Avastin alone or combined to Campto® reduces local Blood Oxygen Saturation in an orthotopic human glioblastoma model (U87-MG) in nude rats

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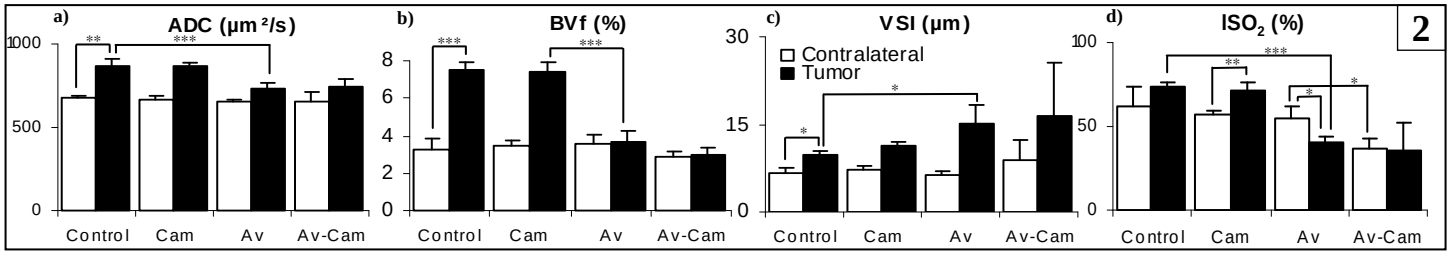
Introduction: Despite aggressive surgery, radiotherapy and chemotherapy, malignant gliomas remain fatal. As these tumors are highly angiogenic, therapies directed against tumor vasculature or preventing angiogenesis have been developed. Monitoring changes in structural and functional microvasculature should help to evaluate the efficiency of these therapies. Previous results suggest that MRI follow-up of early modifications of microvascular parameters (blood volume fraction (BVf), vessel size index (VSI) and blood brain barrier permeability to a contrast agent (BBBperm.)) permits monitoring the effects of anti-angiogenic treatment on gliomas (1). Recently, it has been shown that local Blood Oxygen Saturation (lSO_2), a possible indication of tumor oxygenation status, could be measured by MRI (2). Antioangenic therapies should alter the tumor oxygenation status. In this study, structural (BVf and VSI) and functional (BBBperm. and lSO_2) microvascular parameters were thus measured to further characterize the effect of an anti-angiogenic therapy (antibodies against Vascular Endothelial Growth Factor, Avastin®, Roche) used alone or combined to a chemotherapy (topo-isomerase 1 inhibitor, Campto®, Pfizer) on a orthotopic human glioma model (U87-MG) xenografted in nude rats.

Material and methods: Sixteen nude rats were orthotopically injected at day 0 (D0) with 10^5 U87-MG glioma cells. At D16, T_2-weighted images were acquired to measure tumor size. Rats were randomized at D17 in 4 groups (n=4/group) with similar tumor volume (7±4mm3, data not shown). Treatment started at D18. The first group (Control group) received no treatment. The second group (Av group) received 5 injections of 5 mg/kg of Avastin i.v. every 4 days (D18, D22, D26 and D30). The third group (Cam group) received 3 injections of 40mg/kg of Campto every 7 days (D18, D25 and D32). The fourth group (Av-Cam group) received both treatments (same schedule as for independent treatment). In the Av-Cam group, one rat died before the MRI session. BVf, VSI, apparent diffusion coefficient (ADC), BBB perm. to a P846 (Gd-based contrast agent, 3.5kDa, obtained from Dr P. Robert, Guerbet, France) and lSO_2 imaging were performed, at 4.7T (Bruker Avance 3 system), at D34 (after the end of both treatments). Tumor volume was computed from T_2-weighted images (T_{2w}). ADC, BVf and VSI were mapped using diffusion-weighted and multiple gradient-echo/spin-echo MR sequences applied before and after intravenous injection of ferumoxtran-10 (Sinerem®/Combidex®, 200µmol Fe/kg, obtained from Dr P. Robert, Guerbet/AMAG Pharmaceuticals) (2). BBB perm. was assessed based on T_1-weighted images acquired before and 5-min. after injection of P846 (50µmol Gd/kg). lSO_2 was mapped using a modified vesion of the method proposed by He and Yablonskiy (3). Voxel size was 234x234x1000µm^3, except for lSO_2 maps (468x468x1000µm^3). Data, averaged across rats, are presented for 2 regions of interest (whole tumor and contralateral striatum (Contra)) and each group. Student t-tests (after assessment of variance homogeneity) were used to assess differences (*:p<0.05, **:p<0.01, ***:p<0.001).

