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PFG NMR Study of Intra-cellular Drug Uptake in *Xenopus laevis* Oocyte

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Abstract: Intra-cellular drug uptake in *Xenopus laevis* oocyte has been elucidated using localized MR spectroscopy (MRS) and PFG NMR techniques at a 600 MHz (Bruker, 14.1 T) NMR spectrometer. The localized MRS has been done with a home-made probe, and shows the intra-cellular uptake of nicotinamide. The self-diffusion of the molecule in *Xenopus* oocyte was obtained by PFG NMR technique. The measured data are well fitted with a linear combination of two exponential functions, which shows that there are two types of drug molecules, intra- and extra-cellular molecules. Diffusion coefficients of intra- and extra-cellular drug molecules are 3.7×10^{-11} m^2/s and 6.4×10^{-10} m^2/s, respectively. In the weighting factors there is shown that about 5% of drug molecule is inside the cells. These techniques can be used for drug screening in molecule-, cell-, and tissue-based preclinical test.

Keyword : MRS, PFG NMR, diffusion coefficient, drug screening

INTRODUCTION

Lots of biological reactions are controled by molecular diffusion which is the main effect on the principles of reaction and interaction rate in biological system. Apparent diffusion coefficients (ADC) of water is used to investigate the physiological status of the brain tissue, which has given us informations on the origin and the change of the ADC values in the early stage of brain infarction. The change of the ADC during brain ischemia is

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supposed to be based on the transport of water from extra- to intra-cellular space,[1] and on the changes in the membrane permeability.[2]

Pulsed field gradient (PFG) NMR is a widely used method to measure ADCs of water, ions, and molecules as a non-invasive method in biological cells and tissues.[3] In order to obtain the ADC values separately from two compartment system, intra- and extra-cellular molecules, we have to trace the signal intensity versus the strength of the PFG using a so-called PFG-NMR pulse sequence (Fig. 1). This technique has a powerful sensitivity to molecular displacement in the range of 0.1~10 ㎚, and to the diffusion coefficient of 10^{-8}~10^{-12} m^2/s depending on the physiological condition.[4,5,6]

^1H NMR lines are arising from water and all metabolite molecules in the given system, and contain informations in molecular types, relative weights of the molecules, relaxation times, and other physical parameters. Using a localized MR spectroscopy (MRS) technique, it is possible to obtain NMR spectrum from the desired volume (voxel) of the sample, which gives us the spatial information of the metabolites in the biological systems.

Pulse sequence widely used in PFG NMR is a stimulated echo (STE) pulse sequence (Fig. 1) with magnetic field gradient pulses. Diffusion coefficient is obtained by the equation given by

$$log[S(g)/S(0)] = -I^2 g^2 D\delta^2 (\Delta - \delta/3) \equiv bD \qquad (1)$$

where $S(0)$ is signal intensity without diffusion gradient, $S(g)$ the intensity with the strength of gradient g, r gyromagnetic ratio, D the diffusion coefficient, Δ the interval between two gradient pulses, δ duration of the gradient.

