Evaluation of the BCNU chemosensitivity of a panel of glioma models in relation with vessel size index, blood volume and diffusion MR imaging parameters

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Introduction

The response of gliomas to chemotherapy varies widely. Although some genetic alterations have been identified (1), there is no clear understanding of the mechanisms underlying the chemosensitivity of these tumors. In previous *in-vitro* and *in-vivo* studies (2), we showed that the sensitivity to BCNU differed between glioma models. The sensitivity to BCNU may depend on the intrinsic sensitivity of the cells and on functional properties of the tumor microvasculature. The vessel size index (VSI) and blood volume (BV) magnetic resonance imaging technique provides non-invasive quantitative insight on the microvascular architecture. We applied here VSI, BV and Diffusion imaging to characterize the vascular and tumor tissue status of a panel of six different glioma models in the rat brain.

Materials and Methods

- GV1A1, C6 and 9L and CGL3, CGL9 and U87-MG glioma cells were inoculated by stereotactic injection in 12-20 rats per glioma model (BDIX, Wistar and Fisher rats for the murine gliomas and Nude rats for the human gliomas).
- For each model, multislice T2-weighted MRI (2.35T) was performed on 10 rats to determine the tumor volume and compose groups of 5 or 6 rats bearing gliomas of identical volumes (50-75 mm3).
- The MGESE imaging sequence (3) was applied and repeated 4 minutes after injection of the intravascular contrast agent Sinerem®(Guerbet, France, 200µmol Fe/kg) in the tail vein. VSI maps were derived from water apparent diffusion coefficient (ADC), ΔR2 and ΔR2* maps while BV maps are proportional to ΔR2* maps only.
- ADC maps were obtained using a Stejskal-Tanner MRI sequence.
- Regions of interest (ROIs) were drawn manually over the tumor (3 slices at the center) and the contralateral area in the same slices. VSI and BV histogram analyses were performed.
- The animals were sacrificed after the MRI examination, 1 minute after injection of a Hoechst saline solution for histological analysis by fluorescence microscopy of the functional microvasculature.
- 6 -10 remaining rats per glioma model were randomized on the same day the VSI imaging was performed and kept for survival follow-up: 3-5 of them received 2 IV bolus injections of 10 mg/kg BCNU at 2 weeks interval. The mean and median survival time (FigB) and increased life span (ILS) (FigC) were calculated .

Conclusions

- *In-vitro* and *in-vivo* tests revealed different sensitivities to BCNU of the different glioma cell lines. Previous measurements were validated (2).
- The CGL9 glioma was the most sensitive to BCNU treatment *in-vivo* whereas U87-MG was the least sensitive.
- ADC values in tumors were significantly higher than in contralateral areas
- The ADC of the CGL9 glioma was significantly smaller than the ADCs of all the other gliomas and comparable to the ADC of its contralateral area
- Previous VSI and BV measurements were confirmed in the present study showing heterogeneity within gliomas (Fig E) using pixel by pixel mapping. Higher VSI values and lower BV values were found in gliomas compared to their controlateral area.
- The ADC could be an appropriate parameter to predict the tumor response to BCNU treatment. VSI and BV values confirm their ability to distinguish differences between tumor and contralateral tissue and seem to indicate variable blood volume distributions and vascular arrangements between glioma models.

A. *In vitro* cytotoxicity of a 96h BCNU treatment by MTT assay

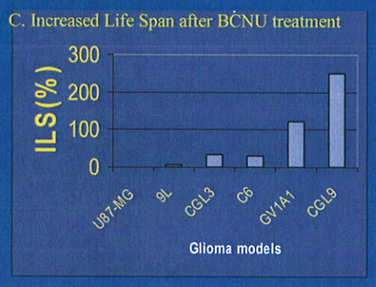
Cell lines	Tumor origin	IC50 (µM)
9L	Rat	31.2 ± 18.4
CGL-1	Human	35.7 ± 13.2
GV1-A1	Rat	59.7 ± 14.2
CGL-3	Human	60.5 ± 13.9
C6	Rat	112.2 ± 72.7
U87-MG	Human	138.5 ± 74.2
CGL-9	Human	173.2 ± 65.5

