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Physics Procedia 82 (2016) 3 – 7

Physics Procedia

International Baltic Conference on Magnetism: Focus on Biomedical Aspects, IBCM 2015, 30 August – 3 September 2015, Kaliningrad, Russia

Multinuclear Magnetic Resonance Imaging and NMR Spectroscopy in Biomedical Investigations

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Abstract

Biomedical investigations with small animals using 0.5-T and 7-T MRI scanners adjusted on the Larmor frequencies of different nuclei ^{1}H, ^{2}H, ^{11}B, ^{13}C, ^{19}F, ^{23}Na, ^{31}P, ^{35}Cl are described. Experiments on registration of signals 19F from the fluorocarbons injected in laboratory animals are discussed. They give presentation on the application of fluorocarbon compounds as blood substitutes and contrasting preparations in MRI diagnostics. A blood substitute product fluorocarbon Perfluoranum® has shown effectiveness in oxygen delivery to the tissues of living organisms, and cardioprotective effect which does not depend on the patient's blood group. Inclusion of paramagnetic atoms (gadolinium, iron, etc.) to the Perfluoranum® chemical formula creates a new compounds with high MRI contrast efficiencies at Larmor frequencies of protons so and fluorine-19 nuclei.

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Peer-review under responsibility of the organizing committee of IBCM 2015

Keywords: magnetic resonance imaging, NMR spectroscopy, biomedical investigations, Perfluoranum®, fluorine-19, molecular imaging

1. Introduction

Magnetic resonance imaging (MRI), based on the effect of nuclear magnetic resonance (NMR), has become an integral part of the medical diagnosis. MRI scanners and NMR spectrometers are used widely in numerous biomedical applications. MRI can reveal different morphological changes of the internal organs and tissues. MRI signal in all medical imaging systems is formed on Larmor frequency of protons. It's not a coincidence that the

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doi:10.1016/j.phpro.2016.05.002

protons as hydrogen nuclei in water molecules have a maximum concentration in most tissues of living organisms. This makes proton MRI suitable for high resolution imaging of majority of the organs.

2. Multinuclear investigations

If the 7-T scanner BioSpec 70/30 could work on the frequencies of the heavy nuclei ^{13}C and ^{31}P, the 0.5-T MRI scanner had only adjustment on the protons. To register signals from other heavy nuclei, we created a special receiver-transmitter infrastructure and have got possibility to observe MR images and NMR spectra still for the nuclei ^{2}H, ^{11}B, ^{19}F, ^{23}Na, and NQR (nuclear quadruple resonance) signals for the nuclei ^{35}Cl. Creation of ^{19}F channel was the most important result for us since the fluorocarbon investigations were by the main line of the studying responses from heavy nuclei. The data of MR images and NMR spectra of nuclei other than proton received at 0.5 T MRI scanner are shown on the Fig. 1. Curves are supplied with either a frequency scale or information on spectrum width (SW) in ppm. Spectral line widths at half height in Hertz are given also.

