POSSIBILITIES OF PHOSPHOROUS MAGNETIC RESONANCE SPECTROSCOPY (31P MRS) IN BRAIN DIAGNOSTICS

MOŻLIWOŚCI FOSFOROWEJ SPEKTROSKOPII REZONANSU MAGNETYCZNEGO (31P MRS) W DIAGNOSTYCE MÓZGU

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ABSTRACT

Phosphorous Magnetic Resonance Spectroscopy (31P MRS) is an interesting technique with a great potential in medical diagnostics. It is a perfect supplement to the examinations concerning information on the biochemical composition of the examined tissue, the level of metabolites containing phosphorus atoms, and indirectly, the metabolism of the tissue. Although the 31P MRS was historically the first known MR spectroscopy technique, it is currently not used as a diagnostic standard in spite of its numerous advantages. The purpose of this work is to present the 31P MRS method, particularly regarding its wide diagnostic capabilities.

Keywords: phosphorus magnetic resonance spectroscopy, 31P MRS, MR spectrum, human brain

STRESZCZENIE

Fosforowa spektroskopia rezonansu magnetycznego (31P MRS) jest interesującą techniką o dużych możliwościach w diagnostyce medycznej. Stanowi idealne uzupełnienie badań o informacje dotyczące składu biochemicznego badanej tkanki, poziomu metabolitów zawierających w swoim składzie atomy fosforu, a więc pośrednio o metabolizmie tkanki. Choć historycznie spektroskopia 31P MRS było pierwszą znaną metodą spektroskopową MR, obecnie nie jest stosowana standardowo w diagnostyce, mimo swoich licznych zalet. Celem pracy zaprezentowanie metody 31P MRS, szczególnie jej szerokich możliwości diagnostycznych.

Słowa kluczowe: fosforowa spektroskopia rezonansu magnetycznego, 31P MRS, widmo spektroskopowe MR, mózg człowieka

1. Introduction

Magnetic resonance (MR) examination methods are widely known and used in both: medicine and physics, and many related sciences. In addition to basic structural examinations, these methods include Functional Magnetic Resonance Imaging (fMRI), Diffusion Weighted Imaging (DWI), Diffusion-

Tensor Imaging (DWI), and Magnetic Resonance Spectroscopy (MRS) [1]. Particularly interesting is the last technique, since it does not produce images as in the case of the other methods. The results of spectroscopic studies are the spectra, in which the individual peaks correspond to the different biochemical substances in the examined tissue [2]. It is possible because in each substance of interest the atoms are surrounded by other atoms in a specific spatial configuration. [3]. As a consequence, their chemical shift of signal frequency changes and the magnetic resonance is sensitive to these differences [4, 5, 6, 7]. Thanks to this phenomenon, the method enables obtaining information on metabolic composition in a non-invasive manner [8, 9].

Among the MR spectroscopic studies, five types of studies using the properties of different atoms under the magnetic field can be distinguished: 1H, 31P, 19F, 13C and 23Na [4, 10]. Due to the fact that hydrogen atoms are most abundant in the human body, and therefore the most MR signal comes from the hydrogen atoms, in the clinical practice the Proton Magnetic Resonance Spectroscopy (1H MRS) examinations are commonly used [2, 11, 12, 13]. The less frequently used, and thus less well-known method is Phosphorus Magnetic Resonance Spectroscopy (31P MRS), though it was historically the first MR spectroscopy technique [14]. It is not a standard clinical procedure approved by the FDA, thus it requires the consent of the bioethical committee [12]. Nonetheless, it can deliver useful diagnostic information on the biochemical composition of the examined tissue metabolites containing phosphorus atoms [15].

2. 31P MRS spectrum and metabolites

31P MRS results are presented in the form of spectra, where the peaks correspond to individual metabolites. At the ordinate (OY), there is information on the intensity of the signal from these metabolites, while at the abscissa (OX), there is information about the chemical shift of the signal frequency expressed in ppm (parts per million), which is characteristic for the metabolite [16]. As a consequence, it is possible to calculate the concentration of the substance in the examined tissue, basing on the area under the peak. Figure 1 shows an example of 31P MRS adult brain spectrum with the marked metabolites.

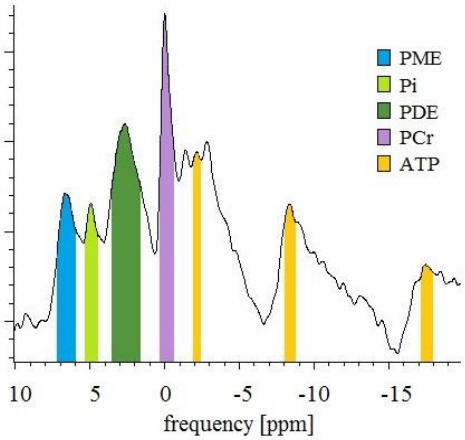

Fig. 1. Metabolites in the 31P MRS brain spectrum of healthy adult (own material)

In the 31P MRS spectrum there are peaks corresponding to biochemical substances containing phosphorus atoms [17]. All of these substances play an important role in cell metabolism, including the energy production and release, so the knowledge of their presence and their level in a tissue is very important in the diagnostic process [18]. A summary of information on the metabolites visible in the 31P MRS spectrum is presented in table 1.

