In vivo metabolite compartmentalization probed using intracellular GdDTPA

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Introduction

Fast trans-membrane water exchange enables intracellular relaxation enhancement of water by contrast agents in the extracellular space. For metabolites not in fast exchange across membranes, intracellular metabolite relaxation enhancement will only occur if the contrast agent and metabolite are in the same compartment. Extracellular contrast agents may be delivered intracellularly using electroporation techniques in vitro and in vivo. Consequently, this study has utilized electroporation methods to deliver GdDTPA into the cytosol of rat muscle in vivo in order to probe the intracellular compartmentalization of MR-visible metabolites.

Materials and Methods

1 ml of Magnevist (n=4) or saline (n=4) was administered via a cannulated tail vein 5 minutes prior to electroporation. Electroporation was performed using a Cyto Pulse PA-4000 electroporation device using a needle array system consisting of 2 rows of 4 27G syringe needles. The rows were separated by 9 mm and each needle in the row was 2 mm apart. 8 x 800 V/cm pulses at a rate of 1 Hz was applied to the rear limb of anaesthetized male Sprague Dawley rats (324±30g), as described previously[1]. The animals were allowed to recover and anaesthetized 24 hours later for MR investigation. In addition, 4 untreated limbs were investigated. The rear hind limb was fixed and aligned along z. Imaging and spectroscopy measurements were performed using a Varian 4.7T system, utilizing a home built 2 cm surface and 9 cm volume coil. Localized longitudinal relaxation time measurements were performed using an IR-PRESS sequence on a 4 x 4 x 4 mm voxel. The voxel was positioned using images acquired from a multi-slice T1W SE sequence. Water T1 measurements were performed using 25 inversion times ranging from TI=0.1 to 5s with TR=10s, TE=17ms. Metabolite T1 measurements were performed using 5 inversion times (TI= 0.15, 0.3, 0.45, 0.8 & 1.4s with TR=6s, TE=17ms together with a M_0 measurement (uninverted, TR=10s, TE=17ms). Resultant spectra were fitted using Matlab 7.3 and JMRUI v3[2] .All procedures were performed with permission of the Danish Animal Inspectorate.

Results and discussion

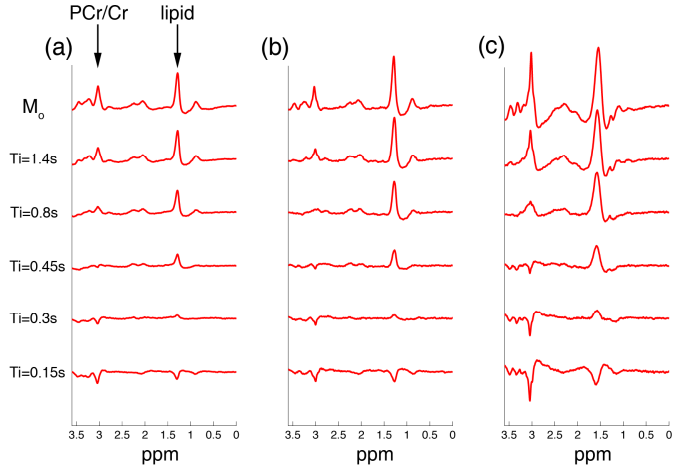

Figure 1: Averaged (n=4) ^{1}H MR spectra acquired with different Ti values (M0 - Tr=10s, Te=17ms, else Tr=6s) from a 4x4x4 mm voxel using PRESS. Individual spectra were normalised using unsuppressed water spectra prior to averaging. Spectra obtained from (a) electroporation & GdDTPA, (b) electroporation & saline and (c) untreated rat muscle.

	H_2O T1 (s)	PCr/Cr (CH_3) T1 (s)	lipid (CH_2) T1 (s)
Untreated	1.4±0.02	1.1±0.01	0.5±0.10
Electroporated (saline)	1.5±0.01	1.3±0.2	0.4±0.1
Electroporated (GdDTPA)	0.9±0.03	0.9±0.1	0.4±0.1

Table 1: Localized water and metabolite longitudinal relaxation times. Water T1s were significantly different in all combinations (P<0.05). For PCr/Cr, only the saline and Gd electroporated were significantly different (P=0.04). There were no significant differences between the lipid T1s.

Using in vivo electroporation, GdDTPA has been delivered into the cytoplasm of rat muscle cells with the aim of probing the compartmentalization of MR-visible metabolites via paramagnetic modulation of T1 relaxation times. The presence of significant contrast enhancement in the T1W images together with reduced water relaxation times within the spectroscopic voxel, 24 hours after intravenous delivery, suggests high concentrations of trapped contrast agent. GdDTPA has been shown to enhance metabolite relaxation in vitro[3], although its intracellular relaxivity is unknown. This study, however, does not provide direct evidence that GdDTPA remains intact and is located within the cytoplasm. Furthermore, in vivo muscle spectra are complicated by orientation, compartmentalization and other factors resulting in complex relaxation effects[4]. In addition, the spectra are influenced by the electroporation, which appears to reduce PCr/Cr concentrations by almost 75%. Despite the complexity of the system, significant differences in the spectra acquired from animals receiving GdDTPA or saline were determined (table 1).

To enable estimates of the concentrations of trapped gadolinium to be performed, it is assumed that the Gd remains chelated and exhibits a water relaxivity of 4.5 $mM^{-1}s^{-1}$. Using this value gives a intra-voxel Gd concentration of 94µM. If a cell density of 85% is assumed this gives an intracellular Gd concentration of 110µM. The PCr/Cr relaxivity of Gd has been shown to be 3.3 $mM^{-1}s^{-1}$ at 1.5T[3] which translates into an intracellular concentration of 104µm. Whilst these values are estimates, the similarity suggests that the change in PCr/Cr relaxation is due to intracellular contrast agent and that the MR-visible PCr/Cr signal may predominantly arise from metabolite in the cytosol.

Conclusion

This study has shown that the in vivo relaxation times of intracellular metabolites may be influenced by the intracellular delivery of GdDTPA 24 hours after electroporation. Despite the significant effects of electroporation on PCr/Cr levels, data from this study suggest that significant components of the MR-visible PCr/Cr resonance reside in the cytosol, supporting current knowledge. Using this approach, the sub-cellular compartmentalization of PCr/Cr and other metabolites in other tissues may be investigated.

Acknowledgements

Financial support from the Lundbeckfonden and the loan of the electroporator from Cyto Pulse Inc. are gratefully acknowledged.

References

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