Extravasation of a New High Molecular Weight Contrast Agent in Tumour Vasculature, Probed by MRI & Histology

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INTRODUCTION: Tumour tissue is highly heterogeneous with disordered vasculature that is characteristically highly permeable relative to other normal tissue blood vessels. Non-invasive techniques for monitoring vasculature are required, both to assess the tumour and to monitor response to anti-angiogenic treatment, which can be achieved using Dynamic Contrast Enhanced (DCE) MRI. Pharmacokinetic modeling of contrast agent uptake can provide information about blood flow and vessel permeability but many models do not have the ability to separate the two, due to the ability of typical contrast agents such as Gd-DTPA to extravasate and accumulate in tumour tissue. A truly intra-vascular contrast agent could provide a means to measure blood flow in tumours. Measuring both high and low molecular weight contrast agent uptake may allow both perfusion and permeability to be assessed distinctly from each other.

In this pilot study, we investigate a novel, high molecular weight (HMW) contrast agent comprised of hyperbranched polyglycerol (HPG) molecules [1] doubly labeled with Gd and a fluorescent marker. The attached Gd allows for the assessment of HPG uptake using conventional DCE-MRI methods, while fluorescence allows for histological confirmation that the agent remains intra-vascular. The objectives of this study were to determine if HPG causes quantitative MRI changes, and to validate the hypothesis that its high molecular weight (448 kDa) will cause it to remain intravascular for sufficient time such that assumptions can be made regarding the relationship between parameters acquired from pharmacokinetic models (such as K^{trans} [2]) and perfusion. Further, we investigate how long it remains in tissue following administration. We compare this new agent to a FITC and Gd-labeled albumin (Glowing Galbumin, BioPAL), a 74 kDa commercially available fluorescently tagged HMW contrast agent.

METHODS: *Contrast Agents:* HPG (synthesized in the Faculty of Pharmaceutical Sciences at UBC) contained 20µg Gd per mg HPG and was tagged with Alexa 647. It was administered as a 5 or 10µl/g bolus i.v. dose from a 60mg/ml solution. Galbumin was administered as a 5µl/g bolus i.v. dose at 25mg/ml. Gadovist (Bayer Healthcare) was administered as a 5µl/g bolus i.v. dose at 60mM.

Groups: HT29 human colorectal carcinoma xenografts in eight NOD/SCID mice were randomly assigned to four groups of two when tumours reached 300mm^3. Group 1 received a single HPG injection during a DCEMRI protocol on day 0, and was repeat imaged for T_1 mapping on days 3, 6 and 14 days. Groups 2 and 3 received the DCE protocol twice: on day 0 with Gadovist and on day 3 with HPG (group 2) or Galbumin (group 3); these mice were euthanized 60 min post contrast agent injection. Group 4 was not imaged, but received HPG via the tail vein while under anesthesia for a 2min HPG exposure.

DCE Imaging Protocol: Baseline images were obtained: a Tofts flip angle experiment (FLASH, TR/TE=100/2.77, FA=10,20,50,60) [3], and a variable flip angle experiment to obtain baseline T_1 (FLASH TR/TE=400/2.75, FA=125, 180, 215). This was followed by a 40 min DCE experiment (FLASH, TR/TE=9/2.660, FA=25), then a repeat T_1 measurement. Imaging was performed on a Bruker Biospec 70/30 using a combination of volume (Tx) / surface (Rx) coil.

MRI post-processing: Regions of interest were drawn in ImageJ to select a centre slice of each image. Using Matlab, T_1 maps at noted time points were obtained from variable flip angle data, and HPG concentration maps were calculated using T_1 maps, assuming 2% blood volume, and that HPG remained intravascular.

Histology: At indicated time points animals were euthanized and tumours were immediately collected and frozen. A 10 µm cryosection was obtained from the central region of each tumour and imaged for native fluorescence of contrast agents (HPG at 625nm; Galbumin at 488nm) using a robotic microscope and camera to obtain tiled images of whole tumour sections [4]. Sections were subsequently stained for endothelium using a PECAM/CD31 antibody and Alexa 546 tagged secondary. Images for individual tumours were overlayed and cropped to tumour tissue boundaries with artifacts and non-viable tissue removed. Image analysis was done using ImageJ software and user-supplied algorithms [4]. Briefly, whole tumour section fluorescence was measured as a reflection of contrast agent accumulation in tumour tissue. Each tumour tissue pixel was then sorted based on its distance relative to CD31-positive vasculature, and the average intensity of contrast agent fluorescence measured for each distance.