Table 1. Summary of information on metabolites visible in 31P MRS spectrum

Metabolite	Abr	ppm	Occurrence in healthy tissue	Function	Visibility in the MR spectrum
phosphomonoesters	PME	6.5	phosphocholine + phosphoethanolamine	MARKER: cell membrane synthesis	at lower field strengths visible as one peak
inorganic phosphate	Pi	4.9	in the form of conjugated pair of anions: HPO_4^{2-} and $H_2PO_4^-$	ATP synthesis	location of the peak depending on the tissue acidity
phosphodiesters	PDE	2.7	glycerophosphocholine + glycerophosphoethanolamine	MARKER: sell membrane degradation	at lower field strengths visible as one broad peak
phosphocreatine	PCr	0	always present in healthy tissue	energy source in the initial phase of the effort	the highest peak in the 31P MRS brain spectrum
adenosine triphosphate	ATP	−2.3 −2.7 −8.3 −8.7 −17.4 −18.1	created continuously in the mitochondria	energy carrier	in the form of three double peaks: γ-ATP, α-ATP, β-ATP; localization of β-ATP peak depends on Mg^{2+}

2.1. Phosphomonoesters

Phosphomonoesters (PME, 6.5 ppm) are phosphocholine (PC) and phosphonethamamine (PE), which are products and markers of cell membrane synthesis [15, 19, 20, 21].

PC reacts with ATP in the replacement process to produce adenosine diphosphate (ADP) and phosphatidylcholine in the presence of creatine kinase (CPK), which is an enzyme catalyzing this reaction. PE participates in the synthesis of phosphatidylethanolamine, as well as in various stages of cell metabolism such as acetylcholine synthesis [22].

2.2. Inorganic phosphate

Inorganic phosphate (Pi, 4.9 ppm) is necessary for the ATP synthesis [2, 20]. The location of the P_i peak in the 31P MRS spectrum is closely related to the acidity of the examined tissue. In the neutral environment, P_i exists as conjugated pairs of hydrogen phosphate (HPO_4^{2-}) and dihydrogenphosphate ($H_2PO_4^-$) anions with the chemical shifts of 5.57 ppm and 3.08 ppm, respectively. Due to the very rapid chemical reaction $H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$, instead of two distant peaks one average peak appears in the spectrum. Its position is dependent on the equilibrium of the reaction and thus the pH index [9, 23, 24].

2.3. Phosphodiesters

Phosphodiesters (PDE, 2.7 ppm) are glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE), which are associated with cell membrane degradation [15, 20, 25]. GPC in healthy cells most likely is the primary metabolite of choline and acetylcholine, the neurotransmitter responsible for memory. However, despite the fact that phosphodiesters occur in the brain at high concentrations, their function is not fully known. Due to the fact that both PDEs and PMEs play a role in cell membrane mechanisms, its status can be indirectly monitored by the concentrations ratio of these two substances: PME/PDE [26].

2.4. Phosphocreatine

Phosphocreatine (PCr, 0 ppm) as a high energy molecule is an energy source in the initial phase of exercise due to the high energy binding in this compound [4, 15]. The presence of PCr accelerates the process of ATP synthesis, thereby increasing the efficiency of a tissue. PCr peak is the highest peak in the human 31P MRS spectrum and is found at 0 ppm, thus it is a reference to the location of other metabolites peaks [2, 7].

2.5. Adenosine triphosphate

Adenosine triphosphate (ATP) contains adenosine and three phosphate groups in its molecule. Consequently, in the 31P MRS spectrum three double peaks are visible: gamma adenosine triphosphate (γ-ATP, –2.3 ppm and –2.7 ppm), alpha adenosine triphosphate (α-ATP, –8.3 ppm and –8.7 ppm) and beta adenosine triphosphate (β-ATP, –17.4 ppm and –18.1 ppm) [2, 7, 11].

It is a carrier of chemical energy used in cell metabolism [27]. This compound is not stored, but it is created continuously in the cellular mitochondria by ADP phosphorylation, which is catalyzed by the ATP synthase enzyme [19, 28, 29].

3. pH measurement

The 31P MRS sequence not only provides information on the concentration of metabolites containing phosphorus atoms, but also allows the non-invasive measurement of the intracellular pH of the examined tissue. It is an important parameter for maintaining optimal functioning of the central nervous system, and its changes may be attributed to the pathology of cellular metabolism [30]. Tissue acidity is influenced by homeostatic mechanisms in nerve and glial cells, e.g. removal of products of metabolic processes, as well as bioenergetic regulation [23, 31].

