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Quantification of brain creatine concentration using PRESS sequence and LCModel: comparison with HPLC method

Y.Lin[1,2], Y.P.Zhang[1], Z.W. Xiao[2], H.Li[3], Z.W.Shen[1], X.K.Chen[1], K.Huang[1], R.H.Wu[1,2], *Member, IEEE*

Abstract—To investigate the accuracy for quantification of brain total creatine (Cr) concentration using in vivo long echo time (TE) PRESS sequence with an external standard and LCModel. Ten swine and an external standard containing a detectable compounds of known concentration were studied by using 1.5T GE Signa scanner and the standard head coil; the single-voxel proton magnetic resonance spectroscopy (1H-MRS) data was acquired from the 20-mm cubic VOI which was placed in the swine brain and external standard respectively by using the PRESS sequence with TE = 135 msec, TR = 1500 msec, and 128 scan averages. The quantification of Cr was accomplished by the linear combination of model spectra (LCModel). After MRS examination, each animal was sacrificed, and in vitro Cr concentration was analyzed by high performance liquid chromatography (HPLC). In the MRS group, the mean concentration of Cr was 9.37 ± 0.137mmol/kg; in the HPLC group, the mean concentration of Cr was 8.905±0.126 mmol/kg. There were no statistically significant differences between two methods (P=0.491), which indicated that long TE PRESS sequence with an external standard can accurately detect the brain Cr concentration. The application of LCModel introduces more convenience for the MRS quantification.

***Keywords*—PRESS sequence; total creatine; external standard; LCModel; HPLC**

I. INTRODUCTION

Total creatine (Cr), resonating at 3.03 ppm and 3.94 ppm chemical shift, represents the quantity of phosphocreatine (pCr) and creatine(Cr) involved in neurones and glial cells[1]. As the storage and transmission of phosphate-bound energy, Cr plays essential roles in energy metabolism. It can undergo phosphorylation-dephosphorylation reaction catalyzed by the enzyme creatine kinase: ADP+PCr⇔ATP+Cr. If oxidative phosphorylation cannot be maintained to supply ATP, PCr can provide the phosphate group to ADP to form ATP to reduce the extent of non-oxidative glucose consumption, which can reduce neuronal death mostly due to a delayed decrease of ATP under hypoxic stress and protect the normal brain function against the accumulation of Lactate [2]. Decreased brain Cr has been linked to anoxic seizure [3]. An inborn deficiency of guanidinoacetate methyltransferase lead to creatine deficiency which exhibited mental retardation, movement disorder, developmental delay and epilepsy. Oral Cr can replenish a large proportion of the cerebral Cr and PCr pool which buffers the cellular ATP concentration and delays its depletion under situations of energy compromise and led to a marked clinical improvement and recovery[4]. Increased levels of Cr may be associated with ageing-related mild cognitive impairment as well as, in more extreme cases, frank dementia [5]. Cr or Cr analogy have also been reported to contribute to anti-tumor, anti-virus and anti-diabetes [6]. In summary, non-invasive detection of brain Cr concentration using ^{1}H-MRS has an important clinical significance for the diagnosis and treatments of brain diseases related to the change of Cr content.

In localized brain MR spectroscopy, the measurement results had been expressed as metabolite ratios for a long period. However, dilemma existed because metabolite ratios were not comparable with quantitative results obtained with biopsy samples and in vitro animal studies. Internal standard such as water have been utilized to acquire Cr concentration [7,8]. However, this method was also reported to have a number of potential errors, so the measurement result was not accurate. For this reason MR external standard method is preferable. It was previously used in short echo time (TE) STEAM sequence[9] and short TE PRESS sequence[10] to quantify brain Cr concentration. Concerning signal to noise ratio, PRESS sequence is better than STEAM sequence. The use of short TE has a advantage to investigate the short T2 matters, such as glutamate + glutamine (Glx) and myo-inositol (Ins) [11], Cr is the long T2 metabolite, long TE PRESS sequence can be used to detect Cr peak and obtain maximum signal-to-noise ratio. LCModel is an automated, user-independent curve fitting software, for absolute quantification of cerebral metabolites from MR spectra. LCModel has also been employed in external standard method [10], but in mainland China, the application of this software was not reported. High performance liquid chromatography (HPLC) method in vitro is a reliable technique to analyze metabolite concentrations, and suitable to assess the accuracy of metabolite quantification detected by in vivo ^{1}H-MRS.

