$R_{1\rho}$ dispersion in rat brain during global ischaemia and gene therapy of an experimental glioma

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MRI contrast based on T_1 in the rotating frame ($T_{1\rho}$) relaxation has been shown to be a sensitive and early marker of cerebral ischaemia and successful gene therapy of a rat glioma [1-4]. $T_{1\rho}$ MR contrast can be modified by changing the amplitude of spin-lock (B_1) field and the relaxation dispersion offers additional information to that obtained by a single B_1 field $T_{1\rho}$ in cerebral ischaemia [1]. In the present study, we have quantified $R_{1\rho}$ (=1/ $T_{1\rho}$) dispersion in the B_1 field range of 0.01 to 6.0 G in normal and ischaemic rat brain as well as during gene therapy of a BT4C rat glioma. Our aim was to study the effects of plausible physico-chemical factors, such as chemical exchange and tissue water content, on $R_{1\rho}$ dispersion as well as to estimate the optimal B_1 range for detection of these pathological conditions.

Methods

For ischaemia experiments male Wistar rats (n=3, weighing 250-350 g) were anesthetized with 1.0 % halothane in N_2O/O_2 (75%:25%) and a femoral vein was cannulated for injections. After acquisition of control $R_{1\rho}$ dispersion curve, animals were sacrificed with 0.5-1 ml injection of saturated KCl and acquisition of $R_{1\rho}$ dispersion data was started immediatedly after cardiac arrest. For brain tumor MRI experiments, BT4C gliomas transfected with HSV-*tk* were induced by implanting 10^4 HSV-*tk* positive cells into the corpus callosum of female BDIX rats (n=8) as described previously [3,4]. Rats in the treatment group (n=3) were injected with GCV (25 mg/kg, i.p., twice daily) for the duration of the study (8 days). Untreated tumor-bearing animals (n=5) served as controls. MR data were collected on days 0,2,4,6 and 8 of GCV treatment. Core temperature was monitored on-line in the magnet. The experiments were performed in a 4.7 T magnet (Magnex) interfaced to a Varian ^{UNITY}INOVA console, using a quadrature surface coil (diameter 16 mm). A LASER localised [5] spectroscopic voxel (approximately 3x3x3 mm^3) was placed either to fronto-parietal cortex (ischaemia) or inside the tumor avoiding contamination from surrounding tissue. $R_{1\rho}$ relaxation times were measured with a spin-lock pulse consisting of 4 ms adiabatic half passage and variable-amplitude (B_1 0.01-6.0 G, 250-160000 rad/s) cw pulse and five spin-lock pulse lenghts between 10 and 90 ms (TR 4 s, TE 56 ms) in front of localisation part. Water peaks were analysed using AMARES [6] and $R_{1\rho}$ were fitted using monoexponentials. $R_{1\rho}$ dispersion was characterised using a model decribed by Korb&Bryant [7]. The model describes R_1 relaxation in a system of bulk water and a single, well-characterized protein. In our system, the solid pool is more complex and most of the fitted parameters are strongly correlated and cannot be interpreted directly. However, the "dispersion shape parameter" b, arising primarily from the rigidness and degree of hydration of the protein, is independent of other four parameters in the fit and therefore usable marker also in our system. In addition, relative changes between relaxation times in control and ischaemic brain, and untreated and treated tumours were calculated for each B_1 field. All values are shown as mean±SD.

Results

In normal cortical tissue, an inherent dispersion was evident with $R_{1\rho}$ approaching ~16 1/s at lowest B_1 fields and reaching value ~6 1/s at B_1 of 6.0 G (Fig A). Following induction of global ischaemia, $R_{1\rho}$ of low-B_1 region apparently increased (by ~1%), whereas the high-B_1 region (>0.2 G) $R_{1\rho}$ markedly decreased (Fig B), with maximal change of 9% observed close to 2.0 G. Minimal contrast between control and ischaemic conditions was observed around B_1 of 0.2 G. Dispersion parameters b from dispersion model were 0.49±0.01 and 0.55±0.01 ($p<0.05$) for control and ischaemic tissue, respectively.

