance peaks (of two selected layer in non-cancerous stomach tissue, average ± SD were as follows; 49.31±5.53 msec at 1.33 ppm in the MC/SMC layer, 54.78±7.15 msec at 1.33 ppm in the MUS layer, 45.09±12.57 msec at 2.02 ppm in the MC/SMC layer, 50.70±6.67 msec at 2.02 ppm in the MUS layer, 50.09±16.87 msec at 3.21 ppm in the MC/SMC layer, and 57.23 ±16.07 msec at 3.21 ppm in the MUS layer (Table 2), respectively. The T_2-relaxation times did not show statistically significant differences between the MC/SMC and MUS layers.

Cancerous specimens: The statistical T_2-relaxation time at three resonance peaks of two selected layers in cancerous stomach tissue, average ± SD were as follows; 35.70±4.98 msec at 1.33 ppm in the MC/SMC layer, 38.49±5.44 msec at 1.33 ppm in the MUS layer, 34.02±9.11msec at 2.02 ppm in the MC/SMC layer, 38.93±7.49 msec at 2.02 ppm in the MUS layer, 31.98±6.50 msec at 3.21 ppm in the MC/SMC layer, and 36.62±8.29 msec at 3.21 ppm in the MUS layer (Table 2). As the results of non-cancerous gastric specimens, the T_2-relaxation times of cancerous gastric tissue do not exhibit significant differences between the MC/SMC and the MUS layers. But the T_2-relaxation times of three metabolite peaks in cancerous specimens were prominently shorter than non-cancerous ones (Table 2). As shown in Fig. 1, the statistical analysis (one-away analysis of variation: ANOVA) in the choline and lipid peaks indicated that the differences between the corresponding T_2-relaxation time of the non-cancerous and cancerous gastric specimens were statistically significant (choline peak: $p < 0.01$, lipid peak: $p < 0.05$).

C. Color Mappings of the Spectral Peak Intensities and the T_2-relaxation Time Distribution

MR spectroscopic images were reconstructed from acquired MRSI raw data and overlapped over the T1-weighted images as shown in Fig. 2 (a) and Fig. 3 (a). The voxel or region (= group of voxel in this study) of interest were chosen on a case by case basis and their spectra were processed to get metabolite peak intensities, as shown in Fig. 2(b) and Fig. 3

Table 2. Comparison of measured T_2-relaxation time using ex vivo ^{1}H CSI between non-cancerous and cancerous gastric tissue

[msec]

Metabolite (PPM)	MC/SMC		Tumor remark	MUS		Tumor remark
	Non-cancerous	Cancerous		Non-cancerous	Cancerous	
Lipid (1.33 ppm)	49.31	35.70	↓	54.78	38.49	↓
NANA (2.0 ppm)	45.09	34.02	↓	50.70	38.93	↓
Choline (3.2 ppm)	50.09	31.98	↓	57.23	36.62	↓

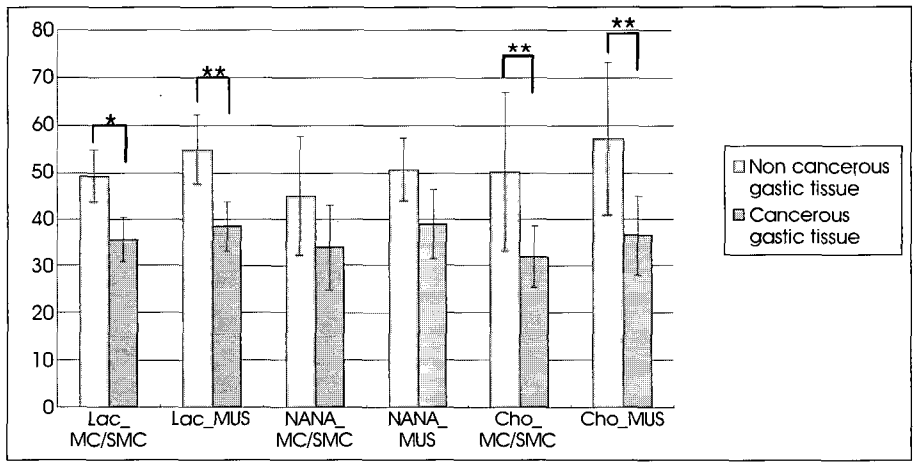

Fig. 1. The statistical analysis result of the measured T_2-relaxation time at 64-MHz MR experiments (*: $p<0.05$, ** : $p<0.01$)

(b) and T_2-relaxation time of the lipid spectral peaks (Fig. 2 (c) and Fig. 3(c)).

