

Chapter III – B. Soussi

Basic principles of MR Spectroscopy in Neurosciences

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AIM OF CHAPTER

The aim of this chapter is to provide a comprehensive introduction to the new possibilities that Magnetic Resonance Spectroscopy (MRS) offers in clinical neurosciences. Focus will be on what MRS can do rather than what MRS is. For simplicity, basic physical and chemical principles will not be much explored and are referred to elsewhere.

INTRODUCTION

For over half a century, interest in Nuclear Magnetic Resonance (NMR) has been continuously increasing. From structural analysis in smaller organic molecules, to biochemical macromolecules, tissue extracts, isolated intact organs and in vivo studies in animals and humans.

For almost two decades, in vivo MRS has been a revolutionary technique in biomedical research. Today, it is a powerful tool in neurosciences giving noninvasive access to the chemistry of the human brain in health and in disease.

Nuclei like ^{31}P , ^1H , ^{13}C , ^{19}F and ^{23}Na have been studied in various organs. However, early applications of in vivo MRS began with the measurements of ^{31}P metabolites in isolated organs and surface regions like skeletal muscles from intact animals.

Historically, ^{31}P has been the most studied nucleus. However, MRS of the brain today relies mostly on ^1H examination due to its relative ease i.e. high natural abundance (99.9%) and sensitivity (100%). Numerous studies have shown that MRS can detect pathophysiological changes in the brain tissue in a number of diseases. Therefore, this chemically specific technique with its ability to examine the mechanisms of disease is continuously gaining attention from clinicians.

In vivo MRS should be seen as complementary to the well established clinical MRI, providing quantitative nondestructive analysis of the biochemistry of the brain cells without the use of radioactive tracers.

It is possible to integrate spectroscopy with conventional MRI equipment of 1.5T or higher magnetic field by adding appropriate hardware and software available from MR manufacturers.

Theoretical background

MR theory is described elsewhere. For more detailed physical and chemical aspects of the technique see references.

The basic principles for MRS are the same as for MRI. It is suitable however, to mention some aspects that are related to spectroscopy. Briefly, and put in its simplest form:

The interaction between atomic nuclei (possessing a spin that gives a magnetic moment) and radio waves when an external static magnetic field is applied gives rise to an electromagnetic signal.

The electromagnetic signal obtained after the application of a 90° radiofrequency pulse is called free induction decay (FID).

At the same time, each nucleus is characterized by the time constants T_1 (longitudinal relaxation) and T_2 (transversal relaxation).

The decaying signal is the result of the relaxation of the nuclei from their excited state to their relaxed state.

The FID is then converted to a spectrum by a Fourier transformation (mathematical algorithm).

The “spectral” chemical shift (δ) is measured in parts per million, (ppm) and is a characteristic of the variation in resonance frequency. Its specific dependency on the chemical environment of a particular nuclei makes it like a “finger print” of the analyzed substance. Figure 1 shows the conversion of a FID to a spectrum by Fourier transformation.

Localization

“Image guided spectroscopy”

Figure 2 (A, B) illustrates the selection of a volume of interest (VOI) based on a topographical MR image in order to acquire a proton MR spectrum.

The same strategy is used in the example in figure 3 to get a ^{31}P MRS localization based on a topographical MRI.

Localization methods

Early localization methods started with surface coil localization which is based on RF pulses and the use of surface coils for spatial localization.

A disadvantage of this procedure is surface tissue contamination of the spectra.

Multi-shots methods

ISIS

Image -selected in vivo spectroscopy (ISIS) uses a combination of 8 pulses. The VOI is pre-selected, based on MRI scan and is repeatedly excited. The ISIS method has been applied to both ^{31}P and ^1H . One advantage of this method is that it can be used without T_2 weighing. However, the eight phase cycles used in localization might make shimming difficult.

Single-shot methods

Two methods are widely used and basically similar.

1) STEAM

Stimulated Echo Acquisition Mode (STEAM) uses a stimulated echo generated by three 90° pulses (90° - 90° - 90°). It is mostly used in ^1H spectroscopy. Signal loss due to motion sensitivity at long echo times is a disadvantage. This method is suitable for short TE acquisitions.

