Journal of Magnetic Resonance 294 (2018) 71–82

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Multiparametric quantification of heterogeneity of metal ion concentrations, as demonstrated for [Mg^{2+}] by way of ^{31}P MRS

Norbert W. Lutz *, Monique Bernard

Aix-Marseille Univ, School of Medicine, CRMBM, 27 Bd Jean Moulin, F-13005 Marseille, France

ARTICLE INFO

Article history:
Received 16 April 2018
Revised 18 June 2018
Accepted 30 June 2018
Available online 30 June 2018

This paper is dedicated to the memory of William E. Hull, Ph.D.

Keywords:
^{31}P NMR
Lineshape analysis
Statistical distributions
Heterogeneity descriptors
Histograms
Free Mg^{2+} concentration

ABSTRACT

Magnesium(II) is the second most abundant intracellular cation in mammals. Non-invasive ^{31}P MRS is currently used to measure intracellular free Mg^{2+} levels in studies of magnesium deficiency disorders. However, this technique only provides one [Mg^{2+}] value for a given tissue volume (or voxel), based on the chemical shift of the ATP-β (or NTP-β) resonance. We present here an approach for quantifying tissue heterogeneity in regard to [Mg^{2+}], by way of multiple ^{31}P MRS-derived descriptors characterizing the statistical intra-volume distribution of free [Mg^{2+}] values. Our novel paradigm exploits the fact that the lineshape of the ATP-β ^{31}P MRS resonance reflects the statistical distribution of [Mg^{2+}] values within the observed volume (or voxel). Appropriate lineshape analysis reveals multiple quantitative statistical parameters (descriptors) characterizing the [Mg^{2+}] distribution. First, the ATP-β ^{31}P MRS resonance is transformed into a [Mg^{2+}] curve that is used to construct a histogram with our specially developed algorithms. From this histogram, at least eight [Mg^{2+}] descriptors are computed: weighted mean concentration and median concentration, standard deviation of concentration, range of concentration, concentration mode(s), concentration kurtosis, concentration skewness, and concentration entropy. Comprehensive evaluation based on *in silico* and experimental models demonstrates the validity of this new method. This basic feasibility study should open new avenues for future *in vivo* studies in physiology and medicine.

1. Introduction

Magnesium(II) is the second most abundant intracellular cation in mammals after potassium (K^+) [1]. A number of magnesium deficiency disorders, among these congenital disorders, are known, which are frequently due to decreased gastrointestinal or renal magnesium(II) absorption. In addition, diabetes and endocrine causes may result in hypomagnesemia, while magnesium(II) decline has been shown to play a critical role in the development of irreversible tissue damage following direct or indirect neurotrauma [2]. Moreover, drugs including antibiotics, anticancer chemotherapeutics and immunosuppressive agents have been linked to magnesium(II) depletion [1]. In such contexts, determining Mg^{2+} levels present in body tissue (rather than blood) allows a direct assessment of variability of intracellular tissue Mg^{2+} concentrations.

More than 30 years ago, a non-destructive and non-invasive method to measure intracellular free Mg^{2+} levels by ^{31}P MRS has been introduced [3]. (Note that in the following, we exclusively deal with the determination of the concentration of free magnesium(II) cations; these are in fast to intermediate exchange with ATP.) This technique uses naturally occurring, endogenous ATP as an intrinsic Mg^{2+} reporter molecule, thus rendering any administration of an exogenous Mg^{2+} probe superfluous. Predominantly based on Gupta et al.'s seminal work on Mg^{2+} quantitation in human blood and cancer cells [4,5], early applications also included Mg^{2+} measurements in intact frog skeletal muscle [6] and perfused heart muscle [3,7], pointing to the *in vivo* potential of this ^{31}P MRS technique. Since the 1990ies, this method has been applied to determine Mg^{2+} levels in vivo in experimental animals and humans, notably in the brain [8–13] and in skeletal muscle [11–13], but also in tumors [9,14] (an overview of Mg^{2+} measurements in these and other tissues has been presented elsewhere [15]). In all these studies, one [Mg^{2+}] value has been determined for the tissue volume (or each voxel) examined, based on the chemical shift of the maximum of the ATP-β (or NTP-β) resonance of the measured ^{31}P MR spectrum. However, this approach does not reflect intra-volume (or intra-voxel) tissue heterogeneity with respect to intracellular Mg^{2+} levels, as it only provides a single value that may or may not be close to the mean. This is also true

