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Proton and Phosphorous MR Spectroscopy in Squamous Cell Carcinomas of the Head and Neck

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Abstract

Rationale and objectives—Phosphorous magnetic resonance spectroscopy (³¹P MRS) has been used to evaluate and predict treatment response in squamous cell carcinomas of the head and neck (HNSCC). Several studies have also shown the potential of proton MR spectroscopy (¹H MRS) in assessing response in HNSCC. In view of the inherent limitations associated with performing ³¹P MRS in clinical settings, the current study was performed to explore as to whether ¹H MRS could provide similar or complimentary metabolic information in HNSCC.

Materials and Methods—Fifteen patients with HNSCC underwent pretreatment MR imaging. Both ¹H MRS and ³¹P MRS were performed on viable and solid parts of the metastatic lymph nodes of these patients. Peak areas of total choline (tCho) and unsuppressed water, as observed on ¹H MRS and phosphomonoester (PME) and β -nucleotide triphosphates (β -NTPs) from ³¹P MRS were computed. The Pearson correlation coefficient value was used to correlate tCho/water and PME/ β -NTP ratios.

Results—In all the patients, the metastatic nodes appeared hyperintense on T2 weighted images and hypointense on T1 weighted images with variable signal intensity. A prominent resonance of tCho on ¹H MRS and a resonance of PME on ³¹P MRS from metastatic nodes of all patients were observed. A moderate correlation coefficient of 0.31 was observed between tCho/water and PME/ β -NTP ($p > 0.05$).

Conclusion—The biochemical pathways involved in ¹H MRS of tCho may be different from the phospholipids metabolites seen by ³¹P MRS of head and neck cancers and thus the two MRS techniques may be complimentary to each other.

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Keywords

Squamous cell carcinoma of head and neck; phosphorous MR spectroscopy; proton MR spectroscopy; phosphomonoester; choline

Introduction

Squamous cell carcinoma of the head and neck (HNSCC) is a malignant tumor arising most frequently in the non-keratinized epithelial tissue of the upper aero digestive tract. This disease accounts for about 5% of all cancers and about 90% of all malignant tumors of the head and neck region (1). These tumors are characterized by a multiphasic and multifactorial etiopathogenesis (2). Despite recent progress in diagnosis and advances in local and systemic therapeutic approaches, HNSCC remains a clinically challenging disease (3). It is expected that development in treatment modalities along with consistent, reliable and reproducible prognostic biomarkers that monitor and predict therapeutic response, may improve the clinical outcome of patients with HNSCC.

Phosphorous magnetic resonance spectroscopy (^{31}P MRS) provides a window for assessing tissue bioenergetics and metabolism of membrane phospholipids (4). Specifically, the ^{31}P MRS spectrum demonstrates signals from phosphomonoesters (PME, phospholipid precursors) and phosphodiester (PDE, phospholipid catabolites), inorganic phosphate (Pi), phosphocreatine (PCr), and nucleotide triphosphates (NTPs) related to energy metabolism. Using ^{31}P MRS, it has been reported that head and neck tumors exhibit elevated PME/ β -NTP ratios (5,6) and this ratio has been used as a response indicator for chemo and/or radiation therapy in head and neck tumors (7).

While ^{31}P MRS studies have reported alterations in phospholipid metabolism, energetics and pH in tumors, the low sensitivity of this technique limits its application to relatively large and mostly superficial tumors in the clinical settings (8,9). On the other hand, proton MR spectroscopy (^1H MRS) has a 15-fold higher sensitivity over ^{31}P MRS (10) and can detect much smaller tumors with better signal-to-noise ratio (SNR) than ^{31}P MRS (11). ^1H MRS provides information about cellular metabolism that enables understanding of the underlying biological and pathophysiological events associated with tumors (12). A resonance of choline containing compounds (tCho) as observed on ^1H MRS includes signals from free choline, phosphocholine (PC) and glycerol 3-phosphocholine (GPC). The tCho signal is thought to be a marker for increased membrane turnover or cell proliferation (4,13). Several promising *in vivo* and *in vitro* ^1H MRS studies have reported elevation of the total choline (tCho)/creatine (Cr) ratio as a consistent finding for head and neck tumors including metastatic squamous cell carcinoma (SCC) lymph nodes (14–16), and SCC cell cultures (17). Previous reports also indicated that ^1H MRS can differentiate between normal and malignant tissues (14,16,18–20). More recently, a ^1H MRS study reported assessment of response to treatment in HNSCC with a sensitivity of 83% and specificity of 82% (21).