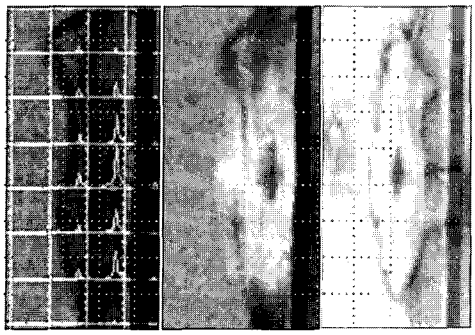

Fig. 2. (a) Multi-voxel spectrum overlapped on T1 image, (b) concentration and (c) T2 map of lipid peak in non-cancerous gastric tissue.

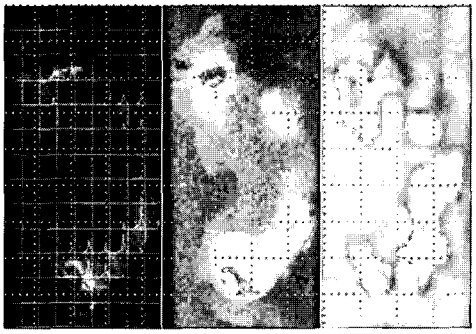

Fig. 3. (a) The CSI spectra overlapped on the T_1-weighted image, (b) the metabolic peak intensity distribution and (c) the T_2-relaxation time map of the lipid peak of the abnormal gastric tissue.

Normal specimens: The layers of non-cancerous tissue are usually so thin that mucosa and sub-mucosa layers cannot be separately selected, as shown in Fig. 2. The distributions of the lipid metabolite peak intensity and its T_2-relaxation time were relatively uniform compared with those of abnormal stomach tissue (Fig. 2). Both the concentration and T_2-relaxation maps were reconstructed at each three metabolite peaks, lipid, NANA and choline, respectively. Authors believe that the change of the T_2-relaxation time reflects the variation in the intercellular environment, involvement of the paramagnetic material, and the effect of magnetic susceptibility within the volume of interest (VOI) may contribute to such alterations.

Abnormal specimens: According to the stage of gastric carcinoma, the layers of cancerous tissues are usually distorted and thick. This study shows that the T_2-relaxation time of the MR spectral peak intensities in abnormal tissue regions were severely decreased. As shown in Fig. 2 and Fig. 3, the distributions of the lipid metabolite peak intensity and its T_2-relaxation time of abnormal stomach tissue were not relatively uniform compared with those of normal one.

Ⅳ. DISCUSSION

In this study, we have demonstrated that the T_2-relaxation time of cancerous stomach tissue has distinct characteristics compared with non-cancerous tissue. We evaluated the T_2-relaxation time of three metabolite peaks in lipid at 1.33ppm, NANA at 2.0 ppm and choline at 3.21ppm using 1.5-T clinical MRSI system. We discarded the spectrum data obtained from six stomach specimens in the experiments because they were broad, featureless and their standard deviation of the VOI data in the MRSI study were too large. This was most likely due to the poorer shimming across a larger selected volume during the MRSI measurement compared with a smaller volume on the single voxel-MRS (SV-MRS) study. We acquired 980 spectra from non-cancerous stomach tissues and 1291 spectra in cancerous tissues using the MRSI technique. We increased the resolution about 139% using this MRSI method compared to the previous SV-MRS method. That is, the voxel size of 3×3×3 mm^3 (27μl) in the previous SV-MRS study was reduced to 2.2×2.2×4 mm^3 (19.36μl) in this study. Albeit the reduction of the voxel size, the mucosa and sub-mucosa layers of the normal gastric tissues could not be clearly separated because these layers are usually thinner than 2 mm of the voxel size. We believe, however, that a more clear classification between mucosa, sub-mucosa and muscle layers is possible using a higher main magnetic field (e.g., 4.7- or 9.4-T MRI/S) than the 1.5-T used in this study.

