Galbumin, a new blood pool agent, extravasates from tumour vasculature

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Introduction

Tumour tissue is highly heterogeneous and characterized by a vastly disordered vasculature along with severe deregulation of normal cell processes. While immuno-histological investigation of tumour vasculature is precise, there is an urgent clinical need to develop accurate, non-invasive imaging techniques to improve the characterization of tumor angiogenesis and the monitoring of the response to anti-angiogenic therapy. To obtain live, vasculature information, a novel protocol was developed using a new contrast agent (Galbumin, BioPal) with fluorescent tags attached for subsequent histological analysis. We explore the possibility of using Galbumin, a blood pool agent in HT29 and HCT116 tumours to exploit blood vessel permeability differences in HCT-116 and HT29 tumour cell types. Contrasting properties of the two tumour models with respect to vessel permeability and propensity for inherent necrosis are obvious from histology as well as MRI.

Materials and Methods

Mice: 5 NOD/SCID mice with subcutaneous HCT-116 (3-mice human colorectal cancer) and HT29 (2 mice) xenografts received an initial scan (under anesthesia) for water-diffusion. The mice then received a baseline MRI scan prior to Galbumin injection and a second scan for 30 minutes starting during Galbumin (contrast agent) injection. Mice were then injected with Hoechst (histology perfusion marker) and tumours were excised 5 minutes after second scan.

MRI: Imaging was performed on a 7T Bruker Biospec 70/30 using a custom-built 4 turn distributed-capacitor solenoid. Diffusion-weighted EPI with TR/TE= 3000/26.9, b-values 0,500 was performed and apparent diffusion coefficient (ADC) maps calculated. 30 minutes of the T1-weighted FLASH (TR/TE=113/2.145) FLASH were acquired following a 150¬µl/mouse bolus of Galbumin (25 mg/mL). A two-TR FLASH protocol (TR=226 ms and 113 ms, TE= 2.145 ms) was used for calculating contrast agent concentration with time resolution of 14.5s.

Immunohistochemistry: An automated microscope with x-y-z slide stage was used to acquire entire tumour-section images. Hoechst (perfusion), CD31 (vasculature), Haematoxylin (nuclei) and Galbumin (contrast agent) were fluorescently imaged independently from tissue sections corresponding to MR scan slices. Galbumin and Hoechst distribution was mapped as a function of distance away from blood vessels in the two tumour types. Extravascular extracellular space, marked with Coll IV was measured and correlated with ADC from diffusion-weighted EPI.

Results and Discussion

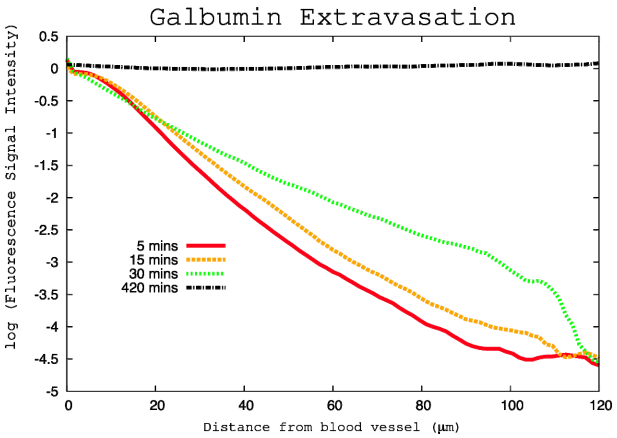

Figure 1. Plot of Galbumin extravasation as a function of distance from blood vessel in HCT-116 tumour xenografts. Red line shows Galbumin intensity for mice injected with Galbumin 5 minutes before sacrifice and the black line for mice injected 420 minutes before sacrifice. Galbumin vessel half-life is in between.

Our histological data analysis suggests that in HCT-116 tumour xenografts, Galbumin tends to extravasate out of blood vessels at a very low rate. Fig.1 shows galbumin as a function of distance from blood vessel from fluorescent microscopy. Initially, the majority of Galbumin is found in tumour cells less than 40 µm away from the nearest blood vessel. Subsequently, galbumin concentration in the tumour tissue spreads out and equilibrates at ca 7h.

The data from dynamic contrast enhanced MRI supports this observation (Fig.2) Fig.2A and Fig2B are T1-weighted images aquired pre- and 20min post- Galbumin injection. From the image clear enhancement of vessels but also a distinct sharpening of tumour tissue heterogeneity becomes obvious. Fig.2C shows the gradient of signal enhancement in a DCEMRI study. Peripheral tumour areas normally well perfused show a pronounced positive enhancement gradient. This slow enhancement is not compatible with an assumption of a blood pool agent. Rather the agent must slowly extravasate and give rise to T1-weighted signal enhancement. Little progress has been done in attempting to extract flow and permeability simultaneously but we use pharmacokinetic models for this purpose.

Conclusions

Galbumin is an effective contrast agent to investigate vessel architecture and vessel permeability. In tumour tissue we have shown that galbumin does not stay in the vasculature as it would in normal tissue. Rather, galbumin leaks out as shown on histology as well as MRI. As such it is a useful tool to study permeability of vessels. It has been shown that the HT29 tumour types have a more organized vascular network relative to HCT-116 and are thus, less "leaky".

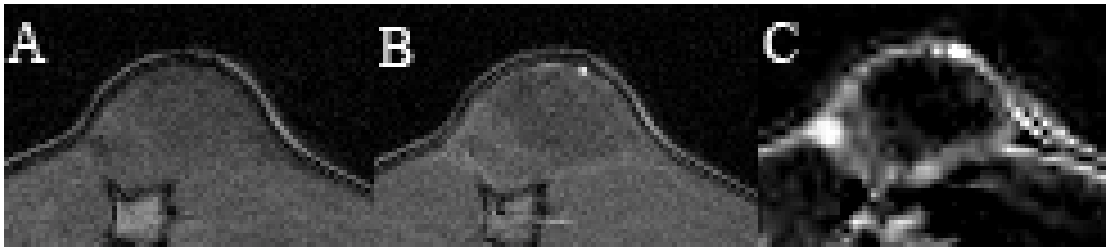

Figure 2. **A** & **B** T1-weighted gradient echo before (A) and after (B) galbumin administration. **C** shows signal enhancement parameter map from DCEMRI acquired during Galbumin administration. Note areas of peripheral enhancement.