MATERIALS and METHODS

Oocyte Preparation

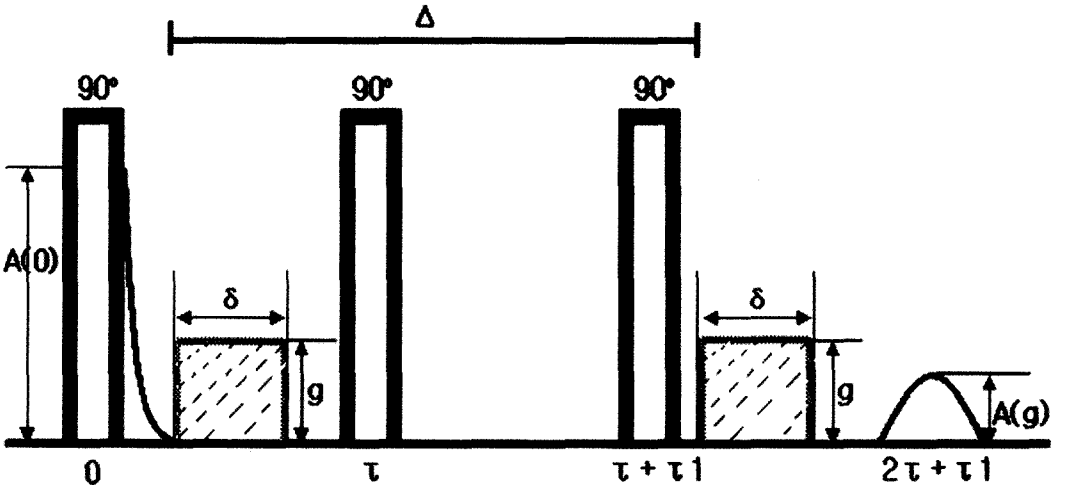

Fig. 1. Stimulated echo (STE) pulse sequence with the magnetic field gradient pulses. Here τ is the time interval between the first and second RF pulse, τ_1 the interval between the second and the third ones, Δ the interval between the gradient pulses, δ duration of the magnetic field gradient pulses, g the amplitude of the gradient pulses. The NMR signal intensity A(g) dependent on the g value is obtained.

Ovarian lobes were excised from the anesthetized *Xenopus laevis* (Xenopuone) through a small abdominal incision. Oocytes were chemically defolliculated by 0.2% collagenase in media (110mM NaCl, 2mM KCl, 1mM $MgCl_2$, 2mM $CaCl_2$, 2mM $NaHCO_3$, 5mM HEPES pH 7.5, gentamycin 50 μg/2mM pyruvate) with a manual method,[7] then stored in 0.33×MR media at 17℃. Healthy oocytes were selected under a optic microscopy by morphology and pigmentation.

Drug Molucule

We used nicotinamide (vitamin B3, Sigma N4126) drug molecule with 100 mM concentration, in MR media, which is the maximum concentration for oocytes not to be distorted. There are four peaks (7.4, 8.1, 8.5, and 8.8 ppm) in ^{1}H NMR spectrum of the drug. Vitamin B3 has an anti-pellagra factor and is called as niacin, nicotinic acid, and nicotinamide depending on the chemical reaction condition. Pellagra, the classic niacin

deficiency disease, is characterized by bilateral dermatitis in sun exposed skin, glossitis, diarrhea, and dementia. Often associated with a largely cereal diet such as maize or sorghum, the disease is now rarely seen in industrialized countries but still appears in India, China, and Africa. Pellagra is often associated with other micronutrient deficiencies and may also develop in cases of disturbed tryptophan metabolism (carcinoid syndrome, Hartnup's).[8]

PFG NMR and MRS Experiments

All measurements has been done with the Bruker 600 ㎒ (14.1 T) NMR spectrometer. using MRS and PFG NMR techniques. We used a 5 ㎜ micro-imaging commercial probe for PFG NMR, and a home-made imaging probe, with 1.7 mm inner diameter of solenoid rf coil, for MRS experiment, respectively. In order to suppress the water signal from the media, the 3-9-19 WATERGATE is added to the STE pulse sequence.

RESULTS & DISCUSSION

Fig. 2 shows the ^{1}H MR Image of *Xenopus laevis* oocyte in a MR media containing 100 mM nicotinamide, and the voxel chosen for the localized spectroscopy (MRS). Conventional SE (spin-echo) pulse sequence added with fatty acid suppression was used, in order to obtain the image, with parameters: repetition time 2 s, echo time (TE) 10 ms, 256×256 matrix, 2.3×2.3 mm^2 FOV, 200 ㎛ slice thickness, and 2 averages, which gives us a high spatial resolution (~9 ㎛). In MRS experiment we have to choose a voxel from which we obtain NMR spectrum except for the outer space of the voxel. The cubic in the oocyte is 0.5×0.5×0.5 ㎜^{3} that was chosen to obtain a localized ^{1}H NMR spectrum from only the intra-cellular space. It was difficult to obtain ^{1}H localized spectrum from each compartment: nucleus, animal pole and vegetal pole, so we used a bigger voxel including