Therefore, the purpose of this study is to investigate the accuracy for absolute quantification of brain Cr concentration using in vivo long TE PRESS sequence with an external standard and LCModel. In vitro Cr concentration was measured by HPLC method. Through correlation evaluation between in vivo MRS method and in vitro

1. Department of Medical Imaging, the 2nd Hospital, Shantou University Medical College, Shantou , Guangdong , 515041, China
2. Guangdong Key Laboratory of Medical Molecular Imaging, Shantou University Medical College, Shantou , Guangdong , 515041, China
3. Central laboratory, Shantou University Medical College, Shantou, 515041, China

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method, an accurate MR spectroscopy technique can be determined.

II. METHODOLOGY

Ten swine (3.13 ± 0.59kg, mean ± SD) were investigated in this study by using 1.5T GE Signa scanner and the standard quadrature head coil. All studies were performed in accordance with animal protection guidelines and approved by the governmental authority. Prior to the MRI examination, all animals were intravenously anesthetized with 1 ml/kg mixed liquor including chlorpromazine hydrochloride and procarbazine hydrochloride, then immobilized with a restraint system and placed supine on the scanner bed with the head firmly fixed. To accurately determine the brain Cr concentration, an external standard method was used in this study. A 125ml spherical phantom containing 5mmolNAA，5mmol γ-aminobutyric acid，2.5mmol glutamine，2.5mmol glutamate，4mmol creatine，1mmol choline chloride， and 2.5mmol myo-inositol with the highest degree of purity (Sigma chemie) in physiological saline was used for the external standard. The phantom was placed adjacent to the animal's head in the detection coil with its axis of symmetry parallel to the static magnetic field during MR scanning and was within the image field of view. ^{1}H-MRS data were acquired from the two 20-mm cubic VOI which were placed in swine brain and external standard solution (Figure 1) by using PRESS sequence with TE = 135 mses, TR = 1500 msec, and 128 scan averages.

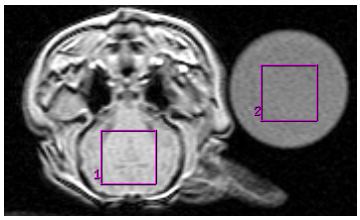


Fig. 1. Voxel (2 x 2 x 2 cm^3) were placed in the swine brain and external standard solution

After data acquisition, the spectroscopic data was transferred to the SGI/O2 workstation, fully automated and user-independent Cr quantification was accomplished by the LCModel with the imported basic set of 135. In order to measure the swine brain Cr concentration accurately, LCModel requires a calibration on a standard of known concentration [12]. Cr analysis data acquired from the external standard was used to calculate a factor for calibration. Let C_{lcm} be the concentration output by LCModel, and let C_{true} be the true concentration (32mmol/L) in the external standard phantom, then the calibrate factor is:

$$F_{calib} = \frac{C^{S}_{true}}{C^{S}_{lcm}}$$

So the in vivo swine brain concentration C of creatine in the VOI was calculated as the following equation:

$$C^{b}_{true} = F_{calib} \times C^{b}_{lcm} = \frac{C^{S}_{true}}{C^{S}_{lcm}} \times C^{b}_{lcm}$$

(where the superscript s and b represent standard solution and brain, respectively).

Finally, the concentration is converted to millimoles per kilogram wet weight by dividing with P_{brain} =1.00Kg/L for the specific gravity of swine brain tissue measured in our lab. The amount of CSF in the VOI also should be corrected according to Decarli C [13].

$$C^{b}_{true} = \frac{C^{S}_{true} \times C^{b}_{lcm}}{C^{S}_{lcm} \times (1 - f_{csf}) \times P_{brain}}$$

After MRS examination, each animal was sacrificed, the brain was rapidly removed and a 2x2x2 cm3 brain sample corresponding to the location of the voxel defined by MR spectroscopy was dissected using a sharp knife. All specimens were wrapped with plastic paper and immediately immersed in liquid nitrogen and stored at -70°C until preparation for HPLC measurement as described by shun ai ming with some modifications [14]. Brain tissues were dissolved in 20ml 0.42 mol/L perchloric acid and homogenized while cooled on ice. The homogenates were centrifuged at 4000 rpm for 10 min at -10°C. An aliquot of 0.2ml supernatant was pipetted into a 1.5-ml plastic microcentrifuge tube and 0.085ml 1mol/L KOH was added into the tube, which was capped tightly and vortex-mixed for 1min and centrifuged at 4000 rpm for 10 min at -10°C again. Then 0.02ml supernatant was ready for HPLC analysis.

