NMR INVESTIGATION OF BORON COMPOUNDS FOR BNCT

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ABSTRACT

BNCT is an experimental cancer treatment modality based on the n-α capture process. The success of the therapy requires that the boron carriers concentration in the tumor cells is much higher than in normal cells. Methods to visualize the boron distribution in tissues are therefore important to establish the best therapeutical conditions. The problems related to the imaging of boron compounds for BNCT are discussed in the present work, and the feasibility of imaging the mostly used boron compounds by means of MRI is presented.

Introduction

The knowledge of the distribution and of the kinetics of boron compounds in tissues is of overwhelming importance for an optimization of the therapy; several methods have been proposed up to now for this purpose. Techniques like MRI and PET possess evidently a superior capability of furnishing these kinds of information, due to their inherent characteristic of imaging a whole section of the sample.

Some drawbacks still do not allow selecting one or the other as an election method: PET is characterized by a not completely satisfactory spatial resolution, and also Magnetic Resonance Imaging (MRI), when applied to the imaging of boron compounds, is affected by some limitations.

At present, usual MRI methods cannot be easily utilized for imaging of boron compounds, due to the very low ^{10}B gyromagnetic ratio and to the high quadrupole moment of this nucleus, which broadens the NMR line at a point where it conflicts with the requirements of MRI.

In the past this problem has been circumvented by employing boron compounds enriched in ^{11}B instead of ^{10}B, taking advantage of the lower quadrupole moment, and of the higher gyromagnetic ratio of the former.[1-2] More recently, images of ^{10}BSH in vivo have been obtained with a low spatial resolution.[3]

The purpose of our work is to develop alternative methods for imaging the boron compounds mostly used in BNCT, i.e. boron phenylalanine (BPA) and mercaptoborane $Na_2B_{12}H_{11}SH$ (BSH)

Methods

BSH

The aim of this part of the research is to obtain images representing the distribution of BSH in the tumor by means of double resonance techniques.

Almost all the studies realized so far with NMR employed B-11 enriched boron compounds; in fact B-10 in BSH is not easily detectable due to its extremely low gyromagnetic ratio, to the low concentration and to its high quadrupole moment, which causes a great broadening of the NMR line.[2, 4]

A different approach, which allows to map directly the ^{10}B compound exploits the modulation of the NMR signal when an indirect J-coupling interaction is present between two resonant nuclei in the molecule, in this case ^{10}B and hydrogen.

Images of the protons bound to B-10 atoms can in fact be realized exploiting the scalar nucleus-nucleus coupling interaction (J-coupling), which discriminates them from the protons of the bulk.[5] Such an image represents the distribution of the boron atoms, as the two distributions (B-10 and hydrogen coupled to B-10) are the same.

The procedure consists in observing the modulation caused by the J-coupling in the abundant spin echoes amplitude envelope through a double resonance sequence named SEDOR (Spin Echo Double Resonance).[6] This leads to the definition of the best delay T, i.e. the one corresponding to a maximum difference between the spin-echo signal and the SEDOR signal. Once that this delay has been chosen, an image obtained with a SEDOR sequence is subtracted from an usual spin-echo image with the same time parameters. The result is thus characterized from the S/N of the usual proton image, and at the same time is representative of the B-10 nuclei distribution. Actually, the difference image represents only the distribution of the protons J-coupling to the B-10 nuclei and, as the spin-spin J-coupling interaction is intramolecular, and therefore short ranged, the image is representative of the B-10 nuclei distribution.[7-8]

The Double Nuclear Magnetic Resonance techniques which are effectively employed for an indirect imaging of BSH are actually different from the one just described, which can instead give an idea of the principle underlying the indirect imaging. There is a common problem for all the difference techniques: if the two subtracting signals are very strong, and their level is much higher than their difference, the sensitivity of the apparatus could not be sufficient to discriminate this small difference.

This is the case of the imaging of the boron compound, where the ratio of the concentration is of the order of tens of PPM. To avoid this problem, other techniques have been developed, which allow to reveal directly the signal generated from the protons coupled to the boron nuclei.[9-10]

BPA

p-boronophenylaline (BPA) is another boron compound widely employed in BNCT. In BPA double magnetic resonance techniques cannot be exploited because there are not direct bonds between hydrogen and boron.

It has been reported that the cellular distribution of 4-borono-2-[^{18}F]fluoro-L-phenylalanine (^{18}F-BPA, an analog of p-boronophenylaline), has been imaged in malignant melanoma[11] and that the kinetics of the same compound has been studied in glioblastoma cells[12] by means of Positron Emission Tomography (PET). Quantitation of BPA by Nuclear Magnetic Resonance (NMR) has been done with volume selective spectroscopy techniques,[13] but such an approach does not allow to image its distribution.

A possible strategy to map BPA distribution is to use the analog ^{19}F-BPA,[14] in a way analogous to PET studies: ^{19}F is a resonant nucleus, has a high gyromagnetic ratio, it has not quadrupole moment and all the standard imaging techniques could in principle be applied with small modifications.

Experimental setup

The apparatus consists of a Bruker spectrometer, with a horizontal magnet of 15 cm bore and operating at a field of 7.0 T (300 MHz proton frequency), where small animals of the size of rats can be introduced. The BSH measurement have been performed with a home built resonator, a standard Alderman&Grant resonator tuned at the proton frequency, coupled to a wire coil tuned at the ^{10}B frequency.

Results

BSH

First of all the sensitivity of the technique has been tested by imaging a tube of water containing BSH with a BLISS sequence;[16] from this it has been found that a concentration as low as 0.5 mMol can be detected. Other in vitro experiences have been performed to verify that the signal is originated only from the BSH, and that the water signal is suppressed.

The technique has then been applied to a C6 tumour bearing rat. The brain has been explanted after approximately 90 minutes from the BSH injection. Two images have been realized: a T1 contrasted proton image showed evidences of the tumor, while an image obtained with a double resonance

sequence[17] shows the BSH distribution. If the two images are superimposed, an evident correspondence between the tumor and the BSH distribution is observed, thus demonstrating the feasibility of the method.

BPA

Several preliminary results have been obtained on *in vitro* samples containing ^{18}F-BPA. The NMR parameters of the compound have been completely characterized, and preliminary images of phantoms have been obtained. These experiment have allowed to check the sensitivity of the apparatus; experiments on excised tissues are in progress, to define the protocols to be applied for an *in vivo* imaging on animals.

Conclusion

Imaging of boron compounds is a fundamental task to determine the success of BNCT; the possibility to non invasively map the distribution of boron will allow to assess the most useful compounds, their pharmacokinetics in various tissues, so establishing the conditions to perform irradiation at the time of maximum difference in the concentrations between healthy and pathological tissues, together with the greatest possible concentration in the tumor.

MRI, due to its intrinsic non-invasiveness and its highest capability of distinguishing between tumors and healthy tissue, is a natural technique to investigate the distribution of boron and to compare it with the tumor extension. Boron is very difficult to image, on account of its chemical characteristics, and subtle alternative methods must be devised. Our work shows that in the future it will be possible to perform, through different strategies, MRI of boron compounds.

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