With the inherent problem of relatively low sensitivity and the necessity of additional hardware in performing ^{31}P MRS in mind, it is highly desirable to assess as to whether similar or complementary information from ^1H MRS can be obtained that may obviate the need to perform ^{31}P MRS in future. Thus, the present study was performed to ascertain if tCho, levels as observed on ^1H MRS, have any correlation with PME levels as observed on ^{31}P MRS.

Materials and Methods

Subjects

The study was approved by the Institutional Review Board and written informed consent was obtained from all patients. Fifteen patients (all males, range=31–76 years), who were newly diagnosed with HNSCC were included in this study. All patients were assessed by a neuroradiologist and a radiation oncologist for the presence of metastatic cervical lymph nodes, based on clinical reports and a physical exam. All patients underwent MR examination prior to surgery, radiation, or chemotherapy.

Data Acquisition

MR Imaging

Diagnostic MR imaging, ^1H MRS and ^{31}P MRS were performed on a 1.5 T Siemens Sonata scanner (n=7) or on a 3T Siemens Trio scanner (n=8) (Siemens Medical Systems, Iselin, NJ). A neck array coil or a neurovascular coil was used on 1.5 T or 3 T MR system respectively. The diagnostic imaging protocol on both MR systems included three-plane scout localizer, axial T2 weighted images [Repetition time (TR)/echo time (TE)=4000/131ms], field of view (FOV)=260×260mm², matrix size=384×512, slice thickness=5 mm, flip angle (FA)=120°, bandwidth (BW)=130Hz, number of excitations (NEX)=1; axial T1 weighted images (TR/TE=600/10ms), field of view (FOV)=260×260 mm², matrix size=384×512, slice thickness=5 mm, FA=90°, BW=130 Hz, NEX=1.

^1H MRS

Single-voxel ^1H MRS was performed using a spin echo (point resolved spectroscopy) sequence with water suppression by means of a chemical shift selective saturation (CHESS) sequence. Sequence parameters included: TR/TE=1500/135 ms, BW=1200 Hz, NEX=256. The ^1H MRS voxel was placed in the center of the metastatic node, based on T2 weighted images. Six outer volume saturation slabs (20 mm thick) were placed outside the voxel to suppress lipid signals from the surrounding normal tissue. Acquisition time (TA) for the ^1H MRS sequence was 6:30 minutes. In order to account for the differences in the voxel size between patients, the data were normalized to the water signal from the voxel, which was acquired by an additional spectrum from the same voxel without water suppression using NEX=8.

^{31}P MRS

A single slice or a multi slice two-dimensional (2D) ^{31}P chemical shift imaging sequence with proton decoupling and nuclear Over Hauser enhancement (NOE) was used to acquire the ^{31}P MRS data using a custom-built 7-cm (outer diameter) transmitter/receiver dual tuned $^1\text{H}/^{31}\text{P}$ surface coil. Sequence parameters for the single slice 2D sequence included: TR/TE=1000/2.3 ms, NEX=64, BW=4000 Hz, TA=9:24 minutes. The sequence parameters for the multi slice 2D sequence were: TR/TE= 1000/0.04 ms, NEX=12, BW=4000 Hz and TA=13:54 minutes. The typical voxel size varied from 1.33 mm³ to 15.62 mm³. The decoupling pulse parameters included: pulse type=Waltz-16, pulse duration=1.0 ms, pulse angle =180°. The NOE parameters included a single rectangular pulse for 5 ms with a pulse angle of 90°. The data set was acquired using elliptical k-space sampling with weighted phase encoding to reduce the acquisition time. To minimize the effect of increased nominal voxel size by elliptical k-space sampling, a Hamming filter (50%) was applied in the spatial dimensions.

