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Infiltrative glioma discrimination by FFC-NMR and quadrupolar peaks ^{14}N-^{1}H origin: a study of three glioma animal models

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Introduction

This study is focused on glioma, an aggressive cerebral tumor. Using Fast-Field-Cycling NMR (FFC-NMR) at low regime, we observed from human glioma resections, different T_1-dispersion behaviors, reflecting the well-known glioma heterogeneity. One clinical case has caught our attention. Indeed, two resections from the same patient exhibit distinct T_1-dispersion curves: one from the core of the glioma (confirmed as solid tumor) and the other from peritumoral region (confirmed with cell invasion). Thereby, using relevant animal models of glioma invasion, we aim to assess the role of FFC-NMR in detecting glioma invasion. We also aim to determine the origin of the QP peak signals: are the proteins involved in their formation intracellular or extracellular?

Methods

Animals: 3 human glioma mouse models were used: U87, a solid standard model (used as reference) and Glio6 and Glio96, both developed in our laboratory[1] and characterized as migration/invasion models models[2]. These models are obtained on immunodeficiency nude mice, by injecting corresponding glioma cells into the right caudate nucleus. At the late stage of the tumor growth, (under MRI control), brains were removed and glioma tissues were extracted (30-210mg weight) and stored at -80°C. Hematoxylin/Eosin (HE) histology was used to control the nature of glioma. All procedures including the intracerebral injection of glioma cells were approved according to the French and the European Guidelines for the Protection of Vertebrate Animals (decree 87–848 of 19 October 1987, licenses C3818510003 from the French Ministry of Agriculture).

Cells: After cell growths in their appropriate cultures, cell pellets were collected at 37°C by centrifugations (5min, 1200rpm). U87 cells were grown in DMEM supplemented with glutamax, 10% fetal bovine serum and penicillin-streptomycin (100 U/ml) in 5%CO_2, while Glio96 and Glio96 cells were grown in untreated flask with DMEM(50%)-F12(50%) supplemented with bFGF and EBF growth factors (20ng/ml) in 3%O_2 and 5%CO_2. Cell viability was assessed using trypan blue exclusion method before and after FFC-NMR.

Acquisitions: FFC-NMR was performed at 37°C with Stelar SpinMaster FFC-2000 relaxometer. Tissue samples (weighting 36-75mg) have been put in 5mm NMR tubes filled with Fomblin a fluor oil, while cell pellets were put directly in the tube. T_1-dispersion curves were acquired in a range [0.12mT-0.7T], using 12 evolution times for each magnetic field value (n=30). QP peaks were acquired with a high sampling (n=30) in [0.035-0.082T]. The total of the both acquisitions lasted 90min.

Modeling and analysis: T_1-dispersion curves were analyzed using power model[3]: $R_1=A.(\nu_0)^{\beta}$ in 2 regimes at low (L) and high (H) magnetic fields, and Fries model[4] for (QPs) signals. Parameters were

selected using the non-parametric Kruskall-Wallis test at $p<.05$ level. This test is derived from the parametric Wilcoxon rank sum, and it is appropriate when using more than two populations.
All analyses were achieved using FitLike[5] software developed under MATLAB©.

Results

T_1-dispersion curves of U87 compared to Glio6 and Glio96 show distinct behaviors. QPs were present and accurately fitted to the Fries model. Three power model parameters: A (offset amplitude) and β_L and β_H (components at low and high regimes) and the QPs amplitude (A_{QP}) were shown to discriminate infiltrative glioma region from solid one.
Comparison of QP peaks from cell pellets (only intracellular compartment) to their corresponding glioma tissues (both compartments extra and intracellular) have similar parameters, hence demonstrating that QP signals predominantly correspond to intracellular proteins. This result was confirmed by a second experiment, which consists of acquiring FFCNMR of U87 glioma cell pellets with and without added trypsin, a serine protease that hydrolyzes large proteins and does not cross the cellular membrane.

Conclusion / Discussion

Peritumoral regions invaded by infiltrative glioma cells is not diagnosed by MRI or by any current medical imaging. Its detection should guide neurosurgeons to remove it as largely as possible, in order to avoid glioma recurrence. Also this is a functional region which could be targeted by appropriate therapies.
In conclusion, our results demonstrate the high potential of low magnetic fields accessible by FFC-NMR to detect infiltrative glioma tissues. Three potential glioma biomarkers were identified: the offset, the slopes at low fields and the QPs amplitude which was demonstrated an intracellular FFC biomarker. Our preclinical results are in line with the clinical reported case and should support the high interest of FFC-MRI in neurooncology.

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