In vitro, cell lines show different sensitivities to BCNU. The CGL9 cell line is the least sensitive to BCNU treatment

B. *In vivo* results on rats bearing different gliomas.

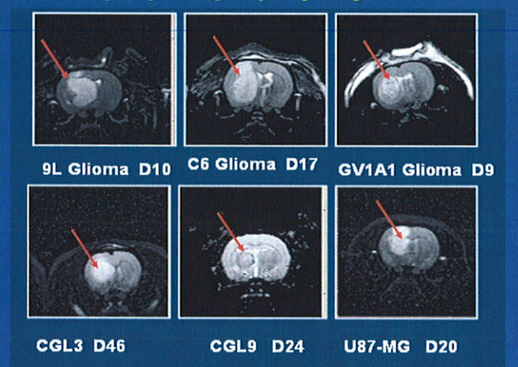
Tumors	Groups	Nb of Rats	Mean Tumor Volume at Treatment Start (mm3)	BCNU Treatment start	Median Survival (days)
GV1A1	CTL (Surv)	3	48 ± 5		13
	BCNU	4		D9	29
9L	CTL (Surv)	5	72± 15		15
	BCNU	5		D11	16
C6	CTL (Surv)	5	62± 20		26
	BCNU	5		D17	34
CGL3	CTL (Surv)	3	75± 10		67
	BCNU	3		D49	89
CGL9	CTL (Surv)	5	52 ± 20		34
	BCNU	5		D23	>120
U87-MG	CTL (Surv)	4	52 ± 20		26
	BCNU	4		D19	26

C. Increased Life Span after BCNU treatment

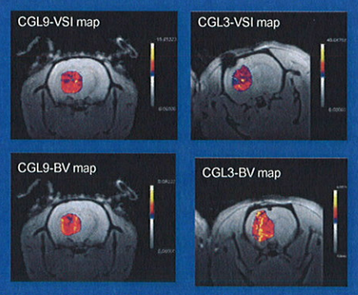


Rats bearing CGL9 showed complete remission upon BCNU treatment

D. Morphological images depicting each glioma model



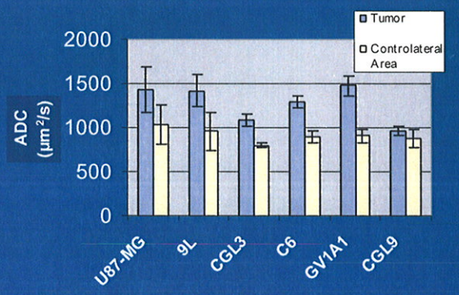
E. Examples of VSI and BV pixel-by pixel maps on CGL9 and CGL3 tumors



G. Collagen IV and Hoechst staining at the center of the CGL9 and CGL3 gliomas (delineated areas of figure E).

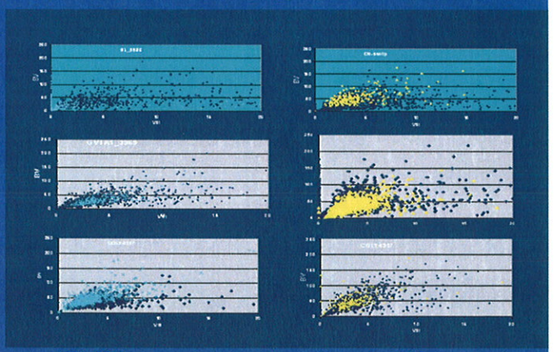
Different histological patterns can be observed on comparing gliomas

F. ADC values of tumor and healthy contralateral part of the brain



•ADC values are significantly higher in the tumors than in their contralateral areas except for the CGL9 gliomas
•The CGL9 glioma demonstrates significantly lower ADC values than all the other glioma models

H. BV-VSI Correlation for a glioma of each model.



This 2D histogrammic representation could reveal useful features upon treatment. Light colors (yellow and light blue) represent the BV versus VSI for the contralateral area of the glioma.

References

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3.Troprès et al, MRM 45, 397,2001