Results: Tumor growth in Av and Av-Cam groups were significantly inhibited compared to the Control group (Table1). In the contralateral striatum, there were no significant differences in ADC, VSI and BVf between groups (Table1). Contralateral lSO_2 in Control, Av and Cam groups was similar but was significantly reduced in Av-Cam group (Table1). Tumoral ADC, BVf, VSI and lSO_2 did not differ between Control and Cam group and were higher than in contralateral striatum (Table1). Tumoral ADC, BVf and lSO_2 in Av and Av-Cam groups were significantly lower than in control group while tumoral VSI was higher than in the Control group (Table1). In all groups, BBB remained permeable to P846, although in Av-Cam group vessel appeared less permeable to P846 (data not shown).



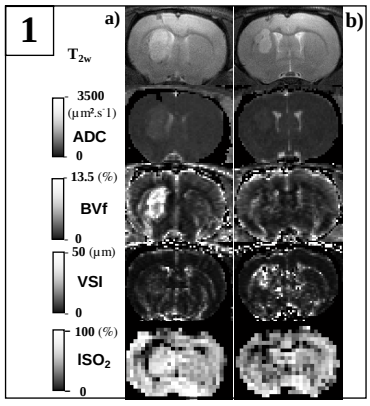


Table 1 (mean ±SD)	Control (n=4)		Cam (n=4)		Av (n=4)		Av-Cam (n=3)	
	Contra	Tumor	Contra	Tumor	Contra	Tumor	Contra	Tumor
Volume (mm^3)	-	264±249	-	119±73	-	78±58	-	38±10
ADC (µm²/s)	682±7	870±40	665±22	862±27	654±10	739±24	652±60	748±43
BVf (%)	3.3±0.6	7.5±0.5	3.4±0.3	7.4±0.5	3.5±0.5	3.6±0.6	2.8±0.4	2.9±0.4
VSI (µm)	6.5±1.2	9.6±0.7	7.3±0.4	11.3±0.8	6.3±0.5	15.0±3.4	9.0±3.2	16.4±9.1
$lS0_2$ (%)	62.2±11.1	74.1±2.5	57.4±2.3	71.8±4.6	55.3±6.7	40.1±3.4	36.7±5.6	35.2±17.3

Figure 1: Representative T_{2w} image and maps of ADC, VSI, BV and lSO_2 in Control (**a**) and Av-Cam group (**b**).
Figure 2: ADC **(a),** BVf (**b**), VSI (**c**) and lSO_2 (**d**) measurements in a U87-MG tumor in Control group or in groups which received either Avastin alone (Av group), Campto alone (Cam group) or a combination of Avastin and Camtpo (Av-Cam group). Mean±SD. *: p<0.05, **: p<0.01, ***: p<0.001
Table 1: Data, averaged across rats (mean±SD), are presented for 2 regions of interest (whole tumor and contralateral striatum) (Contra)) and for each group.

Conclusions: Campto alone (chemotherapy) has no significant effect on tumor growth, ADC, BVf, VSI and lSO_2 as compared to Control group. Both Cam and Control groups exhibit larger tumoral BVf and lSO_2 than contralaterally. Avastin, used either alone or in combination with Campto, has a significant effect on tumor growth and on tumor microvasculature. In both Av and Av-Cam groups, tumoral and contralateral BVf values are similar while tumoral VSI are higher than contralaterally, suggesting a lower vessel density in the tumor. In the Av group, this lower vessel density is associated with a lower lSO_2. In the Av-Cam group, contralateral lSO_2 is also reduced (compared to control values). This reduction could be explained by a systemic toxic effect of combined treatments. Indeed the tumor volume was strongly reduced under Av-Cam treatment but the survival was comparable to that of control animals (data not shown). Our results suggest that MRI follow-up of modifications of microvascular parameters (BV, VSI, BBB perm. and lSO_2) permits monitoring the effects of anti-angiogenic treatment alone or combined with chemotherapy on gliomas. lSO_2 seems to be a sensitive reporter of antiangiogenic therapeutic effect and to provide independent information from BVf, VSI and BBB. Perm. However, lSO_2 real physiological meaning remains to be determined.

References:

[1] ISMRM (2008), Poster #2200. [2] Magn Reson.Med. (2001) 45:397-408. [3] Mag. Reson. in Med. (2007) 57:115–126