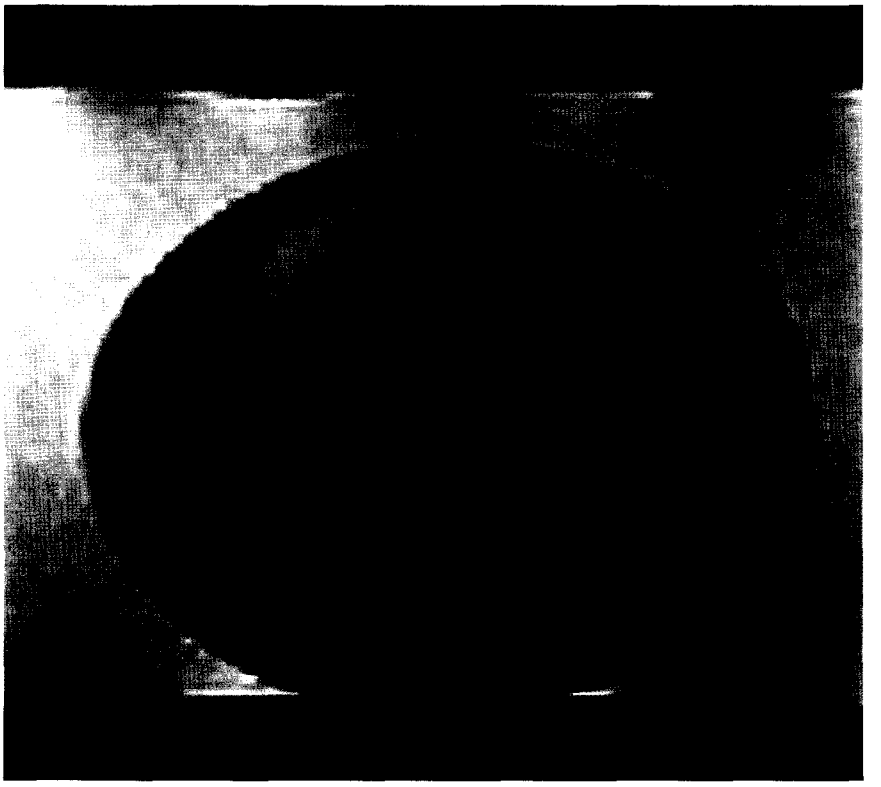

Fig. 2. ^{1}H MR Image of *Xenopus laevis* oocyte. The parameters are: aquisition time 13 min 20 sec, TR (time to repeat) 2 sec, TE (echo time) 6.46 ms, the number of average 400. The cubic is the voxel with the dimension of 0.5×0.5×0.5 ㎜ ^{3} taken for the MRS experiment, in which three components, animal pole, vegetal pole, and nucleus, are partly included.

parts of the three compartments. Same sized voxel in extra-cellular space was used to compare the signal with that from the inner space.

It was impossible to detect ^{1}H localized NMR signal from the voxel in 100 mM nicotinamide media, so we added a water suppression to the conventional MRS pulse sequence of PRESS (point-resolved spectroscopy). In the Fig. 3, there are shown two relative localized NMR spectra, from extra- and intra-cellular spaces, in which there are four

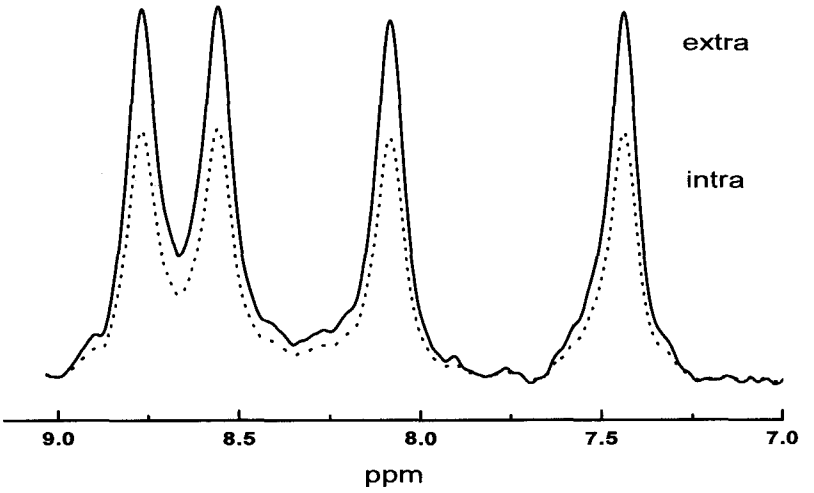

Fig. 3. ^{1}H localized NMR spectra from extra- (solid line) and intra-oocyte (dotted line) voxels in 100 mM nicotinamide media using the MRS technique. There are four peaks (8.80, 8.58, 8.12, and 7.47 ppm) from nicotinamide, and the intensity of the line from intra-oocyte is about 70% of that from extra-oocyte.

nicotinamide peaks at 8.80, 8.58, 8.12, and 7.47 ppm. The signal intensity of the nicotinamide in intra-oocyte is about 70% of that in extra-oocyte.

