A feasibility study of diffusion MRI for early detection of xenograft models in mice.

Paola Porcari[1,2], Monika E Hegi[3], Hongxia Lei[1], Marie-France Hamou[3], Irene Vassallo[3], Silvia Capuani[4,5], Rolf Gruetter[1,6], and Vladimir Mlynarik[1,7]

[1]Center for Biomedical Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [2]Newcastle Magnetic Resonance Centre, Newcastle University, Newcastle upon Tyne, United Kingdom, [3]Clinical Neurosciences, Laboratory of Brain Tumor Biology and Genetics, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland, [4]CNR-IPCF UOS Roma Sapienza, Physics Department, Sapienza University of Rome, Rome, Italy, [5]Center for Life Nanoscience@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy, [6]Departments of Radiology, Universities of Lausanne and Geneva, Switzerland, [7]High Field MR Center, Medical University of Vienna, Vienna, Austria

TARGET AUDIENCE

Neuroradiologists and biologists studying development of brain tumors.

INTRODUCTION

We investigated whether diffusion MRI might be useful for early detection of slow growing and diffuse infiltrative tumours, such as the investigated LN-2669GS and LN-2450GS glioma sphere xenografts, which are otherwise not visible in standard T1 and T2-weighted MRI images[1]. Diffusion MRI protocols, either diffusion weighted imaging (DWI) or diffusion tensor imaging (DTI), were optimized for contrast by exploring long diffusion times (Δ) suitable for probing the microstructural alterations induced in mouse brain by the slow infiltration of glioma sphere cells.
In addition, proton MR spectra of lesions and immunohistochemical assessment were correlated with imaging results.

MATERIALS AND METHODS

LN-2669GS and LN-2540GS human glioma-derived sphere lines[3,4] were cultured under stem cells conditions and used for orthotopic implantation (10^5 cells) into nude mice (n=4 per sphere line). MRI measurements were performed using a 14.1T/26cm scanner (Varian/Magnex Scientific) equipped with a two-loop quadrature surface coil used as a radio-frequency transceiver. The MRI protocol included T2-weighted turbo-spin-echo images, DWI and DTI, both acquired using the pulse gradient stimulated echo (PGSTE) sequence (TR/TE=3920/22ms, Δ/δ=80/4ms, in-plane-resolution=156x156μm^2). Different Δ values ranging from 20 to 200ms were explored at fixed as well as at variable gradient amplitude ranging from 0 to 18G/cm for optimizing glioma detection. DWI acquisition was performed using eight b-values ranging from 294 to 2700s/mm^2 with the diffusion gradient applied along the readout direction. DTI required diffusion-weighted images acquired at the same b-value=1352s/mm^2 but diffusion gradient applied along six different spatial orientations. DTI was reconstructed using FSL DTIFIT routine. Mean values of diffusion indices were calculated in tumours and in the corresponding brain regions of controls (n=4) using a custom MATLAB script. Statistical analysis was performed using two-tailed, unpaired Student's *t*-test. Proton spectra of gliomas were acquired during the last MRI session using the SPECIAL sequence[4] with a VOI located within tumours. Finally, mice were sacrificed and sections stained underwent immunohistochemical assessment.

RESULTS

Tumours were properly identified by diffusion-weighted images (Fig 1) and diffusion maps (Fig 1, 2) acquired using Δ=80ms, whereas the lesions were not visible in the corresponding T2-weighted images (Fig 1, 2). The first evidence of tumour presence was revealed for both xenografts three months after tumour implantation, while no necrosis, oedema or haemorrhage was shown by MRI monitoring, and confirmed by histology. Proton spectra of lesions showed metabolite changes (Fig 3) similar to those previously reported[2] for slowly growing tumours, thus confirming tumour presence in combination with immunohistochemical findings. Mean values of diffusion indices, i.e., mean diffusivity (MD) and fractional anisotropy (FA), calculated in LN-2669GS and LN-2540GS glioma sphere xenografts, and in the corresponding brain regions of controls were $(0.71\pm0.04)\cdot10^{-3}$mm^2/s and 0.16±0.02, $(0.66\pm0.05)\cdot10^{-3}$mm^2/s and 0.20 ± 0.03, and $(0.57\pm0.03)\cdot10^{-3}$mm^2/s and 0.19±0.02, respectively. Compared to the controls, a significant increase in MD was observed in tumours grown as LN-2669GS xenografts but not in the tumours grown from LN-2540GS cells. FA significantly decreased in tumours grown as LN-2669GS xenograft and remained constant in those grown from LN-2540GS cells. These values reflect microstructural differences between LN-2669GS and LN-2540GS xenografts, confirmed by histology.

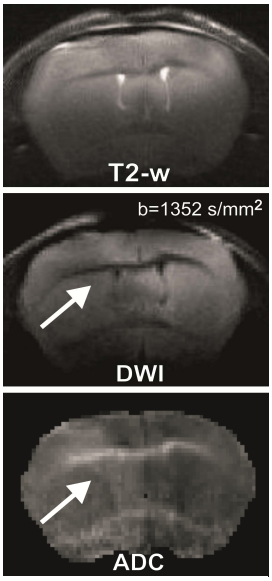

Fig. 1 T2-w and DWI images, and ADC map from LN-2669GS glioma sphere xenograf. Lesion indicated by arrow.

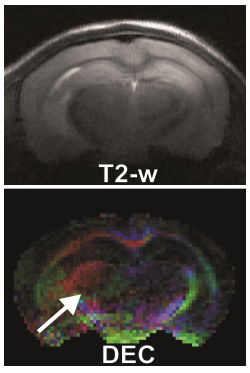

Fig.2 T2-w image and FA modulated directionally-encoded color (DEC) map from LN-2540GS glioma sphere xenograft. Lesion indicated by arrow.

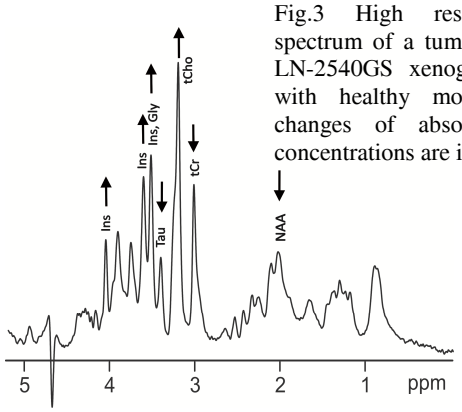

Fig.3 High resolution proton spectrum of a tumour grown from LN-2540GS xenograft. Compared with healthy mouse, the main changes of absolute metabolite concentrations are indicated.

DISCUSSION

Compared with T2-weighted MRI, tumours could be properly identified and investigated by using a long diffusion time. Moreover, different values of diffusion indices between LN-2669GS and LN-2540GS xenografts highlighted diverse tumour microstructure for each xenograft, reflected by histology. In conclusion, this study demonstrates the high sensitivity and specificity of the diffusion MRI techniques for early detection of glioma sphere xenografts, thus providing a potential imaging biomarker for early detection of slowly growing tumours in humans.

ACKNOWLEDGMENTS

This study was supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL and the Leenaards and Jeantet Foundations.

REFERENCES

1. Mlynárik V, Cudalbu C, Clement V, et al. NMR Biomed. 2012;25(4):506-13.
2. Sciuscio D, Diserens AC, van Dommelen K, et al. Clin Cancer Res. 2011;17(2):255-66.
3. Bady P, Diserens AC, Castella V, et al. Neuro Oncol. 2012;14(6):701-11. 4. Mlynarik V, Gambarota G, Frenkel H, et al. Magn Reson Med. 2006;59(6):965–97