Comparison of Cr concentration between in vivo MR method and in vitro HPLC method was conducted by the single-factor analysis of variance (ANOVA). Differences were deemed significant if the p value was less than 0.05.

III. RESULTS

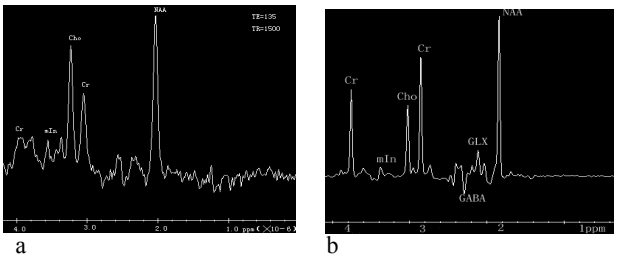
Fig. 2. shows examples of proton magnetic resonance spectra for metabolites in swine brain (a), and external standard solution (b). Major metabolites resonances were seen clearly.

Fig. 3. shows example of the chromatogram for the brain homogenate. Similar chromatograms were found among all the brain samples in the study.

Table.1 lists the Cr concentration using both MR spectroscopy method and HPLC method for each swine. In the MR spectroscopy group, the mean concentration of Cr was 9.37 ± 0.137 mmol/kg. In the HPLC group, the mean concentration of Cr was 8.905 ± 0.126 mmol/kg. There were no statistically significant differences between these two methods (p = 0.491), which indicated that long echo time PRESS sequence with an external standard and LCModel software can accurately quantify the brain Cr concentration.

The in vivo experiment was also performed on 27 healthy subjects aged 20-72 years，three elderly people with subtle cognitive decrement aged > 65 years. The voxel was placed

in the left frontal lobe for the healthy subjects and the elderly people. No evidence of abnormal signal was found on T1WI, T2WI, FLAIR and DWI in all healthy subjects, but higher Cr concentrations in frontal lobe occur in the elderly people compared with the young subjects. Subjects with subtle cognitive decrements appearing normal on conventional imaging had significantly higher Cr concentration in frontal lobe compared with controls.



a b

Fig. 2. proton magnetic resonance spectra for metabolites in swine brain (a), and external standard solution (b) using PRESS sequence with TE 135 msec , TR 1500 msec and 128 averages

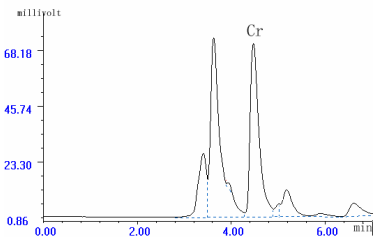


Fig.3. The chromatogram indicated a good baseline, the peak of Cr was well separated from other peaks.

Table 1: comparison of swine brain Cr concentration（mmol/Kg, VOI） measured by PRESS sequence with an external standard method and in vitro HPLC method.

Subject number	MRS method	HPLC method
1	9.16	8.74
2	9.39	8.77
3	9.26	8.92
4	9.45	9.08
5	9.22	8.78
6	9.29	9.12
7	9.39	8.93
8	9.41	8.96
9	9.62	8.88
10	9.48	8.87
Mean	9.37	8.905
SD	0.137	0.126
P	0.491	

IV. DISCUSSION

Total Cr plays essential roles in energy metabolism in the brain, non-invasive detection of Cr concentration using ^{1}H-MRS has an important clinical significance for the diagnosis and treatments of brain diseases related to the change of Cr content.