2) PRESS

Point Resolved Spectroscopy (PRESS), involves a double spin echo scheme (90° - 180° - 180°) which theoretically gives improved S/N. This method is most suitable to ^1H spectroscopy where small volumes and/or metabolites with long relaxation times T_2 are of interest.

Characteristic patterns seen in STEAM and PRESS spectra in patients with acute brain injury are shown in fig 6.

Spectroscopic imaging

Spectroscopic imaging is the simultaneous acquisition of spectra from many volumes using phase encoding. It is suitable for both ^1H and ^{31}P . This method offers the advantage of investigating many slices simultaneously. However, the S/N is lower than in single-voxel techniques.

Water and lipid suppression

The ^1H peak from brain water is dominant as well as the resonance from precranal lipids. Since most ^1H signals from brain metabolite are present at concentrations less than 10 mM, water and lipid suppression techniques are essential in ^1H MRS. Water suppression can be done using Gaussian chemical shift selective pulses (CHESS). The water signal is pre-saturated by using frequency selective 90° pulses.

Outer volume selective pulses may be applied to pre-saturate the lipid resonance. However, by using localization technique such as PRESS and STEAM lipid areas can be kept outside the VOI.

Sensitivity

The analytical limit is around 1 mM. MRS is thus not a very sensitive technique. However, many of the 100% naturally abundant ^{31}P and ^1H metabolites are present in cellular concentrations in the mM range.

In localized in vivo spectroscopy, theoretical minimum resolution is around 1 ml for ^1H and 15 ml for ^{31}P . Generally, volumes for brain ^1H MRS vary from 4 - 30 ml at 1.5T and typically used VOI is around 8 ml. Resolution can be improved at longer acquisition times and with increasing magnetic field strength.

Several factors can influence the sensitivity during an MR examination. For example, the presence of paramagnetic species, or the slow exchange between bound and unbound forms of molecules, can cause signal broadening.

Changes in viscosity, inhomogeneity of magnetic field and many exchange processes could also affect the line shape of a resonance.

However, despite this relative insensitivity, no other method can do today what MRS can.

Field strength

Most clinical MRS is performed at 1.5T to this date. Higher field strength permits better resolution of overlapping peaks. Field strengths of 3 and 4 T for clinical research have been available for a few years. Today, in vivo magnets of 9 T for experimental research are commercially available. A comparison illustrating improvement in resolution with increased magnetic field strength is shown in figure 4 (a, b).

Spectral quantitation

For calculation of in vivo metabolite concentrations it is important to apply quantitation methods using internal and/or external standards.

Absolute quantitation is possible but remains difficult. Relative concentrations and areas of peak ratios are also useful and widely used.

Problems associated with spectral quantitation

Common technical problems encountered arise from:

motion artifacts

magnetic susceptibility effects

partial volume effects

Motion artifacts may arise from breathing or any other movement. Susceptibility effects may arise from the variety of adjacent tissue to the VOI complicating shimming and affecting field homogeneity.

Partial volume effects are caused by the region surrounding the VOI affecting adequate metabolite quantitation. This is particularly problematic when large volumes (> 8 ml) are selected. Smaller VOI can be chosen at the cost of lower signal/noise ratio. Higher magnetic field might solve this problem.

Additionally, general factors like lower field strength, poor shimming and the low concentration of a particular metabolite may complicate calculation of peak areas due to, non-Lorentzian lineshapes, base line distortions and resonance overlap.

Metabolic information

1. ³¹P MRS

A representative ³¹P MRS spectrum of the human brain at 1.5T is shown in figure 5a. where peaks of major metabolites observed are assigned.

The peaks of α , β and γ -ATP, and of PCr and Pi can be clearly identified. Phosphomonoesters such as phosphocholine, phosphoethanolamine and sugar phosphates are under normal conditions are also present on both sides of the Pi resonance and might partly overlap the Pi peak at lower fields.

The γ -ATP peak is the most reliable in analyzing ATP concentrations, while the α and β resonances contain contributions from NAD and ADP respectively.

Free Cytoplasmic ATP can be calculated from the creatine kinase reaction



assumed to be at equilibrium:

$$K_{eq} = \frac{[\text{ATP}][\text{Cr}]}{[\text{H}^{+}][\text{ATP}][\text{PCr}]}$$

The intracellular pH is calculated from chemical shift of Pi relative to PCr according to the formula where δ is the chemical shift:

$$\text{pH} = 6.75 + \log \left[\frac{(\delta - 3.27)}{(5.69 - \delta)} \right]$$

2. ¹H MRS

Figure 5b shows a representative ¹H MRS spectrum of the human brain acquired at 1.5T with major observable peaks are assigned.

¹H MRS detects a number of metabolites present in relatively low concentrations (< 10 mM), when water and fat suppression techniques are used.

Major ¹H metabolites observed are commented below:

N-acetyl -aspartate (NAA) produces a large resonance in a H₂O suppressed ¹H spectrum. The peak may contain up to 20% contributions from Aspartyl-glutamate (NAAG). NAA is generally associated with neurons and axons in the adult brain. It has received considerable interest in several disorders where there is neuron loss. However, its function is largely unknown.

The creatine (Cr), resonance originates from intracellular Cr and PCr these are involved in the

creatine kinase reaction and consequently in energy metabolism.

The Choline (Cho) peak arises from a mixture of glycerophosphoethanolamine and glycerophosphocholine. Both phospholipids are present in cellular membranes. This resonance can provide information about cell density and membrane integrity (or peroxidation).

A glutamate and glutamine (Glu, Gln) peak can be detected in the human brain. Glutamine is a precursor of glutamate. Glutamate is involved in neurotransmission. Gamma-aminobutyric acid (GABA), also present but in lower concentrations during normal physiological conditions may overlap with the Glu, Gln resonance at 1.5T field strength.

Myo-Inositol (MI) provides a relatively large resonance and is involved in osmotic regulation across the cellular membrane and could be specific for glial cells. The amino acid glycine may also contribute to the myo-inositol resonance.

Scyllo-inositol, an isomer of inositol appears also as a singlet peak more downfield. Taurine resonates close to the scyllo-inositol region.

Glucose, an important substrate in brain metabolism gives rise a weak but observable coupled resonance. It is more easily detected under hyperglycemic conditions. Lactate can be detected as a doublet resonance in brain tissue. Under normal conditions, lactate is present at around 1 mM concentration and is increased during ischemic conditions as a result of anaerobic glycolysis leading to a more distinct peak.

The brain tissue is rich in lipids. These might be detected as broad resonances with contributions from several fatty acyl chains. Measurement of lipids may be useful in evaluating myelination and membrane breakdown.

The dominant ^1H and ^{31}P biochemicals in the human brain are also listed in tables 1 and 2 respectively. Resonance frequencies are given in ppm. The concentrations and ratios are mean values from the literature and are rather orientational than absolute.

MRS and bioenergetics

High energy phosphates such as ATP and Pcr are markers of cellular ability to perform chemical and mechanical work. The PCr /Pi is a direct thermodynamic measure of mitochondrial oxidative phosphorylation.

Extensive experimental studies during the past 15 years have confirmed the high value of ^{31}P MRS in the understanding of cellular bioenergetics. Numerous studies have used the bioenergetic behaviour as a marker in monitoring disease development and drug effect. Figure 6 illustrates this. The series of spectra show on one hand the behaviour of phosphorous metabolites in an experimental skeletal muscle ischemia and reperfusion model; and on the other hand, the effect of treatment with ascorbate, a potent antioxidant, on the recovery of high energy phosphates during post ischemic reperfusion.

In clinical applications, ^{31}P MRS has been useful for diagnosis and therapy follow-up of metabolic myopathies. Calculation of the intracellular pH and PCr degradation and resynthesis during muscle exercise and recovery from exercise in patients with muscular and metabolic diseases according to suitable protocols has been used successfully.

^{31}P MRS have been helpful in studying metabolic diseases of mitochondrial origin where changes in lactate and PCr/Pi are taken as markers like in KearnsSayre syndrome.

Aerobic oxidation of glucose provides the human brain cells with energy.

^{31}P MRS can register of metabolic changes during brain hypoxia where a reduction in oxygen and substrate supply leads to energetic failure and consequently to neuronal dysfunction and membrane

breakdown. Thus loss in Pcr and ATP can be detected as well as decreases in intracellular pH. Possible structural membrane changes can be demonstrated from changes in PDE and PME. Intracellular pH and/or lactate are useful markers of low oxygen availability in the cell. It is well that anaerobic metabolism leads to lactate accumulation and in the brain tissue the resulting acidosis might in turn lead to neuronal damage.

Metabolic encephalomyopathies

Brain ischemia and hypoxic/ischemic disease in newborns where cerebral energetics can be monitored to study oxidative and glycolytic metabolism where parameters like pH, Pi/ATP has proven to be good markers.

Anaerobic glycolysis in brain is an indication of impairment in mitochondrial function. Decreased PCr/Pi and elevated lactate levels are indications that could help the diagnosis of that metabolic disorders.

In cases of hepatic encephalomyopathy, Kearns-Syde syndrome and pyruvate dehydrogenase deficiency, MRS is used to monitor therapy.

Brain trauma

Posttraumatic brain injuries might affect cerebral energy metabolism. Decreases in ATP and in intracellular pH were shown by ^{31}P MRS. Elevated lactate probably due to increased anaerobic glycolysis and diminished NAA were also reported from ^1H MRS examinations. In neonates with acute brain injury ^1H MRS examination was able to predict outcome through variations in NAA, Glu/Gln and lactate as illustrated in figure 7.

Stroke

Stroke is associated with degradation of high-energy phosphates (ATP, Pcr) and increase in inorganic phosphate (Pi) and intracellular acidosis as documented from early ^{31}P MRS investigations. Additionally, typical ^1H MRS of patients with stroke show elevated lactate and reduced NAA. Follow-up after the acute infarction period might reveal continued loss in NAA as well as acidosis in the ischemic regions of the brain. These parameters are certainly useful in monitoring the effect of medication.

Alzheimer Disease

^1H MRS using short TE STEAM revealed that myo-inositol is increased in AD. NAA is also decreased in the brain indicating diminished number of healthy neurons.

Figure 8 illustrates abnormalities in ^1H MRS spectrum in a patient with AD.

AIDS

Neurologic disorders such as AIDS-encephalitis and AIDS -dementia resulting from HIV infection have been successfully studied by MRS. Reductions in NAA and increases in Cho have been detected.

Brain tumor

MRS can distinguish between recurrent tumor and tissue necrosis.

Adequate tumor diagnosis and therapy monitoring during the various stages of a tumorous disease are important for optimal treatment. Both ^{31}P and ^1H MRS have been utilized for diagnosis and therapy monitoring of brain tumors. NAA is decreased in brain gliomas. Studying changes in tumor-type dependent metabolites is an area of active research.

Lipids and lactate peaks correlate well with necrotic tumor. High-energy phosphate and phospholipid

(ATP, PCr, PDE, PME) levels vary in response to radiation therapy, chemotherapy and even to nutrition (in experimental cancer).

This suggests to utility of ^{31}P MRS in tumor therapy monitoring focusing on cellular bioenergetics and phospholipid metabolism.

However, biochemical heterogeneity within the tumor tissue is still difficult to study because of poor resolution on commonly available clinical equipment (1.5T).

Brain tumor classification through network analysis and pattern recognition might shed further light on the different tumor types and degree of activity.

Multiple Sclerosis

Changes in NAA, cho and lactate correlate with axonal damages, demyelination and inflammation observed in MS patients during various stages of the disease. These metabolites can be monitored to study the outcome of new treatment.

Epilepsy

^{31}P MRS showed that the PCr/Pi is dramatically decreased during seizures and normalized after seizure discharge.

The glutamine and glutamate peak is elevated in the hippocampus while NAA is diminished in patients with chronic epilepsy.

Changes in GABA have been correlated with drugs affecting GABA metabolism.

An increase in lactate has also been reported in focal epilepsy of extratemporal origin.

These biochemical changes in epileptogenic region of the brain indicate that ^1H MRS can be clinically useful in the diagnosis of this disease as a complement to MRI.

Schizophrenia

^{31}P spectroscopy studies revealed increases in PDE and decreases in PME in the prefrontal cortex of schizophrenics. Alterations in these lipids vary with different brain regions and stages of the disease. Reductions in NAA and glutamate have been reported from ^1H spectroscopy investigations. These reductions were largely found in the hippocampal area/mesial temporal lobe

Additional neurological diseases under evaluation include:

Huntington disease

Increases in lactate and in Pi and decreases in PCr in Huntington disease implicate mitochondrial oxidative phosphorylation in the disease process.

Migraine

^{31}P studies showed diminished PCr and increased Pi and ADP which indicates energetic disturbances in brain tissue in patients with migraine.

Parkinson Disease

A decrease in the neuronal marker NAA and an increase lactate/NAA ratio were reported by ^1H MRS.

Psychiatry (mood disorders)

Both ^{31}P and ^1H MRS have been used in investigation mood disorders where changes in energy metabolism, lipids and Cho were observed. This indicates the potential of MRS in monitoring the effect of psychopharmacological drugs.

CONCLUSIONS

MRS is a unique and powerful technique that has been applied to a number of brain diseases. It can be correlated with imaging and other clinical data for confirmation. It is useful in diagnosis and prognosis of disease and mostly in the evaluation of the noninvasive monitoring of response to treatment.

Metabolic information from MR spectra is an emerging component in modern neurochemistry.

In neuroresearch MRS is definitely a revolutionary tool that will help understand the brain biochemistry of mechanisms of disease. MRS if introduced into a clinical practice could be very supportive in clinical decision making.

Spectral quantification is still difficult therefore relative concentrations of metabolites are usually calculated.

Most reports are difficult to compare due variations various parameters in the methodological set up. Additional complicating factors are the diversity in clinical material studied and exact anatomical localization (including gray-white matter separation). Discrepancies in results can thus be expected.

In vivo MRS is a complex technology that requires the simultaneous optimal adjustment of multiple parameters during an examination. The most critical task in MRS however, is not spectral acquisition but rather spectral analysis. This latter is time demanding and necessitates appropriate know-how in order to interpret the results, eliminate artifacts and quantitate data often by complex procedures and finally statistically analyze the findings.

The precise role of many identified metabolites is still unclear. Therefore, along with experimental mechanistic research, incorporation of MRS in clinical practice as much as possible would increase the body of information since what is still needed is the characterization of spectral patterns in disease conditions and in healthy control conditions.

Major benefits

High chemical specificity in studying:
energy metabolism,
lipid metabolism,
amino acid and intermediary metabolism,
noninvasive regional serial measurements of metabolite in patients and controls subjects.
Therapy response.
Mechanistic studies of inherited and acquired brain metabolic diseases.

Generally, MRS is well suited for the exploration of diffuse brain diseases where it provides new insights.

Technical improvements

Major technical improvements by manufacturers in terms of hardware and user friendly software has contributed largely to the increase in the number of clinical studies using ^1H MRS along with conventional MRI.

Automation of methods for shimming, water suppression and peak integration will replace the manual

adjustment of several parameters thus increase reproducibility and certainly spread the use of this technique.

FUTURE STUDIES

Future studies should focus on multidisciplinary multicentre projects for the development of standardized reproducible measurements e.g. :

Instrumental calibration protocols (internal/extern standards)

Protocols for quality assessment

Comparison of methodologies used for data acquisition, analysis and metabolite quantitation between different centres

Collaborative efforts are necessary for the evaluation of the value of MRS in diagnosis, prognosis and therapy monitoring in order to enhance clinical workability.