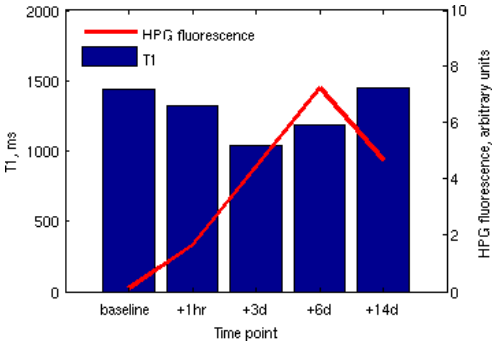

Fig. 1: T1 measurements at multiple time points. Overlaid is a line graph of HPG fluorescence from histology. Baseline refers to pre HPG T1, and to the two minute histology time point

RESULTS AND DISCUSSION: The HPG fluorescence was successfully imaged on histological sections at 625nm, and was found to increase with time. Analysis of the fluorescence intensity as a function of distance from vasculature suggests that HPG remains within or proximal to vasculature at early time points (within 5 & 20µm of CD31 objects at 2 & 60min time points), but does extravasate and accumulate at greater distances from vessels over time (HPG fluorescence greater than background at distances up to 75µm at 6 days post administration). Glowing Galbumin fluorescence was brighter than HPG at 60min post administration, with a similar accumulation profile proximal to vasculature, where fluorescence levels approached background at a distance of about 20µm from vessels.

On average, T_1 decreases by 281 and 173 ms at 40 min post contrast agent administration of HPG and Galbumin, respectively. While the concentrations of Gd in each agent is different, the commercially available Galbumin is not available at a higher concentration, and viscosity would prevent administering it at a higher concentration. HPG is available at higher Gd concentrations, and the increased T_1 effects are evident, and desirable.

Further, in both histology and MRI, we observe an accumulation of HPG over several days, followed by a drop in concentrations after about a week. In Fig 1, we show a bar graph of T_1 at multiple time points. Overlaid is HPG fluorescence (corrected for native tissue background) for matched time points.

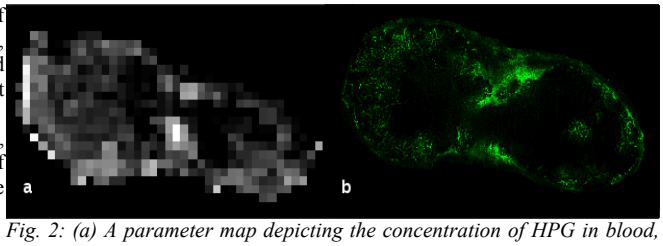

Fig. 2: (a) A parameter map depicting the concentration of HPG in blood, calculated from bookend T1 measurements acquired 40 min post injection. (b) HPG fluorescence image for the same tumour 60 min post injection.

Fig. 1 show that T_1 continues to drop over several days, suggesting an accumulation of HPG in the tumour over this time. At 6 days, T_1 starts to increase again. Histological fluorescence data confirms the HPG accumulation in the tumor tissue with slightly different kinetics: The maximum intensity is seen after 6 days, followed by a decrease at 14 days.

Fig 2a shows a concentration map of HPG in blood in a particular voxel at 60 minutes post HPG injection. In Fig 2b we show the histological section with HPG fluorescence from the same tumour at 60 minute post-injection. Distribution in the MRI suggests that the agent stays intravascular, which is supported by histology.

CONCLUSIONS: HPG causes quantifiable changes in MRI and histology. Fluorescence and MRI demonstrate that at short time points HPG is largely intravascular, though it leaks out over several days. Further, we showed that HPG accumulates in tumour tissue over the course of several days before decreasing. This supports our hypothesis that HPG remains intravascular long enough to extract flow-only data from DCE experiments without contamination due to vascular extravasation. Compared to Galbumin, HPG's effect on T_1 is larger at practical concentrations. In combination with K^{trans} measurement from a low molecular weight agent (Gadovist), flow measurement from HPG could be used to assess contribution from extravasation to K^{trans} in agents such as Gadovist over minutes and large molecules over days.

[1] Kainthan et al, Macromolecules (2006) **39**, 7708-7717 [2] Tofts et al, JMRI (1999) 10(3) 223-232. [3] Dowell & Tofts, MRM (2007) 58:622. [4] Baker et al., Clin Can Res (2008) 14(7); 2171.