* Corresponding author.
E-mail addresses: nlutz@umars.fr (N.W. Lutz), Monique.Bernard@univ-amu.fr (M. Bernard).

https://doi.org/10.1016/j.jmr.2018.06.016
1090-7807/

for ^{31}P MRSI, where spatial resolution is rather coarse [16]. In fact, experimental evidence of heterogeneous distribution of intracellular Mg^{2+} (subcellular microheterogeneity) has been provided by fluorescence microscopy for a number of different cells [15]. In addition, intracellular [Mg^{2+}] is known to depend on malignancy and growth [15], and the latter two characteristics often vary considerably across a given tumor volume [17]. Necessarily, both macroscopic and microscopic intracellular-[Mg^{2+}] heterogeneity within a given tissue volume measured by ^{31}P MRS is reflected in the overall lineshape of the resulting ATP-β resonance. Therefore, information on the underlying Mg^{2+} heterogeneity can be extracted by an appropriate analysis of the ATP-β lineshape. By contrast, the classical method of exclusively evaluating the position of the lineshape maximum disregards most of the information contained in the overall resonance.

We present here the first technique designed to quantitatively characterize the very heterogeneity of tissue [Mg^{2+}]. This method is based on multiparametric statistical analysis of the resonance of the ATP-β phosphate moiety. The new paradigm underlying our technique is grounded in the fact that (i) the chemical shift of the ATP-β ^{31}P MR resonance, $\delta_{ATP-\beta}$, is a function of the Mg^{2+} concentration (based on the dissociation constant, K_d, of the ATP-Mg^{2+} complex [18,19]), and (ii) the overall ATP-β MRS lineshape reflects the statistical distribution of [Mg^{2+}] values in the measured sample volume or voxel, within certain limits. To analyze [Mg^{2+}] heterogeneity with this method, ATP-β ^{31}P MRS resonances are first converted to [Mg^{2+}] distribution curves. Then, these [Mg^{2+}] profiles are transformed into histograms by an appropriate algorithm. Based on these histograms, further mathematical algorithms developed by us are used to calculate at least eight quantitative parameters (descriptors) defining the underlying [Mg^{2+}] distribution, i.e., the [Mg^{2+}] heterogeneity within the measured sample volume.

We exploit, for the first time, the entire MRS lineshape (corresponding to the entire [Mg^{2+}] curve) to quantitatively describe a distribution of [Mg^{2+}] values. We present the paradigm and algorithms underlying our method, together with the proof of principle for the following statistical descriptors of [Mg^{2+}] distribution: weighted mean and weighted median concentrations [20], standard deviation, [Mg^{2+}] range, one or multiple [Mg^{2+}] modes (i.e., [Mg^{2+}] curve maxima), kurtosis (peakedness [20]), skewness (asymmetry [21]) and entropy (smoothness [22,23]), ratios of [Mg^{2+}] mode heights and areas under individual [Mg^{2+}] ranges. We assess the validity of our method based on (i) simulated ATP-β ^{31}P MR spectral lines, and (ii) measured ATP-β ^{31}P MR spectra of well-defined aqueous test solutions. The rationale for our simulations, which are entirely unrelated to conventional NMR lineshape simulations based on spin dynamics or Bloch equations, has been described previously in great detail for an analogous application to thermal heterogeneity [24,25]. Briefly, the main purpose of our *in silico* modeling is to examine questions such as: What are the ranges of the statistical descriptor values for [Mg^{2+}] distribution curves to be expected from actual experiments? How sensitive are the resulting [Mg^{2+}] distribution curves, and their associated descriptor values, to actual [Mg^{2+}] gradients vs. spurious effects, i.e., curve shape contributions unrelated to [Mg^{2+}] distribution? What are the effects of ATP-β ^{31}P MRS lineshape correction by our empirical deconvolution method on the resulting [Mg^{2+}] distribution curve shapes and, consequently, on the derived descriptors of [Mg^{2+}] profiles? How do [Mg^{2+}] distribution descriptors obtained from simulated spectra compare with those from measured MR spectra of model solutions? Note that empirical rather than *ab-initio* simulations are employed; both chemical shift and linewidth values are taken from actual ^{31}P MRS experiments. This bestows high practical relevance upon our calculated models.