Data Analysis

The acquired ^1H MRS signal (free induction decay) was zero-filled (1024 data points), smoothed (Hanning filter, width 200 ms) and Fourier-transformed, followed by phase (zero

and first order polynomial) and baseline correction for optimal linear frequency dependence. The ^1H MRS data were analyzed by taking the peak area ratio of the tCho at 3.2 ppm and unsuppressed water resonance at 4.77 ppm using a Leonardo workstation operating the Syngo software (Siemens, Germany). Peak areas were determined using pre-defined model fits for tCho and water resonances. The fitting of individual metabolite peaks was optimized by adjusting the chemical shift, amplitude and line-width interactively. The quality of spectral fitting was estimated by the difference spectrum (fitted spectrum subtracted from original spectrum). The peak areas for tCho and water were computed and the tCho/water ratio was calculated from all the patients.

It has been suggested that using internal water as a reference causes an inherent source of error (22) since the signal intensity of the unsuppressed water signal may be modulated by variations in spin-lattice (T_1) and spin-spin relaxation times (T_2). Therefore, in addition to the tCho/water ratio, compensation of T_1 and T_2 relaxation effects was carried out by calculating the proton density (ρ) value from the metastatic node from each patient using the following equation.

$$SI = \rho [1 - \exp(-TR/T_1)] [\exp(-TE/T_2)] \quad \text{Eq. (1)}$$

where SI=signal intensity of the unsuppressed water signal; ρ =proton density; T_1 =spin-lattice relaxation time; T_2 =spin-spin relaxation time measured from each patient. At $TR=1500$ ms and $TE=135$ ms, the value of ρ was determined from Eq. (1) using the T_1 and T_2 values from each metastatic node. Quantitative T_2 and T_1 measurements in each case were performed by acquiring a series of T_2 -weighted images [four different TE s; 13, 53, 80 and 120 ms ($TR=2000$ ms)] and T_1 -weighted images [five different inversion times (TI s); 60, 200, 400, 800, 1600 ms ($TR/TE=1880/4.38$ ms)] respectively. The tCho/ ρ ratio were computed from all patients.

The acquired ^{31}P MRS signal (free induction decay) was zero-filled (1024 data points), smoothed (Hanning filter, width 100 ms) and Fourier-transformed, followed by phase (zero and first order polynomial) and baseline correction for optimal linear frequency dependence. The ^{31}P MRS data were analyzed by first selecting a representative voxel encompassing the tumor from the multi-slice data set using the Syngo software. Peak areas were determined using pre-defined model fits for PME and β -NTP resonances. The peak areas for resonances at 6.5 ppm due to PME and at -16.0 ppm from β -NTP were computed and the PME/ β -NTP ratio was calculated from all patients.

Statistical analysis was performed using the SPSS program (version 15.0, SPSS Inc., Chicago, IL). Pearson correlation coefficient values were calculated to correlate both tCho/water and PME/ β -NTP and tCho/ ρ and PME/ β -NTP values.

Results

The metastatic nodes appeared hyperintense on T_2 weighted images and hypointense on T_1 weighted images with variable signal intensity. Of the 15 patients studied, four had partially or completely cystic metastatic nodes (based on signal intensity on T_2 and T_1 weighted images). Cystic/necrotic tumors typically exhibit significantly reduced or absent tCho probably due to the absence of active membrane turnover in fluids (23,24). Therefore, data from these patients were excluded from the analysis. On ^1H MRS, the spectrum from a typical metastatic node exhibited elevated resonance from tCho and in some cases, an inverted resonance of lactate (Fig. 1). However, lactate signal was not consistently observed in all patients. The resonance from tCho was observed from all 11 patients, and a resonance from Cr was observed in 7/11 cases. A typical ^{31}P MRS spectrum from a metastatic node demonstrating resonances from PME, PDE, Pi, PCr and γ -, α - and β -NTPs is shown in Figure 2.

The mean tCho/water and PME/ β -NTP ratios from solid viable tumor nodes were 8.32 ± 3.51 and 0.69 ± 0.31 respectively. A scatter plot of tCho/water and PME/ β -NTP illustrates a modest positive relationship between these two ratios (Fig. 3). The Pearson correlation coefficient between tCho/water and PME/ β -NTP was 0.31 ($P > 0.05$). After compensating for T1 and T2 effects, the correlation coefficient between tCho/ ρ and PME/ β -NTP was 0.29 ($P > 0.05$).

