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

B. Lemasson1,2, T. Christen1,3, N. Pannetier1,3, R. Farion1,3, C. Segebarth1,3, X. Tizon1, P. Provent1, P. Genne4, E. L. Barbier1,3, O. Duchamp5, and C. Rémy1,3
1Inserm, U836, Grenoble, F-38043, France. 2Onco design Biotechnology, Dijon, France. 3Université Joseph Fourier, Grenoble Institut des Neurosciences, UMR-S836, Grenoble, F-38043, France

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 (ISO2), a possible indication of tumor oxygenation status, could be measured by MRI (2). Antiangiogenic therapies should alter the tumor oxygenation status. In this study, structural (BVf and VSI) and functional (BBBperm. and ISO2) 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 I 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 10^6 U87-MG glioma cells. At D16, T1-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 ISO2 imaging were performed, at 4.7T (Bruker Avance 3 system), at D34 (after the end of both treatments). Tumor volume was computed from T1-weighted images (T1v). 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®/Combid, 50μmol Fe/kg, obtained from Dr P. Robert, Guerbet/AMAG Pharmaceuticals) (2). BBB perm. was assessed based on T1-weighted images acquired before and 5-min. after injection of P846 (50μmol GdDTPa). ISO2 was mapped using a modified version of the method proposed by He and Yablonskiy (3). Voxel size was 234x234x1000μm³, except for ISO2 maps (468x468x1000μm³). 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). Contra lSO2 remained permeable to P846, although in Av-Cam group vessel appeared less permeable to P846 (data not shown).

Conclusions: Campto alone (chemotherapy) has no significant effect on tumor growth, ADC, BVf, VSI and ISO2 compared to Control group. Both Cam and Control groups exhibit larger tumoral BVf and ISO2 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 ISO2. In the Av-Cam group, contralateral ISO2 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 (BVf, VSI, BBB perm. and ISO2) permits monitoring the effects of anti-angiogenic treatment alone or combined with chemotherapy on gliomas. ISO2 seems to be a sensitive reporter of antiangiogenic therapeutic effect and to provide independent information from BVf, VSI and BBB. Perm. However, ISO2 real physiological meaning remains to be determined.

References: