Detection of iron oxide particle-containing inflammatory cells in developing infarction by susceptibility contrast MRI by Carr-Purcell T_2

M. Kavec[1], O. Gröhn[1], P. Valonen[1], M. Garwood[2], R. Kauppinen[1]

[1]University of Kuopio, Kuopio, Finland, [2]University of Minnesota, Minneapolis, Minnesota, United States

Introduction

Iron oxide particle-based contrast agents are commonly used as blood pool contrast agents in animal fMRI [1] owing to greater contrast-to-noise relative to BOLD and long plasma half-life. Iron oxide particles have also been used to label specific ligands for cellular and molecular imaging by MRI [2, 3]. More recently, the ability of reticulo-endothelial cells to take up iron oxide particles has been exploited for *in vivo* labelling of circulatory monocyte/macrophage cells [4]. These phagocytotic cells accumulate in damaging tissue, e.g. in ischaemic stroke, and thus this process can be visualised by MRI *in vivo* [4].

Super-paramagnetic iron oxide particles create strong local field gradients and are readily detected by gradient echo (GRE) MRI. However, due to very efficient dephasing, large signal void areas often result loss of anatomical details in GRE MRI. It has recently been shown that Carr-Purcell (CP) T_2 MRI can be used to reveal tissue susceptibility by acquiring data with varying interpulse interval (τ_{CP}) [5]. This method retains anatomical details, yet reveals endogenous susceptibility *in vivo*. In the present work we have used CP-T_2 MRI in imaging ischaemic stroke in rats injected with AMI-227 post-stroke.

Methods

Male Wistar rats (250-350g) were anesthetized with 0.8-1.5% halothane in N_2O/O_2 (70%/30%). The gas composition and the core temperature was monitored on-line and normothermia was maintained either by an electrical heating element during surgical procedures or circulating warm water in a heating pad in the magnet bore. AMI-227 (Sinerem®, Guerbert, Paris) was reconstituted with 0.9% saline to yield 200 μM Fe/ml of solution.

Focal cerebral ischaemia was induced (day 0) by the intraluminal thread method [6]. Animals (n = 9) were transferred into the magnet after complete insertion of occluder on bench. MRI variables were quantified 60 minutes after the MCA occlusion (MCAo), following in-bore retraction of the occluder thread. The animals were briefly reanesthetized with halothane 5 hours post-ischaemia, and 500 μl of AMI-227 solution was injected i.v. The rats were reanesthetised with halothane for further MRI examinations on day 1, 2, 3, 5 and 7 after ischaemia. A subgroup of animals was sacrificed on day 2 (n = 2), 3 (n = 3) and 7 (n = 4).

The MRI experiments were carried out in a 4.7 T horizontal magnet interfaced to a Varian ^{UNITY}INOVA console (Varian Associates, Palo Alto, CA, USA), using a quadrature surface coil as a transmitter/receiver. The transverse images covering striatum were positioned according to T_1-weighted coronal pilot images. D_{av} was quantified using single spin-echo sequence with four bipolar gradients along each gradient axis with b values of 0-1000 s/mm^2 (TR = 1.5 s, TE = 56 ms, 2 averages, voxel size 0.11x0.47x2 mm^3). CP-T_2 was acquired with fully adiabatic multi-echo spin-echo sequence using hyperbolic secant (HS1) pulses in CP train, an adiabatic half passage pulse for spin excitation and a pair of HS1 pulses for slice selection and spin refocusing (TR = 2.5 s, 2 averages, voxel size 0.11x0.31x2 mm^3). Different TEs (15, 55, 95 ms) were obtained either by increasing the inter-pulse delay between the centers of CP pulses (τ_{CP} = 2.5-20 ms; referred to as long τ_{CP}) or increasing the number of inversion CP pulses (0, 16, 32) and keeping τ_{CP} constant (τ_{CP} = 2.5 ms; referred to as short τ_{CP}).

For histology, the animals were sacrificed, transcardially perfused, brains were removed from the skull, postfixed and frozen until histological analysis. Horizontal sections (1-in-5 series) of 30 μm were cut with a sliding microtome and stained using Accustain® (Sigma Diagnostics Inc., St. Louis, MO, USA), labeling iron particles with Prussian blue color.

