A fast method for ^{31}P localised MRS in vivo

Fabio Ginnari Satriani[1], Emiliano Surrentino[1], Alessandro Ricci[1], and Rossella Canese[1]
[1]*Cell Biology and Neurosciences Dept, Istituto Superiore di Sanità, Rome, Italy*

Target Audience – Researchers interested in fast acquisition for in vivo ^{31}P MRS

Introduction – ^{31}P Magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI) offer the most powerful approaches today available to non-invasively measure extracellular pH (pHe) and intracellular/extracellular pH gradient (ΔpH) in intact cancer cells and tissues (1-3). ISIS is the most used technique for ^{31}P spectra localized within a specific body district. Unfortunately, it is a low sensitive technique which requires long acquisition times at the commonly used magnetic fields. Typically, a ^{31}P ISIS measurements at 4.7 T in a volume of about 400 mm^3 lasts a total time of about 50 minutes. pHi can be measured from the chemical shift difference between Pi and α-ATP, because Pi position is pH dependent and it is located mainly in the intracellular compartment. The exogenous cell-impermeant ^{31}P reporter 3-aminopropyl phosphonate (3-APP) can be used as reference for the pHe evaluation because its position in the spectrum is pHe dependent. This probe should be retained within the tumor for the entire duration of the measurements. Unfortunately, this condition is not always fulfilled in highly vascularized tumors where the clearance of the 3-APP within the tumor area is very rapid (~ 30-40 min). We need to adopt a different protocols for highly vascularized tumours.

Pourpose – We developed a method that allows us to perform a fast ^{31}P MRS acquisition on superficial tumours with localization performance similar to that obtained with methods which requires longer acquisition times such as ISIS, by dephasing the signal arising from the mouse body.

Methods – To dephase the spins arising from regions surrounding the tumours we applied a saturation band. This technique requires an homogeneous magnetic field and precise frequency calibration in order to dephase the signal from the unwanted region only. For this reason in combination with the surface coils are generally used adiabatic pulses, which rotate the magnetization along a generic plane by an angle independent from B_1.

Experiments were performed on a VARIAN/Agilent Inova MRI/MRS system operating at 4.7 T, by using a three turn ^{31}P surface coil specifically designed to fit superficial tumour combined with a butterfly ^{1}H coil (RAPID Biomedical) for shimming and positioning of the VOI.

A phantom has been built with two compartments in order to simulate the tumor and the adjacent animal body. The compartments were separated by a sheet of acetate and filled with acrylamide gel containing 3-APP (500 mM) and inorganic phosphate (Pi, 500 mM), respectively. ^{31}P MR spectra were obtained using an adiabatic excitation (sech90, 4000 us, TR 3 s, 64 averages) and a saturation slab covering the mouse body (sech180 or BIR4 followed by a spoiling gradient).

We performed pulse calibrations (shape, power, length) on our phantom in order to obtain the better suppression efficiency of unwanted signal [defined in (4) as the unit minus the ratio between the intensity of signal coming from the "body" compartment, Pi, obtained with and without the saturation band) and the best selection efficiency of the wanted signal (the ratio between the intensity of signal coming from the "tumor" compartment, 3-APP, obtained with and without the saturation band]. Because of the chemical shift artifact, the voxel for the Pi spins is shifted (in all three dimensions) by the voxel for the 3-APP spins of a small amount (which is less than 2 mm in our study). In order to minimize this artifact we choose the excitation frequency at the equal distance between Pi and 3-APP frequency.

We then apply the sequence with an without the saturation band in vivo on a subcutaneous ovarian tumor model (SKOV3.ip) implanted in the dorsum of SCID mouse (5). Mice were anesthetized by breathing a mixture of isofluorane and oxigen, and a heating pad was used to maintain normal body temperature. Multislice gradient echo images were acquired from the tumour followed by a manual shimming in the tumour voxel. We acquired ^{31}P spectra with the new method [adiabatic excitation (sech90, 4000 us, TR 3 s, 64 average, time about 3 min) preceded or not by the saturation slab with parameter optimized on the basis of the best results on phantom] and we measure the selection efficiency and suppression efficiency. We choose the excitation frequency at the equal distance between Pi and 3-APP frequency, again for minimize chemical shift artifact. We do not consider the voxel shift for the α-ATP because we are interested only in its position (as a reference for pH measurement) which is stable for different tissue composition and pH.

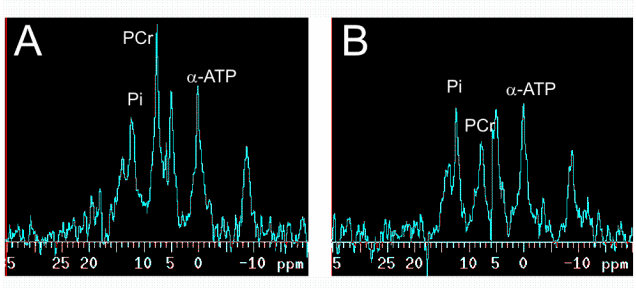
Results – On the phantom we obtained selection efficiency of 81.5 % and a suppression efficiency of 72.5 % by applying a saturation pulse sech180 (2000 us) followed by a sech90 pulse (4000 us). The small amount of 3-APP signal lost due to saturation does not represent a problem because for pH measurement we are dealing with signal positions and not amplitudes.

In in vivo experiment we used the PCr signal to estimate the suppression efficiency (this signal is mainly due to contamination from surrounding tissues, muscles and skin) and Pi and α-ATP to estimate selection efficiency to avoid losses of wanted signal. We obtained a suppression efficiency of 55 % and a selection efficiency of 100% for Pi and 81% for α-ATP. In fact, in the saturated spectrum it is possible to observe that the intense signal of PCr is strongly reduced as well as the hump due to phosphonate present in the bone. An example of a spectrum acquired in the absence and in the presence of the saturation band is shown in Fig.1A and B.

Discussion

Tumour microenvironment may play a key role in tumour malignancy. In particular, acidity has been shown to have a role in resistance to chemotherapy. Although there are several method for pHe assessment, ^{31}P MRS only is able to detect simultaneously pHi and pHe and therefore the intracellular/extracellular pH gradient (ΔpH). In this contest, the proposed method seems to be useful for speed ^{31}P MRS acquisition for pH assessment. This method cannot be used in case of studies on metabolism because signal amplitudes and therefore their quantification is compromised by the application of the saturation band but can be applied in pH measurements because the signal positions only are involved.

This method can be applied to measure pH in vivo in all superficial tumors such as in situ melanomas and breast carcinomas.



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References

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