Discussion

Head and neck tumors exhibit distinctive spectral characteristics compared to normal tissues including elevated PME/ β -NTP and PDE/ β -NTP ratios on ^{31}P MRS (25–27). Elevated PME and PDE levels have also been reported in a variety of *in vitro* cultured tumor cells (28). The PME resonance comprises of phosphoethanolamine (PE) and phosphocholine (PC) components. The importance of PME resonances can be judged by the fact that they have been implicated in predicting tumor malignancy (5,6), monitoring treatment response (8,27) and prediction of treatment response based on pre-treatment values (7). Another prominent resonance visible on ^{31}P MRS is the PDE that is comprised of glycerol 3-phosphocholine (GPC) and glycerol 3-phosphoethanolamine (GPE) with a dominant contribution from GPC (9). A few studies (5,6,25) have investigated the variations in PDE levels (indicators for membrane catabolism) in head and neck tumors. These studies reported significantly higher PDE/ β -NTP in tumors compared to normal tissues. However, the clinical applicability of PDE resonance as a possible biomarker for response to therapy has been limited in human tumors probably because some studies report an increase while other report a decrease in PDE after treatment (5,25).

Despite these promising studies, widespread clinical application of ^{31}P MRS for studying tumors has been challenging. The reason for its limited use is the need for additional hardware and the inherently lower sensitivity (7%) of ^{31}P nucleus in comparison to the ^1H nucleus. The lower sensitivity translates into long acquisition times and the need to study large tumors. A potential means to enhance the sensitivity is the use of proton-decoupling (29) and NOE pulses (30) that leads to a threefold increase in the signal intensity of coupled resonances. However, these additional radiofrequency pulses increase the specific absorption rate (SAR), which may limit their use in some human studies. Therefore, it is more appealing and convenient to make use of metabolite information as observed on ^1H MRS for diagnosis, staging and predicting treatment response in HNSCC.

Several *in vivo* and *in vitro* ^1H MRS studies of head and neck tumors have indicated the role of tCho/Cr ratio in distinguishing HNSCC from surrounding tissue (14,20,31,32) and differentiating residual or recurrent malignancies from post radiation changes (15). In one *in vivo* study, tissue hypoxia and pO_2 levels were correlated with tumor lactate indicating the use of ^1H MRS in monitoring oxygenation of HNSCC tumors (18). An *in vitro* study indicated the potential of tCho/Cr ratio in predicting treatment response in head and neck tumors (21,33). Huang *et al.* (33) also reported a correlation between tCho levels *in vivo* and response to treatment (chemotherapy and/or radiation). These investigators reported that patients, who did not have any evidence of malignancy, demonstrated lower tCho/water ratios.

On ^1H MRS, the combined tCho resonance is mainly composed of components with N-trimethyl protons such as free Cho, PC and GPC. Due to the small chemical shift difference between methyl protons of PC and GPC, it is difficult to resolve these signals using *in vivo* ^1H MRS (34). However, using *in vitro* high-resolution ^1H MRS, Barker *et al.* (35) observed three-fold higher concentrations of GPC over PC with only a small contribution of free Cho from normal canine brain specimens. These observations were in good agreement with an *in vivo* proton decoupled ^{31}P MRS study on normal human subjects (36). In brain tumors, increased membrane turnover leads to elevations in the tCho resonance predominantly due to

increases in the levels of PC as has been observed by Usenius *et al.* (37) in high-grade astrocytomas using high resolution *in vitro* ^1H MRS. Similar trends in PC and GPC concentrations during the S-phase of rat mammary tumor cells were reported (38). Using cultured human mammary epithelial cells, Aboagye *et al.* (39) demonstrated that progression from normal to malignant phenotype is associated with altered membrane choline phospholipid metabolism. Investigators of this study observed a “GPC to PC switch” in breast tumor cell lines and increased levels of PC relative to GPC with progression from normal to immortalized, to oncogene-transformed, and to tumor-derived cells. It has been hypothesized that growth factor-mediated activation of the tyrosine kinase cascade involving receptor-grb 2-sos-ras-raf-1-MEKMAPK leads to an increase in PC levels (40). These studies indicate a dominance of the PC resonance contributing to the tCho resonance in tumors.