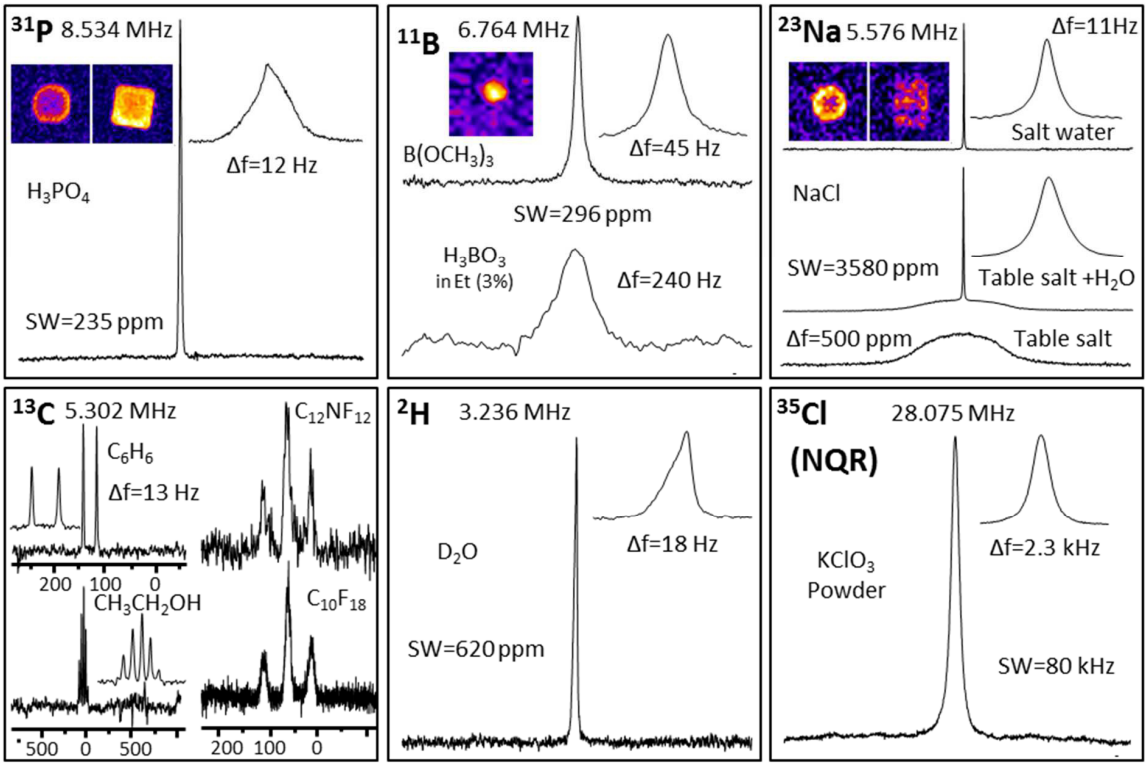

Fig. 1. Examples of NMR spectra of nuclei other than proton received at 0.5 T MRI scanner.

For all nuclei, except ^{13}C and ^{2}H, it was succeeded to receive also MRI. Images of the acceptable quality with the resolution of 2 mm were received after scanning by gradient echo (low flip angle) method in time about 5 minutes. Besides, by means of the self-made probe adjusted on the frequency of 28 MHz and placed out of a scanner magnet, the signal of a ^{35}Cl nuclear quadruple resonance (NQR) from potassium chlorate $KClO_3$ successfully was registered. It should be noted that only for ^{2}H nucleus the isotope enriched sample is used. Other registered isotopes are presented in used samples at the natural abundance.

Registration of signals ^{19}F was successfully carried out with use of both options. However the preference was given to the second option as in the first option of RF generator worked at the power close to maximum for it. It limited possibilities of a variation of RF pulse parameters (amplitude and duration). Besides, there was a risk of

unstable work or even an overheating the equipment at long experiments. These are factors it was considered when planning experiments with laboratory animals which duration could exceed one hour. In Fig. 2 ^{19}F NMR spectra of synthetic fluorocarbon substances – Perfluoranum® and its main components are given (left part of the Fig. 2). Also the ^{19}F MRI of a rat for the second day after an intravenous injection Perfluoranum® is presented. ^{1}H and a combination of ^{1}H and ^{19}F MRI are given for reference of anatomical structures. It is seen that Perfluoranum® is concentrated in two days after injection in the liver and spleen. The curves under MR images demonstrate velocity of perfluorodecaline excretion from the rat liver – the curve with dots is an experimental one, bold one is theoretical line. They show that the excretion time for the main component of Perfluoranum® constitutes about 6 days.

