Physica Medica 29 (2013) 677–683

Contents lists available at SciVerse ScienceDirect

Physica Medica

journal homepage: http://www.physicamedica.com

Original paper

Choline-containing compounds quantification by ^{1}H NMR spectroscopy using external reference and noise measurements

S. Mazzetti [a,*], C. Bracco [a], D. Regge [b], R. Caivano [a], F. Russo [b], M. Stasi [a]

[a] Department of Medical Physics, Institute for Cancer Research and Treatment, Candiolo, Italy
[b] Department of Radiology, Institute for Cancer Research and Treatment, Candiolo, Italy

ARTICLE INFO

Article history:
Received 16 May 2012
Received in revised form
22 June 2012
Accepted 1 July 2012
Available online 24 July 2012

Keywords:
^{1}H-MRS
Metabolite quantification
Choline compounds
In vivo evaluation

ABSTRACT

Proton magnetic resonance spectroscopy (^{1}H-MRS) is largely exploited in clinical settings to non-invasively investigate chemical compounds in human tissues. Applications of ^{1}H-MRS in oncology field are connected to the detection of abnormal levels of choline compounds in more active tumours, providing useful information for cancer diagnosis and treatment monitoring. Since benign lesions may also show presence of a choline peak, implementing absolute evaluation will help differentiating benign from malignant tumours. An external reference procedure was described to provide choline quantification in standard unit of measurements. Spectra were acquired on a 1.5 T scanner using both phantoms and healthy volunteers with a PRESS sequence. The implemented quantification procedure used metabolite and noise measurements on the spectrum to remove large part of scanner settings contributing to metabolites of interest. A standard quantification was also used to compare performances of the noise-based method. *In vitro* quantification had accuracy and precision in the range (95–99)% and (5–13)%, respectively. When applied to *in vivo* studies on healthy volunteers, the method provided very close values of choline concentration, more exactly (1.73 ± 0.24) mmol/l. The method proposed can quantify the proper choline content in phantoms as well as in human structures, as brain. The method is ease of use, computational costless and it can be rapidly calibrated and implemented in any centre.

Introduction

In vivo localized proton single-voxel magnetic resonance spectroscopy (^{1}H-MRS) is an application of magnetic resonance that non-invasively provides chemical information about metabolite content in human tissues [1]. The first clinical usage of ^{1}H-MRS came in the 1980s and since then ^{1}H-MRS has become an important application in oncology to study patients with brain cancer [2].

Recently, ^{1}H-MRS acquisitions have been expanded to cancer investigation in other regions of the body including prostate [3–7], breast [1,8–12] and musculoskeletal lesions [13–15]. The potential value of ^{1}H-MRS in oncology is usually connected to the detection of abnormal levels of choline compounds, typically found in more active tumour masses [16,17]. Such molecular information is expected to be useful for cancer diagnosis, treatment monitoring and patient follow up.

However, some recent studies demonstrated that choline signals could also be detected in benign lesions and in normal tissues [10,12]. For this reason a quantitative assessment is required to accurately establish choline levels and, thus, distinguish between the different pathologies.

Absolute determination of metabolite concentrations by ^{1}H-MRS has been attempted by either internal or external standard references and using known concentrations [18]. The ratio of the area under a metabolite peak to the area of internal reference is commonly used as a surrogate of metabolite concentration since it is very easy to compute [19–22]. Absolute quantification (AQ) by external reference offers some advantages: higher reproducibility of results can be achieved, concentrations can be expressed in mM units, internal reference, such as water or creatine (for brain tumour masses), becomes unnecessary and tracking metabolite variations would be more representative of actual clinical modifications during longitudinal studies. The drawbacks of external reference AQ include time consuming data acquisition (i.e. phantom calibrations) and more extensive data analysis [23].

This work describes an external reference quantification procedure to account for scanner contribution to the signal in order to evaluate choline concentrations in human tissues. The method is based on the observation that scanner settings simultaneously influence signals generated by metabolites as well as thermal noise.

---

[*] Corresponding author.
E-mail address: simone.mazzetti@ircc.it (S. Mazzetti).

1120-1797/$ – see front matter © 2012 Associazione Italiana di Fisica Medica. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ejmp.2012.07.002

Hence, metabolite concentrations, independent of scanner technical settings, can be obtained using the ratio between the metabolite signal and spectrum noise content.

Materials and methods

Acquisition protocol

Phantom studies and *in vivo* measurements were performed on a clinical whole-body scanner (Signa HDx, GE Medical System, Milwaukee) operating at 1.5 T with standard ^{1}H-MRS acquisition software provided by the manufacturer. A body coil was used for signal transmission and a dedicated eight channel high resolution head coil (8HRBRAIN, MRI Device Corporation, Wisconsin) as a receiver for both MR imaging and ^{1}H-MRS. A series of T2-weighted fast gradient echo (FGRE) images were acquired in the three spatial planes to correctly place the spectroscopic voxel. Spectra were collected with a point-resolved spectroscopy sequence (PRESS) [24] using the following acquisition parameters: repetition time (TR) 1500 ms, echo time (TE) 35 ms [25], NEX = 8, total number of scans = 128, spectral width = 2500 Hz, sampling points = 2048.