Chemical shift of PCr is not susceptible to pH changes, whereas chemical shift of P_i is strongly associated with pH of the environment [19, 23]. Measurement of intracellular pH is therefore possible knowing the relative position of the peaks corresponding to P_i and PCr in the 31P MRS spectra [16, 21]. In practice, during data analysis, the PCr peak as a reference for pH calculation is moved along the OX axis in the 31P MRS spectrum to 0 ppm [8]. As a consequence, the entire spectrum is shifted, and the chemical shift of P_i is equal to the difference in chemical shift between P_i and PCr. For calculating pH, the appropriate Henderson-Hasselbalch equations, are used [4]. A number of algorithms used by various research teams are available in the professional literature. They are often developed in a laboratory on the basis of own research and calculations [12]. In the examinations of human brains, two formulas by Petroff et al. are most commonly used [23, 29, 32, 33]:

$$\mathrm{pH} = 6{,}66 + \log\frac{\delta\mathrm{pi} - 3{,}08}{5{,}57 - \delta\mathrm{pi}} \tag{1}$$

$$\mathrm{pH} = 6{,}77 + \log\frac{\delta\mathrm{pi} - 3{,}29}{5{,}68 - \delta\mathrm{pi}} \tag{2}$$

where: δpi – difference of chemical shift between P_i and PCr [34].

Between the results obtained using these two formulas, there are significant statistical differences. Consequently, one method of pH calculation must be used consistently to monitor pH changes, and it is necessary to consider the mathematical formula used when comparing the results with the results obtained in other research centers [12].

4. Mg^{2+} concentration measurement

Another possibility given by the 31P MRS method is the measurement of Mg^{2+} concentration, which cannot be measured in a noninvasive manner by other currently known methods [2, 4, 24]. Knowledge of this parameter may be helpful in medical diagnostics, as Mg^{2+} plays an important role in the regulation of calcium channels and sodium-potassium pumps [23].

The Mg^{2+} concentration can be measured on the basis of the β-ATP chemical shift using a suitable

mathematical formula [2]:

$$\mathrm{pMg} = 64{,}24 - \log \frac{(\delta_\beta + 18{,}58)^{0{,}42}}{(-15{,}74 - \delta_\beta)^{0{,}84}} \tag{3}$$

where: δ_β – difference of chemical shift between β-ATP and PCr.

5. Application of 31P MRS in brain diagnostics

The 31P MRS method can be used in studies of various parts of the human body (muscles, liver, brain, heart), as well as in animal studies [2, 7, 14]. Particular attention has been paid so far to the 31P MRS studies of brain, due to the high energy requirements of the brain compared to other parts of the body. For example, in brain tumors elevated PME/PDE, PME/PCr, and decreased PCr and PDE levels were found, and the magnitude of these changes was dependent on the tumor type [8, 10, 34]. Increased PME along with accompanying ATP decline was seen in depression [22]. The PME decrease along with the PDE, PCr and γ-ATP increase were observed in Alzheimer's disease and neurodegenerative lesions [9]. Changes in β-ATP, PCr and PDE were found in different regions of the brain in schizophrenic patients [13, 19, 25, 26]. Changes in the 31P MRS spectrum were also detected in the brain of patients with hepatic disease [35].

In addition, the presence of changes in the 31P MRS spectrum with aging was confirmed by previous studies: PCr and PDE levels increase, while PME level declines, that may indicate an increase of cell membrane degradation [29].

The brain pH values in healthy subjects given in the literature by individual groups of researchers vary and fluctuate between neutral and slightly alkaline values between 6.96 and 7.11 [10, 21, 23, 35]. The pH decreases with age. Due to the importance of maintenance an adequate pH level in healthy tissue, this parameter may be helpful in the diagnostic process. Increased pH occurs in epilepsy, Alzheimer's disease and brain tumors, while it declines in bipolar disorder, anxiety and depression [9, 10, 12, 34]. In people with head injuries the pH rises in the area of focal lesions immediately after injury, and after three weeks it returns to optimum value [9].

6. Advantages and disadvantages of 31P MRS

Because of its specificity, the 31P MRS has several advantages and disadvantages. It is particularly noteworthy that this is the only non-invasive diagnostic technique for obtaining information on metabolism and energy metabolism in the studied brain region [4, 12]. However, to perform examinations with this method, it is necessary to use special MR coils dedicated to the phosphorus frequency [2, 7, 34]. In addition, the 31P MRS sequences are long and have a low signal-to-noise ratio (SNR) [10].

7. Conclusions

Phosphorus magnetic resonance spectroscopy can be a valuable diagnostic tool to study energy processes and tissue metabolism. The potential of this method is already evident on the basis of prior research using 31P MRS. However, further work is needed on optimizing such examinations and the required equipment.

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