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Evaluation of Early Response to Temozolomide and Radiotherapy with Magnetic Resonance Imaging and Proton Magnetic Resonance Spectroscopy in Human Glioma Models in Nude Rats.

P. Walker[1], X. Tizon[2], S. Parfait[1], J. Miteran[1], G. Crehange[3], P. Maingon[3], N. Hoffman[2], P. Genne[2], O. Duchamp[2]

[1] LE2I, UMR CNRS 5158, Dijon, France; [2] Oncodesign Biotechnology, Dijon, France and [3]Anti-Cancer Centre Georges-François Leclerc, Dijon, France

INTRODUCTION - OBJECTIVES

Glioblastoma is the most aggressive subtype of brain tumors. Validation of more predictive biomarkers of treatment efficacy in experimental human glioblastoma models would greatly benefit from the establishment of additional quantitative endpoints. The aim of this study was to validate proton Magnetic Resonance Spectroscopy (^{1}H-MRS) and Diffusion-weighted MR Imaging (DW-MRI) to evaluate the anti-tumor activity of Temozolomide (TMZ) and radiotherapy (RT) in 2 human glioblastoma models.

METHODOLOGY

Animals and Gliomas:

CGL9 and U-87 MG glioma cells were inoculated by stereotactic injection in the right caudate nucleus of 2 groups of 22 nude rats. Tumor-bearing rats were ranked according to body weight and randomized 12 days (U-87 MG) or 19 days (CGL9) after inoculation. U-87 MG rats received either 5 administrations of 16.5 mg/kg TMZ per os daily or 5 daily fractions of 2Gy in the right cerebral hemisphere, or no treatment (CTL). CGL9 rats received either 5 administrations of 16.5 mg/kg TMZ per os daily or no treatment (CTL). D0 was defined as the day of treatment start. The imaging protocol was performed on 3 rats per group.

Procedures were performed according to ethical guidelines concerning animal care and handling.

MRI and MRS:

All imaging was performed on a Bruker Pharmascan 4.7T at D0, D1, D4, D7, D11 (U-87 MG) and D0, D1, D4, D7, D14 (CGL9). During the imaging protocol, the animals were maintained under anaesthesia via a constant flow of isoflurane at 2-3% delivered by a nose cone. The tumor volume was measured using T_2-weighted (U-87 MG) or T_1-weighted images, contrast-enhanced images (CGL9) using Magnevist®. DW-MRI and ^{1}H-MRS were performed at the same time-points.

(i) Tumor volume measurements using Magnetic Resonance Imaging (MRI)

The T_2-weighted sequence (TSE TR 4000 ms/TE 60 ms/ FOV 3.5 cm/Matrix 256²/0.8 mm slice width/ETL 8) consisted of 20 contiguous slices in the axial plane for a nominal acquisition time of 3min12s. For CGL9, the tumor volume was estimated on images acquired 5 minutes after Magnevist®. The T_1-weighted sequence (TSE TR 740 ms/TE 12 ms/ FOV 3.5 cm/Matrix 256²/0.8 mm slice width/ETL 2) consisted of 25 contiguous slices in the axial plane for a nominal acquisition time of 5min24s.

(ii) Diffusion-weighted MRI and ADC estimation

Diffusion-weighted imaging was performed using an EPI-SE sequence (TR 3000 ms/TE 30 ms/ FOV 3.8 cm/Matrix 192²/1.6 mm slice width). The chosen b values were 0, 100, 600 and 1100 s/mm^{-2}. Apparent diffusion coefficient (ADC) values were calculated using in-house developed plugins of ImageJ [1] as the mean ADC value within a region of interest manually drawn in one MRI slice.

(iii) Magnetic Resonance Spectroscopy (MRS)

Single Voxel Spectroscopy (SVS) was used to evaluate glioma and healthy contralateral brain tissue metabolism. A PRESS sequence with TE 11ms and TR 2.5s was used. Acquisitions with and without water-suppression were acquired. Two voxels were placed: one in the tumor, and the other in the contralateral healthy tissue. Voxel sizes were adapted to the dimensions of the tumor in order to avoid partial volume effects with normal cerebral tissue. Spectral data were analyzed using LCModel version 6.2 [2], and peak areas of total Choline and N-acetyl-aspartate (NAA) were measured.

Fig. 1 **U-87 MG**

(a) T2-weighted anatomical images showing localisation of gliomas along with tumor and contralateral voxel (b) ^{1}H MR spectrum from glioma. (c) Evolution of tumor volume with time in control, RT-treated and TMZ-treated rats. (d) Survival curves for control, TMZ-treated and RT-treated rats. ^{1}H MR spectra from glioma after (e) TMZ treatment and (f) RT treatment. The evolution of the ^{1}H metabolites, NAA over Choline ratios are depicted in figures (g:TMZ) and (h:RT), respectively. Data on the variation of ADC is given in (i) and an example of DW-MRI is shown in (j-k).