A short TR was selected to maintain scan duration unchanged for both phantom studies and *in vivo* acquisitions. In fact, increasing scan time in clinical settings would decrease patient compliance, resulting in motion artifacts and would reduce overall patient throughput. Furthermore, a longer TR would reduce the total number of acquisitions, yielding a lower signal to noise ratio (SNR).

Automatic shimming on the unsuppressed water signal ensured that full width at half maximum (FWHM) was equal to or less than 5 Hz. If this condition was not met automatically, manual shimming was performed to adjust the FWHM to a lower value. Total imaging time, including pre-imaging shimming adjustments and water suppression, was less than 5 min.

Phantoms

In vitro metabolite quantification was carried out on a phantom provided by the scanner manufacturer and on several home-built phantoms. The first was a sphere filled with an aqueous solution of biochemicals typically present in brain tissue. The home-built phantoms were two sets of vials: the first was designed as a training set for the AQ of choline and consisted of five Falcon tubes (each one with a volume of 50 ml), filled with a choline chloride (Sigma–Aldrich, Steinheim, Germany) and bi-distillate water solution with concentrations ranging between 0.5 and 4 mmol/l. The second, referred to as a validation set, consisted of six vials in which choline concentrations were different with respect to the first set and ranged between 0.75 and 2.25 mmol/l. All of the aqueous solutions of choline chloride were prepared in tubes of 10 cm in length and 3 cm in diameter at room temperature.

During acquisitions each sample was kept in a water container to optimize field homogeneity, reducing magnetic susceptibility differences at vial–air interfaces. Coil resistive load was increased to simulate body presence by posing a 15 × 15 × 40 cm^3 box filled with NaCl and $CuSO_4 \bullet 5H_2O$ solution beside the choline-containing Falcon tube, so that measurements were more representative of *in vivo* studies.

In vitro measurements

A pre-scan procedure was run before each acquisition to define optimal settings for analogue/digital receiver gains, transmitter gains and water centre frequency. In fact, radiofrequency excitation caused sample temperature rising and consequent metabolite frequency shifts which, if neglected, might have produced errors during spectra acquisitions. If the pre-scan process had been omitted, the default frequency as set by the scanner for excitation would not have been optimal for the prescribed scan, thus the metabolite content in the examined voxel would not have been correctly stimulated. Before using the phantoms for calibration, the influence of several scanning factors on signal amplitude was investigated: a large quantity of spectra were acquired on the manufacturer's phantom to evaluate signal variations correlated to analogue gain (G1), digital gain (G2) and voxel size (V). Measurements were taken by changing one parameter at a time and holding the others at a constant value. For example, maintaining fixed gain values (G1 and G2) while changing voxel size ensured that all signal variations were ascribable to the different voxel sizes.

Data processing

At the end of the acquisition process, raw data were transferred from the scanner to a dedicated workstation where post-processing took place. Spectra analysis was performed by homemade tools and the reconstruction process consisted of the following steps: phase correction, apodization, zero-filling, Fourier transform and baseline correction. In particular, the parameter to implement zero order phase (φ) correction was obtained by Bolan's approach [12]. Phase was measured and corrected using the average of two different autophasing methods: 1) the phase of the first 100 time domain points was fitted to a straight line to obtain φ at time $t = 0$ and 2) φ was chosen to maximize the smallest value of the real part of the spectrum. A robust estimate of φ was produced using the average of φ found when applying these two methods.

Apodization was carried out using a 1.5 Hz line broadening decaying exponential function, while zero-filling procedure doubled points in the spectrum, increasing the frequency point number. Following these preliminary corrections, water suppressed/unsuppressed FIDs were Fourier transformed and the resulting spectra were frequency referenced by setting the maximum water peak to 4.7 ppm. The procedure was applied to all FIDs acquired by each individual head coil channel.

The final spectrum was constructed from the eight components, each weighted by its SNR [8]. Water peak linewidth was used as the parameter to accept or reject a spectrum before post-processing steps; in this study the water peak linewidth threshold was set to 3 Hz and this value was meant only for *in vitro* studies. Baseline correction was finally applied to the resulting spectrum as shown in Heuer's work [26]: to decide whether the *i*-th point lay on baseline, a rectangular 15 spectral points width window was centred on it. Maximum and minimum values within this window were extracted in order to compare their difference to noise standard deviation. The *i*-th point was classified as baseline if the absolute difference between minimum and maximum values did not exceed twice the noise standard deviation. The final baseline was constructed by spectrum fragments connected with straight lines.

The following analysis was implemented to automatically detect the choline peak and to measure its area. Detection algorithm was based on the analysis of first and second spectrum derivatives. The first derivative was computed in the range (−1, 4.5) ppm; then the first derivative zero crossing points were determined and, where each zero crossing point was found, the second derivative magnitude was compared to a threshold value to avoid spurious peak detection. Peaks found in this step were compared to an adaptive value, i.e. if the peak to noise ratio was greater than three, then the algorithm returned the value in ppm where the peak was detected. Once identified, it was fitted to a Gaussian bell using nonlinear least-square regression. The fitting procedure was included in the post-processing step to remove possible spurious signals superimposed on choline tails. Once the fitting procedure was