Histological validation of the cerebral blood volume quantification in a C6 brain tumor model using RSST1-MRI with an intravascular contrast agent: Gd-ACX

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Introduction: Quantification of the tumor microvasculature is of a great interest to monitor the effects of anti-angiogenic drugs. In brain tumors, the cerebral blood volume (CBV) quantification by MRI remains complex due to possible leakage of contrast agents (CAs) through the blood brain barrier. The α-cyclodextrin (ACX) derivative (1) was shown to complex gadolinium, resulting in a novel CA for MRI (Gd-ACX) with twofold higher relaxivities than Gd-DOTA (2). Gd-ACX has been shown to remain confined to the vascular space in a C6 brain tumor model (2) and has been used in combination with the Rapid Steady State T_1 ($RSST_1$) technique for CBV fraction (CBV_f) mapping in microvasculature permeable for Gd-DOTA (3). In this study, we aim to validate the $RSST_1$-MRI approach using Gd-ACX for tumor CBV quantification with histological vascular morphometric analysis.

Method: At 2.35 T, $RSST_1$ imaging was performed on four male Wistar rats 21 days after intrastriatal injection of C6-tumor cells using an intravenous injection of 0.05 mmol/kg Gd-ACX, as well as, one hour later, of 0.1 mmol/kg Gd-DOTA. The CBV_f was mapped in a 2 mm thick coronal slice according to $CBV_f = (S_{post} - S_{pre})/S_0$, where S_{pre} and S_{post} are the mean signals acquired with a repetition time (TR) of 750 ms and an inversion time of 325 ms prior to and following CA injection, respectively. S_0 is the equilibrium signal from the vascular and extravascular compartments acquired with TR = 10 s. At the end of the MRI experiments, Hoechst 33342, a fluorescent marker having similar diffusion properties as Gd-DOTA, was injected intravenously (6 mg in normal saline) (4) and the brains were removed and frozen one minute after injection for immunofluorescent staining of the microvasculature (anti collagen IV). Twenty 10 µm thick coronal sections from the location of the MRI slice were analysed. Due to technical limitations, only the ipsilateral hemisphere was scanned with a microscope digital camera system. The perfused tumor microvasculature stained by the Hoechst dye was selected and the vascular length density L_V was determined by vascular morphometric analysis and a stereological method (5) using ImageJ software. The vascular volume density V_V, corresponding to the CBV_f, was computed according to:
$V_V = \pi d^2 L_V /4$, where d is the mean vascular diameter.

Results: In the contralateral hemisphere, the constant vascular signal after both CAs was averaged over at least one minute. The CBV_f of 1.02 ± 0.25% measured with Gd-ACX was confirmed with Gd-DOTA yielding 1.09 ± 0.33%.

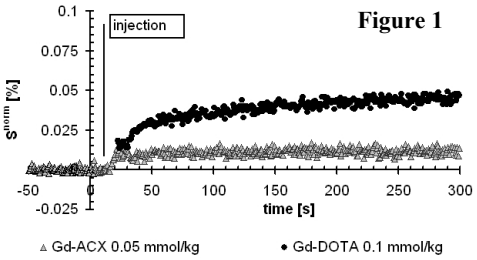

Figure 1

In tumor tissue, which covered 46 ± 11% of the imaged slice, only the constant signal amplitude following Gd-ACX injection allowed the determination of the CBV_f while the signal after Gd-DOTA exhibited a typical leakage profile (Figure 1). The vascular volume fraction obtained by MRI and histology from central and peripheral tumor ROIs are summarized in Table 1. The CBV_f measured by MRI was similar to the V_V of perfused vessels stained by Hoechst 33342. The ratio of perfused to total V_V was about 0.5 in this late stage glioma model.

Table 1	**CBV_f by MRI**	**V_v perfused vessels** Hoechst 33342	V_v (all vessels) Anti collagen IV
Tumor periphery	**1.40 ± 0.50 %**	**1.40 ± 0.16 %**	3,00 ± 0.67 %
Tumor center	**0.46 ± 0.29 %**	**0.49 ± 0.32 %**	1.08 ± 0.74 %

Discussion: In the hemisphere contralateral to the C6 tumor, the CBV_f quantification with the new CA Gd-ACX was validated with Gd-DOTA. The contralateral CBV_f of about 1% is lower than in healthy rat brain 2-3% (6), probably because of the mass effect. Therefore care should be taken when reporting relative vascular volume values. In tumor tissue, the CBV_f quantification can not be validated with Gd-DOTA because the tumor microvasculature is permeable to this CA. Immunofluorescent staining of the total vascular bed does not reflect the CBV_f obtained by MRI which is only sensitive to the perfused vasculature. However, after selecting the tumor vessels perfused by the Hoechst dye the results obtained by histological vascular morphometric analysis correlate very well with the tumor CBV_f obtained by MRI, confirming the low CBV_f measured by MRI in this C6 tumor model as well as the spatially heterogeneous tumor vascularity.

Conclusion: Contrary to Gd-DOTA**,** Gd-ACX can be used for tumor microvasculature quantification and is a potential CA for the preclinical evaluation of vascular targeted cancer therapies.

References: 1. Gadelle et al, Angew Chem Int Ed 1991; 2. Lahrech et al, J Cereb Blood Flow Metab 2008; 3. Perles-Barbacaru and Lahrech, J Cereb Blood Flow Metab 2007; 4. Rijken et al, Microvasc Res1995; 5. Adair et al, Am J Physiol 1994; 6. Perles-Barbacaru et al, ISMRM 2006