Fig. 4 shows the *b* value dependence of PFG NMR signal intensity from 100 mM nicotinamide. The data are well-fitted with a linear combination of two exponential functions (dotted and dashed lines) from which we obtain two different ADC values; D_e = 6.4×10^{-10} m^2/s, D_i = 3.7×10^{-11} m^2/s for the nicotinamide moleculles for extra- and intra-oocyte components, respectively. The relative amount of each component is obtained from the exponential fitting, in which the intra-oocyte component is about 5%. In this MRS experiment we used a 5 mm NMR tube in which there are about 300 oocytes in 100 mM nicotinamide media, then only the inner-part of the RF coil can contribute to the NMR

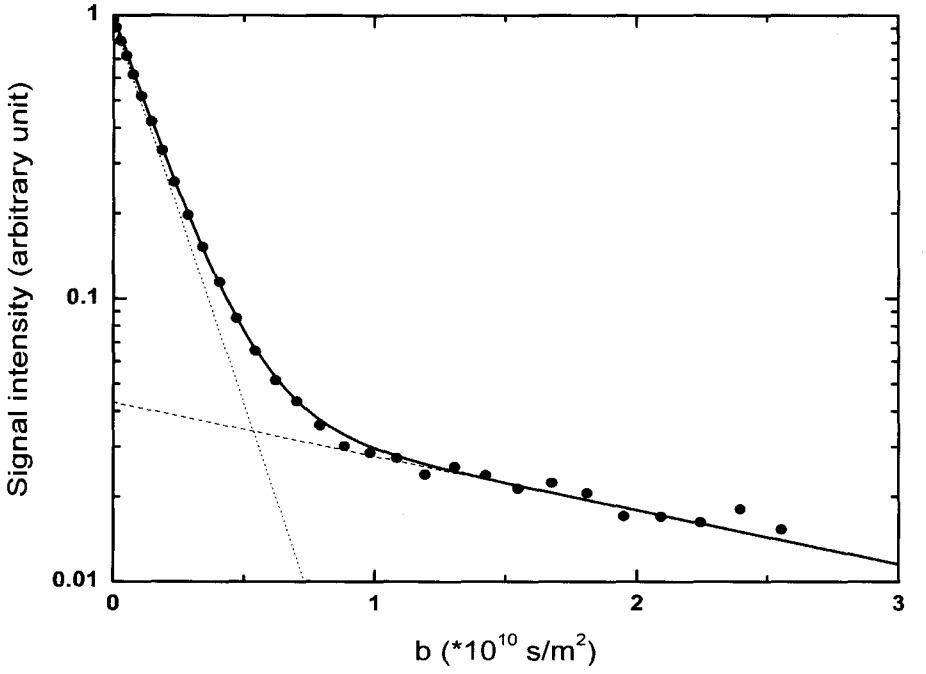

Fig. 4. The b value dependence of nicotinamide signal intensity from *Xenopus* oocyte media with 100mM nicotinamide. The data are well-fitted with a linear combination of two exponential functions (dotted and dashed lines) from which we obtain two different ADCs; $D_e = 6.4\times10^{-10}$ m^2/s, $D_i = 3.7\times10^{-11}$ m^2/s of the extra- and intra-oocyte components, respectively.

signal. The value of 5% is very small compared to the result obtained from the MRS experiment, which may be originated from the experimental condition, but these two techniques can be tools for drug screening in cell-based drug discovery.

CONCLUSION

We obtained preliminary results of localized NMR spectroscopy and PFG NMR in order to obtain how much of drug molecule is inside a living cell. These two techniques, MRS and PFG NMR, can be tools for drug screening in molecule-, cell-, and tissue-based preclinical test.

Acknowledgments

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