BRAIN BYTES

Quantification of brain metabolites

Introduction

Magnetic resonance spectroscopy (MRS) provides a means to non-invasively detect brain metabolites *in vivo*. Traditionally, MRS has been used as a diagnostic tool in the biochemical characterisation of pathological processes such as tumours; however, better access to high-field magnetic resonance imaging (MRI) has led to a greater uptake in the use of this modality particularly in neuropsychiatry (1). The increase in the popularity of MRS may be partly due to the enhanced signal-to-noise ratio (SNR), but equally may be resultant from the fact that many neuropsychiatric disorders are predicated on biochemical/neurotransmitter dysfunctions and as such, in these instances, MRS is the modality of choice. This increase in popularity has translated into a substantial number of research articles now being published, and by far the majority of this published literature presents metabolite results in the form of ratios otherwise referred to as *'relative quantification'* (RQ) as opposed to *'absolute quantification'* (AQ) of metabolites. As the names suggest, the differences between relative and absolute quantification are significant and thus have an impact on the interpretation of data and the conclusions that can be drawn from it. The focus of this issue of Brain Bytes will be on the differences between the two methods and how this can affect the interpretation of data. Lastly, a brief overview is presented on how absolute quantification of brain metabolites is achieved.

Quantification strategies

Relative quantification is a sound method for reporting MRS data whereby metabolite concentrations are presented as a ratio. Typically, one of the measured metabolite concentrations (or peak) is chosen as the internal standard or reference and serves as the denominator in the ratio, with the numerator being the concentration (peak) of the metabolite of interest. There are several advantages of using this type of quantification method. RQ does not require additional image acquisitions or time-consuming post-acquisition data analysis. As such, RQ is simple to implement and metabolite results can be generated rapidly. Additionally, confounds relating to partial volume effects of CSF in the voxel of interest can be avoided with the use of RQ, as both the internal standard (the metabolite that makes up the denominator) as well as the metabolite of interest (the numerator) are both equally affected by partial volume effects and as such, cancel out. In special cases where both the numerator and denominator are predicted to change in opposite directions (e.g. the numerator increases in concentration while the denominator decreases), RQ offers greater sensitivity. Thus, when the metabolite which serves as the denominator in the ratio remains constant (or changes in a predictable manner), then the RQ method can be a robust means of presenting MRS results. However, there are disadvantages in using RQ methods. The greatest limitation of RQ occurs when there is uncertainty about which metabolite concentration has changed, as is the case when the internal standard (denominator) changes in an unpredictable manner. For example, if the concentration of the internal standard decreases while the concentration of the metabolite of interest remains unchanged, the resulting ratio could be ambiguous and any interpretation of the data or conclusions thereof solely based on the behaviour of the metabolite of interest (numerator) would clearly be inaccurate. The assumption that the concentration of the internal standard remains the same through normal and pathological states is erroneous and should be avoided (2). There are also inherent limitations in using RQ when inferring changes to metabolite concentrations as a result of pharmacological interventions. In particular, for treatment trials, MRS data are often compared with biochemically derived concentrations, which are expressed in standard units such as molarity (moles of metabolite per litre of tissue water) or molality (moles of metabolite per kilogram of tissue water). In these instances, data presented as ratios are of limited value. One way of circumventing this problem is to convert the measured metabolite peaks to standard concentrations units by using an empirically derived value from the literature whose concentration remains constant. This technique otherwise known as the endogenous marker method is relatively straightforward; however, it remains an RQ technique and as such, most of the advantages and limitations associated with such still apply.

Absolute quantification on the other hand is the process by which MRS data is collected and post-processed in a manner whereby the final concentrations that are derived are the actual *in vivo* metabolite concentrations for the sampled voxel of interest. The advantage of AQ is that it provides an unambiguous representation of the change in metabolite concentrations independent of any secondary influence of other metabolites. This is important especially in disease states where there may be changes in several observed metabolites or even T1 and T2 relaxation effects. AQ also allows the presentation of

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metabolite concentrations using standard concentration units of molarity as well as molality and thus can be easily compared to biochemically derived concentrations enabling modelling of pharmacokinetics and compartmental analyses. The disadvantage in implementing AQ is that it is significantly more time-consuming compared to RQ, since additional data acquisition and post processing is required to quantify the *in vivo* metabolites. There are several techniques, by which AQ of spectra can be achieved. One method referred to as the external reference technique involves mounting a vial of reference solution, with a known chemical composition and relaxation properties, in the head coil simultaneously with the subject's head. Immediately following the acquisition of the *in vivo* spectra, the reference vial is scanned with the subject's head still in place. *In vivo* metabolites are then adjusted against the reference signal and subsequently quantified. When using this method however, it should be borne in mind that since the reference vial is spatially separate from the subject head, adjustments in acquisition parameters are required to correct for B_1 inhomogeneities.

The byte

MRS is increasingly being utilised to investigate biochemical and neurotransmitter functions in neuropsychiatry. While the MRS modality is robust, appropriate care is required when interpreting *in vivo* spectra to ensure that conclusions derived from the data are valid. One potential source of ambiguity for MRS data is the use of RQ for data analyses where unpredictable changes in the concentrations of metabolites make interpretation of the resulting ratios difficult. A more accurate and reliable method involves determining AQ of measured metabolites, which allows for more precise data interpretations and conclusions. While AQ techniques are more time-consuming, they offer vastly improved diagnostic specificity in comparison.

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