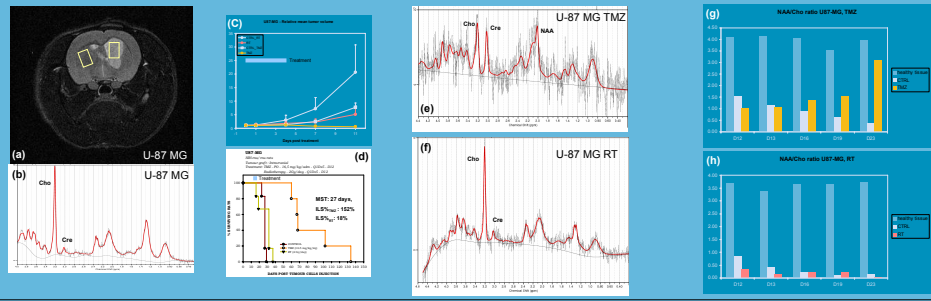
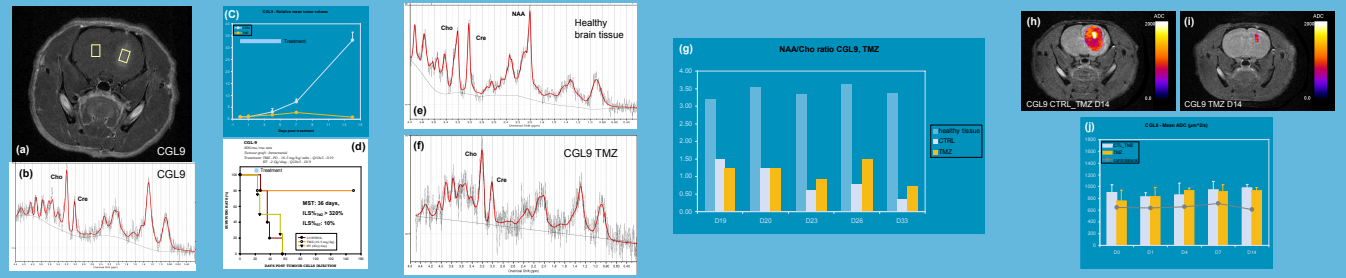


Fig. 2 **CGL9**

(a) post-contrast T1-weighted anatomical images showing localisation of gliomas along with tumor and contralateral voxel (b) ^{1}H MR spectrum from glioma. Figure (c) illustrates the evolution of tumor volume with time in control and TMZ-treated rats. (d) Survival curves for control, TMZ-treated and RT-treated rats. ^{1}H MR spectra from contralateral tissue (e) and glioma after TMZ treatment (f). The evolution of the ^{1}H metabolites, NAA over Choline ratios are depicted in figures (g). Data on the variation of ADC is given in (j) and an example of DW-MRI is shown in (h-i).



RESULTS

- TMZ increased the life span of both U-87 MG and CGL9 tumor-bearing rats (ILS = 152% and >320% for U-87 MG and CGL9 respectively). The TMZ-treated to CTL tumor volume ratios (T/C) were 8 and 2% for U-87 MG and CGL9 at the last imaging timepoint, respectively.
- There was no significant increase of lifespan for rats treated with RT (ILS = 18% and 10% for U-87 MG and CGL9 respectively). The RT-treated to CTL T/C was 58% for U-87 MG at the last imaging timepoint.
- ADC was increased by 34% in TMZ compared to CTL group for U-87 MG tumors at D11, whereas ADC was not modified by TMZ in CGL9 tumors. ADC also increased by 27% at D7 for the U-87 MG RT group compared to CTL group.
- There was no significant difference of the ratio of NAA over total Choline (NAA/tCho) between TMZ and CTL groups in the CGL9 model.
- In the U-87 MG model, NAA/tCho decreased from 1.53 to 0.35 in the CTL group, while it increased from 1.02 to 3.10 in the TMZ group. No difference of NAA/tCho was observed between CTL and RT-treated groups.

CONCLUSIONS

Using MRI, we observed a strong inhibition of tumor growth by TMZ treatment on both models, together with increased survival. ADC increased as a result of treatment with TMZ and RT on U-87 MG, but did not vary significantly in CGL9 tumors. By showing variations of the ratio of NAA over total Choline biomarker, we showed that monitoring tumor metabolism using ^{1}H-MRS is well suited to follow the growth of U-87 MG tumors and allows quantification of the antitumor effect of TMZ.

[1] Abramoff, Biophotonics Intl 11, 7 (2004). [2] Provencher, Magn Res Med 30, 672 (1993).