Electroporation facilitated intracellular delivery of contrast agent to probe sub-cellular metabolite compartmentalization *in vivo*

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Introduction

The permeability of cell membranes may be temporarily increased by exposing cells to pulsed high electric fields. When the trans-membrane potential induced by the pulsed field exceeds a certain threshold, a rearrangement of the cell membrane occurs leading to the formation of trans-membrane pores, dramatically increasing cell permeability to ions, contrast agents and even macromolecules[1]. The ability of electroporation to deliver extracellular paramagnetic contrast agents intracellularly into the cytoplasm[2] makes it possible to probe the compartmentalization of MR detectable metabolites. Whilst water is in fast exchange over the cell membrane, metabolites will only undergo relaxation if the contrast agent and metabolite are in the same compartment. In this study the compartmentalization of creatine and choline is investigated by trapping gadolinium based contrast agents intracellularly in rat muscle cells in vivo.

Methods

Male Sprague Dawley rats (N=21) were anaesthetized and 8 needles (27G, 2 rows (9 mm apart) of four needles (2mm apart)) were inserted into the rat hind leg. The needle arrays were positioned approximately perpendicular to the long axis of the muscle fibres. 8 pulses of 800 V/cm were applied for 100 µs at 1Hz using a Cyto Pulse PA-4000 Electroporation device, 5 minutes after intravenous administration of contrast agent or saline via a cannulated tail vein. In a pilot experiment, four rats were administered 1 ml of Magnevist. In a second set of experiments, each animal was injected with either: 1 ml of isotonic saline (N=5), 1 ml of Gadovist (1M, N=3) or 1 ml of Gadovist (0.5M, N=6) previously diluted 1 to 1 with isotonic saline. Control animals that received neither saline or contrast agent (N=7) were also investigated. Each animal was scanned two days as well as 1, 2, 3, 4 and 8 weeks following electroporation. Anaesthetised animals were positioned in a Varian 4.7T imaging and spectroscopy system and water and metabolite relaxation times obtained from a 4x4x4 mm voxel in the electroporated muscle (Fig 1 - blue squares). Water T_1 values were obtained using the following parameters: TR = 10 s, 3 averages, 25 inversion times ranging from 100 ms to 5000 ms. Water M_0 was estimated in a scan with no inversion and 3 averages. Metabolite T_1s were measured using CHESS water suppression and the following parameters: TR= 6 s, 100 averages, 5 inversion times 150, 300, 450, 800 and 1400 ms. M_0 was estimated in a scan with no inversion using a TR of 10 s and 100 averages. T_1 was fitted after manual annotation of each peak as previously described[3]. Contrast agent concentrations were estimated based on the relaxivity measured in phantoms containing BSA (Table 1) and the measured T_1 values of each metabolite.

Results and Discussion

T_1w images acquired post electroporation are shown for control animals, saline and Gadovist electroporated animals (figure 1). From the images, it is clear that Gadovist enhances the electroporated muscle and remains intracellular for periods up to two months. It is further clear that electroporation causes edema (saline 1-2 days) which gradually decreases and resolves after two weeks. Figure 2 shows the correlation between contrast agent concentration estimated by water signal and concentrations estimated by Cho and tCr measurements. Both fitted slopes were 1.61 and both correlation coefficients 0.89. The standard error of the fit was 0.21 and 0.07 for Cho and tCr respectively. The similarity of these results suggest that the contrast agent responsible for the enhancement remains predominantly intact. In Figure 3, T_1 as a function of time is shown for Cho, creatine (tCr) and water. The solid black line indicates the mean T_1 value of control animals +/- SEM (dotted lines). A circle indicates statistical difference from the saline electroporated animals while a star indicates statistical difference from control animals. Reductions in metabolite and water T_1 values are observed for all contrast agents administered.

Conclusion

Electroporation can be used to deliver contrast agents intracellularly in vivo. This study suggests that the contrast agent remains trapped for periods up to 2 months and reduces the longitudinal relaxation time of metabolites ordinarily considered to be intracellular throughout this period. The delivery of contrast agent into the cytoplasm[2] suggests that a significant component of the MR visible creatine and choline may also reside within the cytoplasm. Consequently, this approach is well suited to study the sub-cellular compartmentalization of metabolites in vivo and may also be used for long term labelling of cells in vivo.

Longitudinal relaxivity values (mM s^{-1})					
Phantoms	**H_2O**	**Creatine ($-CH_2$)**	**Choline ($-CH_3$)**	**Creatine ($-CH_3$)**	**NAA ($-CH_3$)**
Gadovist	5.4 (0.4)	8.2 (0.5) *	2.4 (0.1) *	4.1 (0.2) *	3.7 (0.3) *
Gadovist BSA	5.6 (0.1)	8.2 (0.6) *	2.3 (0.2) *	3.7 (0.3) *	3.5 (0.3) *
Magnevist	4.4 (0.3)	5.5 (0.5) *	8.6 (0.2) * !	3.9 (0.2)	1.7 (0.3) *
Magnevist BSA	3.8 (0.3)	4.1 (0.9)	7.8 (0.3) * !	3.3 (0.4)	1.0 (0.7) *

Table 1: In vitro relaxivities at 4.7T . * =significant difference between metabolite and water. !=signifi difference between BSA and non BSA

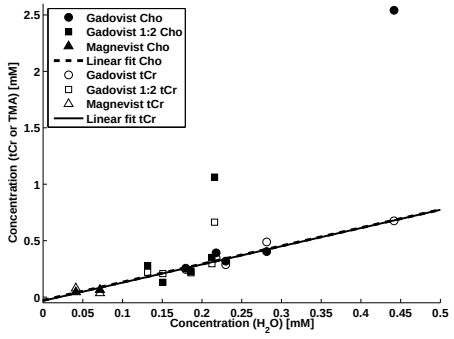

Figure 2: Concentration estimated using Cr/Cho vs concentration estimated using H_2O

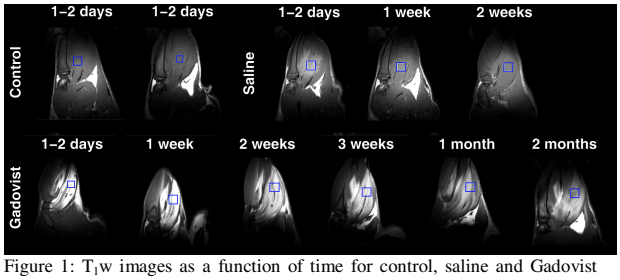

Figure 1: T_1w images as a function of time for control, saline and Gadovist treated animals

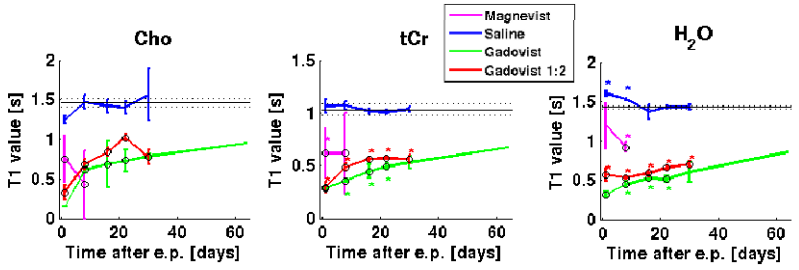

Figure 3: T_1 as a function of time for the different treatments. The black line is control values +/- SEM (dotted)

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