Absolute D_{av}, CP-T_2 and relative susceptibility were analysed in the regions-of-interest (ROI) covering ipsilateral striatum. Relative susceptibilities were determined from long and short τ_{CP} CP-T_2-weigted images at TE = 55 or 95 ms as $(SI_{short\ \tau CP} - SI_{long\ \tau CP})/SI_{long\ \tau CP}$ [5]. The areas of significantly low MR signal, likely originating from leaky blood brain barrier, could potentially bias the results, thus where excluded from the ROI analysis. Prussian blue positive deposits were counted in each animal in 6 representative brain sections and normalized by the count on a day 2. Data are presented as mean ± SEM and Student's t-test was used for statistical analysis.

Results

Low D_{av} was detected in MCA territory as a sign of ischaemia (Fig. 1D) and low diffusion values were detected until day 2 after stroke. In the animals injected with AMI-227 after MCAo, signal void areas were observed both in the infarct and in peri-infarct areas (Fig. 2A). The normalised susceptibility images are shown on the bottom row of Fig. 2A, revealing a well defined area of hyperintensity. The intensity and volume of hyperintense regions peaked on day and 3 (Fig 2A). A quantitative regional analysis of susceptibility contrast is given in Fig. 1C. Prussian blue staining shown (Fig. 2B, C) illustrates scattered distribution of iron deposit containing cells, concentrating in peri-vascular structures and in the periphery of ischaemic lesion. Prussian blue positive deposits were present in highest number on day 2 after MCAo closely matching the intensity of CP-T_2 susceptibility contrast (Fig. 2D).

Conclusions

We show that susceptibility contrast obtained from CP-T_2 MRI reveals AMI-227 containing cells in the infarcting brain with good anatomical precision. Positive contrast obtained by CP-T_2 match well with histology revealing scattered presence of iron particle containing cells. The CP-T_2 method could, in principle, provide quantitative data from the iron concentration in the brain thus greatly superseding conventional GRE MRI for cell tracking. Furthermore, at high field (≥4.7 T) the sensitivity of this method is expected to be high enough to reveal small number of iron oxide particle-containing cells, such as macrophages.

Acknowledgements

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References

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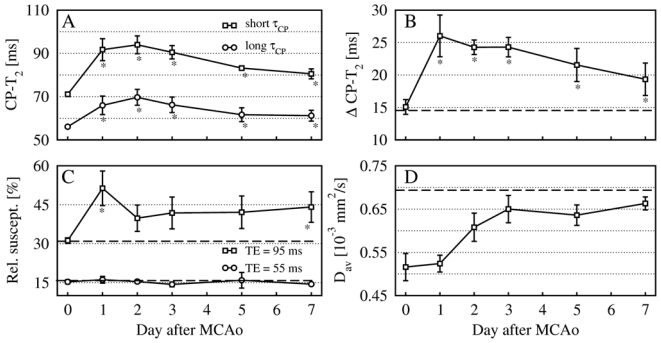

Fig. 1. CP-T_2 (**A**), difference of short and long τ_{CP} CP-T_2 (**B**), relative susceptibility (**C**) and D_{av} (**D**) in ipsilateral striatum. * denotes $P < 0.05$ relative to day 0 value. Dashed line represents the respective value in non-ischaemic tissue.

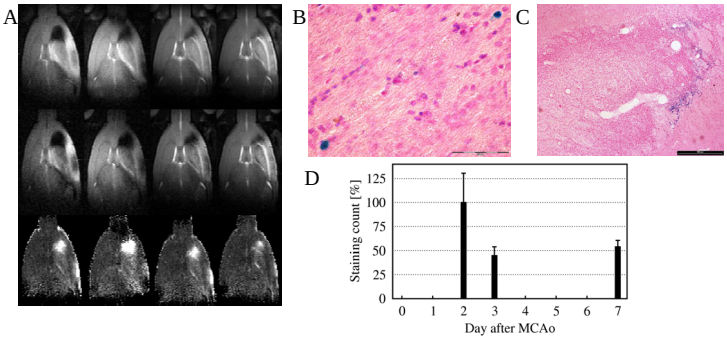

Fig. 2. (**A**) CP-T_2-weighted (TE = 95) short (upper row) and long (middle row) τ_{CP} images and relative susceptibility images (bottom row) collected, from left to right, on a day 2, 3, 5 and 7 after MCAo and AMI-227 injection. Histological sections (**B, C**) of the animal in (**A**) and positive staining reaction count in ipsilateral striatum