Future technological improvements in magnetic field strength, gradients, data processing and analysis will also encourage more applications of ^{13}C and ^{19}F .

And last, envision in vivo non-invasive access to highly localized and reliable chemical information as a routine clinical procedure in health and in disease... Life would become much easier for both patient and clinician.

Until then MRS continues to be an area of intensive investigation.

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Legends to figures and tables

Figure 1.

The free induction decay is converted to a spectrum by a Fourier transformation.
The FID signal (= amplitude vs time) is converted to a spectrum (= amplitude vs frequency).

Figure 2.

Volume selection and spectral acquisition.

A: MRI of normal human brain, illustrating the VOI = 50 x 40 x 50 mm.

B: Proton MRS spectrum of the selected volume showing the major proton metabolites. (Reproduced from 47)

Figure 3.

In vivo ^{31}P MR spectrum localised from rat brain with ISIS (VOI = 10 x 10 x 10 mm). Magnetic field strength = 2.35 T. The peaks of the adenosine triphosphates a-, b-, and g-ATP, the phosphocreatine, PCr, the inorganic phosphate Pi as well as the phosphomonoesters PME and the phosphodiester DPE are assigned.

Figure 4.

Improved resolution with improved magnetic field strength.

a) In vivo ^{31}P MR spectrum of rat skeletal muscle at 2.35T.

b) In vitro ^{31}P high resolution NMR spectrum of skeletal muscle extract acquired at 11.74 T. The peaks of the adenosine triphosphates a-, b-, and g-ATP, the phosphocreatine, PCr, the inorganic phosphate Pi are well resolved. Peaks at 6.3-7.3 ppm are PME including G-6-P at 7.17ppm. The large Pi peak arises from artifactual degradation of PCr.

Figure 5.

Localized MRS spectra of normal human brain illustrating the major metabolites observed.

(A) is an ISIS ^{31}P MR spectrum obtained at 2 T (VOI = 100 ml). The adenosine triphosphates a-, b-, and g-ATP, the phosphocreatine, PCr, the inorganic phosphate Pi as well as the phosphomonoesters PME and the phosphodiester DPE are well resolved.

(B) is a proton MR spectrum at 1.5 T obtained with STEAM combined with CHESSE to suppress the water signal, (VOI = 8 ml). The assigned proton metabolites are: N-acetylaspartate NAA, glutamate and glutamine GLU-GLN, creatine and phosphocreatine Cr-PCr, choline CHO, inositol INS scyllo-inositol Scy-INS, taurine TAU, glycine GLY and glucose. (Reproduced from 3)

Figure 6.

Illustration of the dynamics of cellular energetics by in vivo ^{31}P MRS. The potential of MRS in therapy monitoring is also demonstrated.

The spectra are from a skeletal muscle from a control rat and a rat treated with ascorbate. At rest (A), after 2 h of ischemia (B), after 4 h of ischemia (C) and after 4 h of ischemia + 150 minutes of reperfusion (D). The treated rat showed higher levels of PCr and ATP after reperfusion. Spectra were obtained by accumulating 128 FIDs with a repetition time 1 s at 2.35 T. (Reproduced from 13)

Figure 7.

^1H MRS illustrating patterns seen in STEAM spectra (a, c) and in PRESS spectra (b, d) from the brain of two children with after birth brain injury.

Spectra (a, b) are from a patient with a mild brain injury and show good outcome.

Spectra (c, d) are from a patient with a traumatic brain injury and show poor outcome (Note the low NAA signal and the elevated lactate signal).

Figure 8.

A proton MRS spectrum from the brain of a normal patient (A) compared with a spectrum of a patient with Alzheimer disease (B). (Reproduced from 58)

Table 1. Major proton metabolites with approximate mean concentrations and corresponding resonance frequencies detected in normal human brain by in vivo MRS.

Table 2. Summary of ^{31}P metabolites in normal human brain obtained by in vivo MRS. Relative mean metabolite ratios are also given.