In addition to simulations, experiments performed on a series of specially designed phantoms providing well-defined [Mg^{2+}] gradients are presented, along with a preliminary example of an actual *in vivo* application. Validation results are presented for field strengths commonly used in present-day animal experiments (9.4 and 11.7 T); similar field strengths (e.g., 7 T) are increasingly used in human studies, which renders our validations relevant for future applications in both animal models and patients. All calculations are performed employing a preprogrammed EXCEL spreadsheet provided as Supporting Information.

We present here our approach from a basic research point of view; issues of biological interpretation and clinical relevance are not dealt with in this report, but will be subject of future research projects. Since methods able to measure statistical [Mg^{2+}] distributions have been unavailable up until now, the significance of statistical [Mg^{2+}] descriptors in biomedical research has remained unexplored. Although the current report is clearly focused on the proof of principle rather than on any particular application, our findings are expected to have significant implications for studying the relationship between [Mg^{2+}] variations and other biochemical, biophysical, and physiological properties in a variety of biological tissues in vivo. With future projects aiming at a comprehensive *in vivo* validation being planned, a broad scope of research fields in biochemistry, pharmaceutical and medical chemistry may benefit from our novel approach, with potential applications to cardiology, myology, neurology and oncology.

2. Theory

The use of ^{31}P MR spectroscopy for intracellular [Mg^{2+}] measurement is based on the [Mg^{2+}] dependence of the chemical shift of the ATP-β resonance, $\delta_{ATP-\beta}$. The relationship between $\delta_{ATP-\beta}$ and [Mg^{2+}] is given by the following equation [26]:

$$[Mg^{2+}] = K_d^{MgATP}\left(\frac{1}{\Phi} - 1\right) \tag{1}$$

with

$$\Phi = \frac{[ATP]_f}{[ATP]_{tot}} = \frac{\delta_{ATP-\beta} - \delta_{ATP-\beta}^{MgATP}}{\delta_{ATP-\beta}^{ATP} - \delta_{ATP-\beta}^{MgATP}} \tag{2}$$

where K_d^{MgATP} is the dissociation constant of the MgATP complex; $[ATP]_f$, concentration of free (uncomplexed) ATP; $[ATP]_{tot}$, total concentration of ATP (free and complexed with Mg^{2+}); $\delta_{ATP-\beta}$, measured ATP-β chemical shift; $\delta_{ATP-\beta}^{ATP}$, ATP-β chemical shift for uncomplexed ATP; $\delta_{ATP-\beta}^{MgATP}$, ATP-β chemical shift for ATP complexed with Mg^{2+}. K_d^{MgATP}, $\delta_{ATP-\beta}^{ATP}$ and $\delta_{ATP-\beta}^{MgATP}$ are empirical values that are determined by way of calibration measurements; consequently, K_d^{MgATP} is also denoted as K_{app} (apparent K_d). These values may vary slightly as a function of intracellular parameters such as pH and ionic strength. Besides the basic relationship involving three parameters obtainable through appropriate calibration (Eqs. (1) and (2)), more complex relationships have been developed for specific tissue types, e.g., for brain and exercising muscle. Calibrations for these tissues resulted in five and ten fitted parameters, respectively [13]. The dedicated EXCEL template provided in Supporting Information currently provides the option to choose between these three and five-parameter algorithms for ppm-to-μM Mg^{2+} conversions; validation results for the five-parameter algorithm are presented and discussed in Supporting Information.

The method for quantitative characterization of [Mg^{2+}] distributions presented here is based on the following general paradigm, originally developed by us for applications to two other measurement parameters, viz., pH and temperature [24,25,27]: Suppose that a sample is heterogeneous with respect to a measurement parameter, *p*. Further suppose that the chemical shift of an MR