Tomonori Isobe et al. [30] reported that the T_2 relaxation time of metabolites in the brain tumor seems to change. And similar results were reported in the cerebral infarctions and brain edema by Usenius JPR, et al [31] and Kamada K et al. [32]. However, these studies did not found significant changes in the T_2 relaxation time of metabolites. Nevertheless, we found significantly different T_2 value between normal and abnormal stomach tissue. In statistical analysis as shown in Fig. 1, the T_2-relaxation times of choline and lipid peaks on MR spectra were clearly decreased in cancerous tissues. We infer that the T_2-relaxation time of choline and lipid metabolite peaks can be used as a marker of invasion of gastric cancer cells. However, there were no statistical differences of T_2 relaxation time between two MC/SMC and MUS layers.

Authors expected that T_2 value map of metabolite peak would provide somewhat more clinically relevant information compared with simple biochemical metabolite peak intensity distribution. It is worthwhile to obtain the T_2 relaxation time of the metabolites for a better understanding of the molecular environment of the metabolites in the cancer. T_2 relaxation times for metabolites in cancerous gastric tissue were different than those in normal gastric tissues. This difference may result from

the varying cellular environments of cancer cells. Although the details are yet unclear, various factors, such as changes in the intercellular environment, involvement of the paramagnetic material, and the effect of magnetic susceptibility within the VOI may contribute to such alterations. The changes in the intra cellular environment in cancerous gastric tissue that may cause changes in the T_2 relaxation time including changes in the intracellular energy metabolism, pH, oxygen pressure, and intracellular skeleton, as well as the structural strain in the intracellular space. The involvement of paramagnetic material in gastric cancer tissue such as intratumoral microscopic hemorrhage may also cause susceptibility effect and T_2 shortening. The shortening of the T_2 relaxation time shows that the molecular movement is limited. The prolongation of T_2 means that the molecule can freely move. Our results indicate that the molecular motions of lipid, NANA and choline are limited in the cancerous tissue. Especially, the T_2-relaxation time of choline in cancerous tissue was significantly shorter than non-cancerous one, which suggests that the T_2-relaxation time of choline to be a possible malignancy index.

The analysis of T_2-relaxation time using ^{1}H MR spectroscopy has been limited to stationary human organs. As the gastric wall moves with peristaltic and respiratory motions, it is difficult to acquire in vivo MR spectra from stomach tissue layers. The final goal of this study is to exploit the T_2-value measurement and analysis method using in vivo MR spectroscopy in order to diagnose the gastric cancer disease. The results of this study represent the fundamental data, which may be potential and useful in overcoming the limitations of in vivo MR spectroscopy to diagnose the stomach disease.

V. CONCLUSION

We examined the characteristics of T_2-relaxation time of the biochemical metabolite peak according to the stomach wall layers using ex vivo ^{1}H CSI, and assessed the clinical usefulness of diagnosis for stomach cancer. In comparison with normal tissue, the T_2-relaxation time of lipid at 1.3 ppm, NANA at 2.0 ppm, and choline at 3.2 ppm peaks decreased in the stomach tissues invaded by tumor cells. MR spectrum peak identification and pathological correspondence with this study should be examined in more detail and with regard to analytical aspects in future studies. The T_2 relaxation time changes themselves may aid in understanding of the underlying pathology, which has to be further investigated. It has been recognized for the first time that the T_2 relaxation times of all main metabolites are decreased in cancerous tissue and could be used as an indicator for evaluating the malignancy. These results imply that MR spectroscopy can be used as a robust diagnosis tool for determining the development and progression of human stomach cancer.

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