The increase in PME pool in tumors and cultured tumor cells reflect increased membrane turnover (41) and cell nutritional status (42) in the same way that elevated tCho levels are seen in various malignancies on ^1H MRS (43) and thus intuitively, the same information can be obtained by ^{31}P or ^1H MRS. The PME resonances in *in vivo* ^{31}P MRS are often not sufficiently resolved to separate the contributions from PC and PE. However, a number of studies have indicated that PE is the dominant metabolite contributing to this resonance. High performance liquid chromatography and *in vitro* ^1H MRS of human brain tumor specimens have revealed significantly increased PE concentrations in malignant tumors compared to biopsied or autopsied normal brain specimens (44,45). In a study, four to five fold increases in the concentration of PE were observed in pituitary adenoma, malignant lymphoma and medulloblastoma specimens compared to normal brain specimens (44). Increases in PE levels were also reported in the same study from high-grade astrocytoma specimens (44). Moreover, it has been also been reported that the degree of increase in PE is much greater than PC in breast carcinomas (46), lymphomas (9), neuroblastomas (47), medulloblastomas and high-grade astrocytoma, but not in metastatic hepatocellular carcinomas (48). In a murine lymphoma model infiltrating the liver, Dixon *et al.* (49) observed 10 times higher concentration of PE compared to PC. Using *in vitro* high-resolution ^{31}P MRS, a higher concentration of PE, compared to PC, was also observed in cultured SCC cell extracts (17). Although the mechanism and significance for elevated concentration of PE in tumors are not fully understood, it is likely related to the activation of phosphatidylethanolamine metabolism that plays a key role in the modification of the tumor cell membrane (50). A high concentration of PE has also been observed with stimulation of phospholipase C or D in a variety of cancer cell lines (51,52). The enzymes phospholipase C or D trigger the breakdown of phosphatidylethanolamine to PE and diacylglycerol, and PE acts as a long-term second-messenger system for cellular proliferation and cell growth (53).

In spite of the similar steps involved in the biosynthesis of PE and PC, different regulatory mechanisms by a complex enzyme system and transport mechanism may result in different PE and PC concentrations in tumors. Of importance to this study; the phospholipid metabolites contributing to the increases in tCho in ^1H MR spectra (PC and to a lesser extent GPC), are not exactly the same metabolites contributing to the increase in PMEs in ^{31}P MR spectra (PE and PC). Thus the moderate correlation between tCho and PME levels observed in our study is probably due to the fact that the dominant PC signal in the tCho resonance (observed on ^1H MRS) is only a secondary component of the PME signal observed on ^{31}P MRS. We should also note that differences in metabolite levels may arise from differences in voxel dimensions and co-ordinates chosen for ^1H MRS and ^{31}P MRS studies. While this was true, the results obtained here were from similar tumor areas as the voxels were selected from the viable regions of the tumor and the sizes were selected such to encompass the maximum tissue volume for optimal SNR. Future studies with improved acquisition and processing strategies and with higher numbers of patients may further establish the relationship between ^{31}P MRS and ^1H MRS visible phospholipids.

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Fig. 1.

Representative image and spectrum from a patient with HNSCC. (a) An axial T2-weighted image from the neck region demonstrating a hyperintense metastatic lymph node. The white box overlaid on the T2-weighted image shows the voxel selected for spectroscopy (b) A ^1H spectrum (TE=135 ms) from a single voxel ($20 \times 20 \times 20 \text{ mm}^3$) demonstrating a resonance of tCho and an inverted resonance due to lactate.

Fig. 2.

Representative ^{31}P spectrum from the same patient shown in Fig. 1. T2 weighted MR image demonstrating an overlaid grid obtained from 3D ^{31}P chemical shift imaging acquired with proton decoupling and nuclear Overhauser enhancement pulses using a $^1\text{H}/^{31}\text{P}$ dual tuned surface coil. Corresponding spectrum from a voxel (black) ($23 \times 23 \times 23 \text{ mm}^3$) exhibiting various metabolites.

Fig. 3.

A scatter plot between tCho/water and PME/β-NTP demonstrating a moderate positive correlation ($r=0.31$, $P>0.05$).