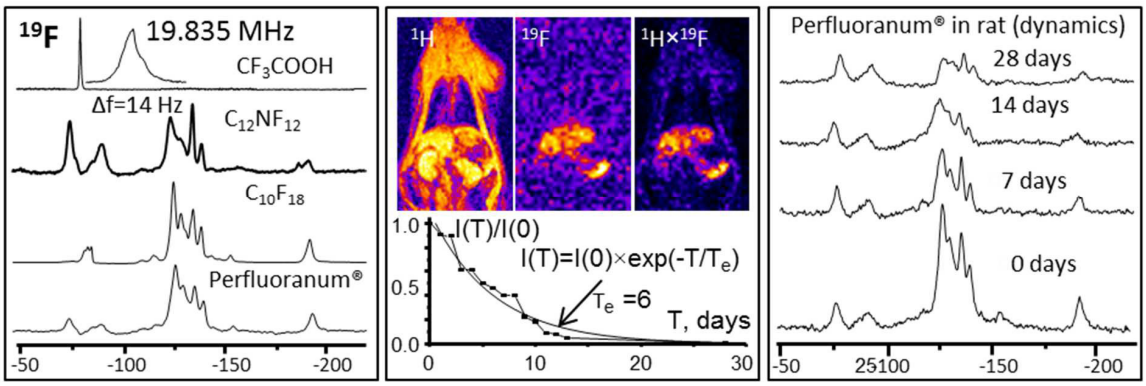

Fig. 2. Left: 19F NMR spectra of fluorocarbons. Center high: MRI of rat for the second day after an intravenous injection Perfluoranum®. Center below: curve of perfluorodecaline in rat liver decay. Solid line is the simulation of decay with Te=6 days. Right: 19F spectra of whole body of rat at different days after Perfluoranum® injection.

3. Perfluoranum® and its properties

There is virtually no fluorine atoms in a living organism (except for weak traces in the tooth enamel). Any injection of fluorine compounds respond to electromagnetic RF exposure of magnetic resonance scanner and can be detected when the excitation frequency of the electromagnetic wave is equal to the Larmor frequency of the fluorine nuclei. Fluorine-based MR images will have absolutely no background in normal tissues where fluorides are absent. Another reason to pursue development of NMR diagnosis on fluorine nuclei is the existence of fluorine-containing medicines, for example, blood substitute Perfluoranum® [Moroz et al. (1995), Maevsky et al. (2003)]. It is critical to follow the distribution of the Perfluoranum® after injection and to monitor the dynamics of its biochemical interactions with tissues.

Fluorine-based MRI can detect fluorine distribution in the body after the injection, which helps to determine concentration of the oxygen carriers, retention in tissues and subsequent removal. A number of technical and fundamental issues have to be addressed for the successful application of MRI visualization of fluorine in medical practice [Moroz et al. (1995)]. First of all, the transceiver transmit path of radio frequency channel has to be modified and adjusted it to the precession frequency of fluorine nuclei. Then, the imaging protocol has to be optimized in the order to form the most intensive fluorine images with high spatial resolution. Quantifiction of fluorine signal is possible with implementing localized fluorine nuclei NMR spectroscopy; this would allow determination of the metabolic profile of the specific tissue. Finally, fluorine images have to be co-localized with anatomical images obtained using proton MRI. Successful implementation of fluorine imaging and spectroscopy techniques is based primarily on the fact that the fluorine nuclei have very close characteristics of their magnetic parameters to protons, on which all known MRI techniques are built. In fact, the Larmor frequency of the fluorine nuclei is only 5% less than the respective frequencies of protons, natural content of fluorine-19 (19F) is 100%, both nuclei have the same spin quantum number of ½ and close relative sensitivity.

Another reason for developing medical NMR fluoric techniques is a desire to create non-toxic fluorocarbon-based MRI contrast materials that can drastically improve imaging of internal organs and visualization of vascular

network. Finally, a structure of fluorocarbon compounds in Perfluoranum® may allow using Perfluoranum® emulsion as bio-containers for the targeted delivery of pharmaceutical products to the zones of pathology. Table 1 demonstrates that the fluorine-19 nucleus and protons have closest NMR characteristics as compared to all other clinically interesting nuclei.

Table 1. NMR properties of clinically interesting nuclei.

Nucleus	Gyromagnetic ratio (MHz/T)	Spin quantum number	Relative content	Relative sensitivity
^{1}H	42,6	1\2	99	1.0
^{13}C	10,7	1\2	1,1	0,016
^{17}O	5,8	5\2	0,1	0,029
^{19}F	40,0	1\2	100	0,83
^{23}Na	11,3	3\2	100	0,093
^{31}P	17,2	1\2	100	0,07

It is known that fluorocarbon compounds can be present long time in the body without causing toxic effects and not altering organ functionality. This is a significant advantage in comparison with gadolinium-based MRI agents, which can cause toxic reactions. Perfluorocarbons (PFCs) are completely inert, not toxic, not metabolized in the body and excreted mainly with exhaled air.