Dispersion in non-treated gliomas was similar to control tissue with b value of 0.47±0.02. In contrast to ischemia, during GCV treatment the $R_{1\rho}$ dispersion curve shifted towards lower $R_{1\rho}$ values and also its shape changed (Fig C,D). This was reflected by a decrease in b (Fig E). Interestingly, the maximal relative change in $R_{1\rho}$ (Fig D) was reached already at 0.2 G and remained practically constant at B_1 fields above that It should be noted, that analysis revealed differences in treatment effectiveness between animals, so that the starting day for the decrease in dispersion varied from day 4 to day 8.

Discussion

The present study shows markedly differing $R_{1\rho}$ dispersion in acute ischaemia and tumour eradication by gene therapy. The data suggest that in ischaemia $R_{1\rho}$, dispersion with B_1 range of 0.2 to 2.0 G will show most significant change so that an optimal single-B_1 is close to 2.0 G, a finding that is consistent with a previous study [1]. In contrast, in gene therapy treated tumors the measurement of $R_{1\rho}$ dispersion with B_1 >0.2 G may not offer additional gain as compared to single-B_1 experiment. Unlike ischaemia, gene therapy alters $R_{1\rho}$ across the entire B_1 range so that low-B_1 $R_{1\rho}$ MRI can be used to detect treatment response with lower SAR values, albeit with slightly lower sensitivity.

As far as physico-chemical mechanisms affecting $T_{1\rho}$ are concerned, it is likely that in tumors accumulation of water as well as biochemical alterations in macromolecular pool lead to overall decrease in relaxation rates [3]. In contrast, in early moments of global ischaemia only minor changes in macromolecule pool occur. Furthermore, it has been previously demonstrated that $T_{1\rho}$ is not directly affected by anoxic depolarisation suggesting that shift of water into cells is not an important factor in the development of $T_{1\rho}$ contrast in ischaemia [2]. It is therefore possible that other B_1-sensitive factors, such as alterations in chemical exchange play a significant role in ischaemia [8]. Furthermore, loss of spin-lock condition at low B_1 fields due to local fields gradients can partially explain the observed increase in $R_{1\rho}$ at low B_1 strenghts [9].

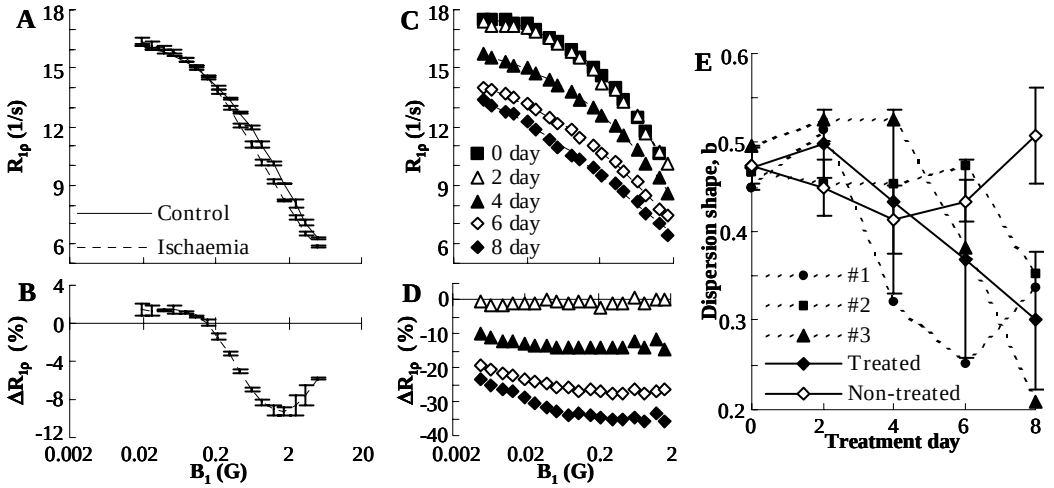

Fig. $R_{1\rho}$ in ischaemia (A,B) and during gene therapy in animal #1 (C,D). Parameter b of the dispersion fit [7] during the therapy. Data for treated animals are shown (dotted lines) as well as group averages (solid lines).

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