In localized brain MR spectroscopy, metabolite ratios have been usually used to express the change of metabolites for a long period. The most compelling reason was that ratios correct for several experimental unknowns, difficult to obtain, or uncontrollable experimental conditions, e.g: B_1 inhomogeneities, instrumental gain drifts, imager and localization method differences, voxel partial volume contamination from cerebral-spinal fluid (CSF) [15]. However, the main drawback of ratios is that the results are lack of objectivity, accuracy and comparability. If the concentration ratio of two metabolites is increased, it may not be possible to know whether this increase is due to a relative increase in the numerator or a decrease in the denominator [7].

Concerning metabolites analysis in clinical MR spectroscopy studies, absolute concentrations have advantages over metabolite ratios. The MRS with internal reference method exploiting the known water content of tissue has been usually used to acquire absolute metabolites concentration [7, 8]. This method has conceptual simplicity and directness, because metabolite and internal reference signals are both acquired from the same VOI under the same loading conditions. Internal reference method was also insensitive to many of the experimental factors affecting the performance of the quantitative techniques, including effects related to loading, standing waves, B_1 inhomogeneities, practical issues of phantom positioning and user expertise and examination duration [16]. However, there is a drawback in the assumption of the constant internal reference: the fraction of NMR-visible water can not remain constant during various physiological and pathophysiological states. Using water as internal reference turned out to be particularly sensitive to baseline distortions of the strong water resonance in the reference acquisition without water suppression, this sensitivity severely complicated the exact definition of the boundaries for signal integration [9]. So, using water as internal reference could not accurately quantify metabolites concentration.

For this reason, using MR external standard method to quantify brain metabolites concentration is preferable. It was previously used in stimulated echo acquisition mode (STEAM) sequence to detect Cr concentration since 1993[9]. Concerning signal to noise ratio, PRESS sequence is better than STEAM sequence. PRESS sequence is based on one 90 degree and two refocusing 180 degree orthogonal slice selective pulses, it acquires the entire signal due to the 180 degree pulses, and hence yields twice the S/N compared to STEAM. External standard method using short TE PRESS sequence to measure brain Cr concentration has been reported [10], and the result trended to be low compared with

quantitative results obtained with biopsy samples or in vitro animal studies. The use of short TE has a advantage to investigate the short T2 matters, such as glutamate + glutamine (Glx) and myo-inositol (Ins) [11], Cr is the long T2 matter, long TE PRESS sequence can be used to detect Cr peak and obtain maximum signal-to-noise ratio.

In our study, we use a long echo time PRESS sequence with an external standard and LCModel software to quantify swine brain Cr concentration. In order to examine the accuracy of in vivo MRS method, in vitro Cr value was detected by HPLC method. Good agreement was obtained between in these two methods, which indicated that long echo time PRESS sequence with an external standard and LCModel could be accurately quantify the absolute Cr concentration. This experiment was also carried out in clinic, further information will be found for the diagnosis and treatments of brain diseases related to the change of Cr content.

However, MRS with external standard method was reported to have a number of potential errors [16]: it produced spectral distortions due to increase magnetic field inhomogeneities and correspondingly impaired water suppression produced by the external phantom itself which can lead to eddy currents and effects related to loading; also the subjects will feel less comfortable due to the phantom positioning. But, in our study, we used the PRESS sequence to acquire the analysis data, which can correct for field inhomogeneities、instrumental gain drifts、imager and localization method difference due to the two refocusing 180 degree pulses. We also use the relatively small phantoms 125ml to eliminate standing wave effects and other uncomfortable phenomena. The external phantom itself can also be used to correct for residual signal variations due to B_1 inhomogeneity [16].

The in vivo Cr value is close to the in vitro value. However, slightly higher value was observed in the MR spectroscopy group compared with HPLC group, because the Cr peaks contain contributions from aminobutyric acid, lysine and glutathion, which could explain the higher Cr values obtained by in vivo MR spectroscopy methods, although the differences were not statistically significant. So, further studies should be done in order to find more detailed information.

V. CONCLUSION

The in vitro HPLC method is reliable and suitable to use to assess the performance for absolute quantification of cerebral metabolites using in vivo ^{1}H-MRS, so the dilemma of comparing in vitro vs in vivo results can be solved. The long echo time PRESS sequence performed with an external standard is demonstrated to be an accurate and scientific technique to detect the brain Cr concentration, which is helpful to provide further insights into the diagnose and treatment of brain diseases related to the change of Cr content. The use of LCModel software introduces more convenience for the 1H-MRS quantification.

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