Medical applications of fluoric compounds originate after discovering for fluorocarbon as an effective gas transporter. Note that fluorocarbons do not exist in nature and can be obtained by chemical substitution of all atoms of hydrogen by fluorine in hydrocarbons, therefore they are called sometimes "fluorocarbonhydrogens", although they do not contain hydrogen atoms. The emulsion of fluorocarbons in the water effectively absorbs oxygen and carbon dioxide, for example, liquid PFCs are able to dissolve more than 50% by volume oxygen and more than 150% carbon dioxide. The scientists of Nesmeyanov Institute of Organoelement Compounds RAS (INEOS RAS) Moroz et al. (1995) and Maevsky et al. (2003) have made a major contribution to the development of this field, they discovered an outstanding gas transmission function of the PFC.

In 1966 year, Clark and Gollan (1966) found that mouse submerged in the liquid PFC could long enough (up to 10 minutes) breathe the oxygen dissolved in PFC. Lungs of mouse were filled with liquid PFC, but in spite of this her breathing functions continued. After cleaning murine lungs from fluid and restoration the normal breathing process – the mouse continued to live, despite the prolonged period of deprivation from atmospheric oxygen.

4. Development of new fluorocarbon compounds for biomedicine

Immediately after this discovery, the United States, Japan and the USSR began work to create a substitute for blood plasma on the PFC basis. The joint research team of the Institute of Biophysics and INEOS RAS has eveloped recipes of Perfluoranum®, as well as its manufacturing technology by Maevsky et al. (2003).

Perfluoranum® is an aqueous micro-emulsion (droplet size 80-100 nm) mixture of two perfluoroorganic compounds, stabilized by polymeric surface-active substance (PSA). It quickly dissolves large amounts of oxygen, ensures the delivery of oxygen from the alveoli to erythrocytes and from erythrocytes to tissues, improves metabolism and gas exchange at the level of tissues, and also has a distinct cardio protective effect. Ministry of Health of the Russian Federation has allowed its use as blood substitute. Perfluoranum® has saved the lives of hundreds of wounded soldiers who have experienced excessive blood loss during Afghanistan war. An important advantage of this artificial blood substitute is its indifference to the blood type.

PFCs are extremely resistant chemically and thermally, biologically stable and not metabolized in the body. PFCs are removed from the body mainly through the lungs with exhaled air. Speed of removal of these compounds is determined by two factors – the elasticity of PFC steam (boiling temperature) and its solubility in lipids, i.e. the ability to penetrate through membranes of pulmonary alveoli.

Fluorocarbon drug Perfluoranum® is a mixture of two PFCs – perfluorodekalin and parametilcyklogeksilpiperidin, the first of which in itself is weak stable and quickly removed from the body, while the second one provides high stability of emulsion droplets in the mixture with greater duration of excretion from the body. The combination of these two compounds ensures stability and efficacy of the resulted emulsion, which

can be stored in refrigerators up to 3 weeks and frozen for up to two years. It is important to outline that detailed studies have not shown any pathological changes in organs and tissues after complete removal of perfluorocarbons in spite of the long duration of their presence in the body.

High concentrations of the Perfluoranum® in living tissues open up the opportunity to use this agent for medical diagnosis by detecting fluorine-19 signal with NMR (19F-MRI). Several different research groups in Netherlands, USA, and Japan applied 19F-MRI on artificial objects (phantoms) and laboratory animals and received high-intensity 19F-NMR three-dimensional images [Sloviter and Kamimoto (1967), Geyer et al. (1968), Riess and Krafft (2006)]. However, due to the fact that none of these countries did not have the drug type Perfluoranum®, passed the full cycle of clinical research and allowed for medical use, these fragmented results are still very far from clinical applications.

The Moscow State University team leaded by academician Alexey R. Khokhlov continues developments of the Perfluoranum®-based MRI contrasting compounds and new reagents for targeted delivery of medicines to the damaged tissues [Gulyaev et al. (2013), Volkov et al. (2014)].

Acknowledgements

MRI and NMR measurements were fulfilled at the Moscow State University on the equipment of the Center of collective application “Biospectrotomography” with using advices Bruker Tomikon S50 and BioSpec 70/30 USR from unique complex of scientific apparatus with the same name “Biospectrotomography”.

These works are supported by the grant from the Ministry of Education and Science of the Russian Federation No. 14.604.21.0060 (RFMEFI60414X0060).

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