Vessel Size Index and Blood Volume Imaging in Pancreatic Cancer Xenograft Model Using Ferumoxide

A. C. Yung[1], J. C. Tso[1], J. Flexman[1], S. Ng[1,2], D. Yapp[1,2], and P. Kozlowski[1]

[1]University of British Columbia, Vancouver, BC, Canada, [2]British Columbia Cancer Research Centre

Introduction. Ferumoxtran is a superparamagnetic iron oxide (SPIO) MRI contrast agent that has been used to estimate blood volume fraction (BV) and the vessel size index (VSI, or average blood vessel diameter) [1] in tumours. The administration of SPIO in blood produces steady-state susceptibility contrast which can be related to BV (proportional to ΔR_2^* due to contrast injection) and VSI (proportional to $D^{1/2}(\Delta R_2^*/\Delta R_2)^{3/2}$) The technique assumes that the SPIO contrast agent remains intrasvascular, which may not be the case in leaky tumours. Ferumoxide (Feridex, Bayer Healthcare) is a SPIO agent which may be an attractive alternative, due to its clinical availability (ferumoxtran is no longer commercially available), and its larger particle size which may improve sequestration in the tumour vasculature (120-180 nm vs. 15-30 nm diameter for ferumoxide and ferumoxtran, respectively [2]). This work describes the use of ferumoxide in measuring BV and VSI in an orthotopic xenograft mouse model of pancreatic cancer.

Methods. CB-17 SCID mice were implanted with human pancreatic adenocarcinoma via direct surgical suture onto the mouse pancreas [3]. All MRI experiments were performed under isoflurane anesthesia on a 7T 30cm-bore Bruker scanner, with an actively decoupled surface coil for reception (1.7x1.4cm) and a Bruker quadrature birdcage coil for transmission (i.d. = 7cm). A voxel size of 0.312x0.312x1mm was used throughout the study (128x128, 10-12 slices) Five tumour-bearing mice were imaged 4-12 weeks after implantation to study ferumoxide stability in the tumour: a multi-gradient echo FLASH (MGE-FLASH) baseline scan was acquired to derive T2* from the multiple echo decay (TE/TR=4/1087ms, 16 echoes, scan time=2min20sec) and was then repeated for 1.5 to 2 hours after a ferumoxide injection via tail vein catheter (200 µmol Fe/kg body weight). Nine mice were used for BV and VSI measurements, four of which were also used in the stability study in the same session. In the pre-injection phase, we acquired a T2-weighted spin echo MSME (TE/TR=6000/50ms), a R2* map (MGE-FLASH, same parameters as before) and a diffusion coefficient map (SE-EPI, TE/TR=23/6000ms, 16 shots, b=0 and 750 sec/mm^2). Post-injection R2* maps and T2-weighted SE images were acquired at least 15 minutes after contrast administration. After imaging, carbocyanine dye was injected intravenously immediately before sacrifice and tumour extraction.

The change in blood susceptibility due to contrast agent $\Delta\chi$ was estimated from blood samples from 3 normal and 2 tumour-bearing SCID mice, according to the method described in [4]. Mice were injected with ferumoxide (same dose as in VSI scans) and blood was collected 15 minutes later via cardiac puncture. Two capillaries filled with the blood were positioned perpendicular and parallel to the main magnetic field and a nonlocalized spectrum was acquired (4 kHz sweep width, TR=1sec, NA=120). The susceptibility of the blood containing ferumoxide was estimated by measuring the separation between two spectral peaks (one from each capillary), which equals half the susceptibility difference between air (χ_{air}=-0.36 ppm) and the blood containing ferumoxide [4]. The susceptibility increase due to ferumoxide in blood was calculated from equations similar to [1], with measured values of hemoglobin oxygen saturation (using a pulse oximeter), and assumed values for hematocrit (42%) and the volume susceptibilities of oxyhemoglobin, deoxyhemoglobin and plasma (-0.74, -0.561, -0.718 ppm in cgs units, respectively).

Vessel size index and blood volume fraction can be calculated in a pixel-wise manner using the equations presented in [1]. Image registrations were performed in the case of misregistration between the pre and post-contrast images. Negative values generated from ΔR2, ΔR2* were not included in analyses and considered artifacts due to motion or absence of contrast agent. Pixels corresponding to a VSI exceeding 156 um and a BV exceeding 17% were also excluded from the computations. For each tumour, whole-tumour microscopic images of immunohistochemical staining (Leica DM6000B) of the central section were processed to evaluate the following biomarkers related to the vasculature: carbocyanine (related to perfusion), CD31 (stains vessel walls), and DAPI (stains cell nuclei). Non-specific staining, artifacts and background areas were removed by manual thresholding and masking. The percentage of staining for each marker was determined by automated image analysis and was normalized to DAPI to account for variation in tumour sizes. Dual-stain coincidence was detected using nearest neighbour analysis. The MRI slice most closely resembling the histology section was manually selected for comparison with the histology measures (distinct lobes of the same tumour mass were considered individually).

Results. Figure 1 shows the average post-contrast T2* values (as a percentage of baseline T2*) in a representative tumour slice, which suggest that the contrast agent concentration remains stable in the vasculature for at least 1.5 hours. The spectroscopy measurements indicated that the average susceptibility change produced by ferumoxide in blood was $\Delta\chi$ = 1.37 ± 0.05 ppm (cgs units). Figure 2 shows a representative BV and VSI map (white represents rejected pixels; VSI values > 100 microns set to yellow to improve visualization). The median BV and VSI in this slice are 0.01 and 11 µm, respectively. Out of the 11 tumors from 9 mice (two mice had two tumors), five tumours were not compared to histology due to failure of the carbocyanine injection and/or post-injection displacement of the tumour in the slice direction. Figure 3 shows good correlation between average BV and the dual-stain percentages of CD31/carbocyanine normalized to number of cells, which relates to the amount of perfused blood vessels in the tumour (R=0.835, p=0.015). Average VSI was also highly correlated with average BV (R=0.898 , p= 0.038).

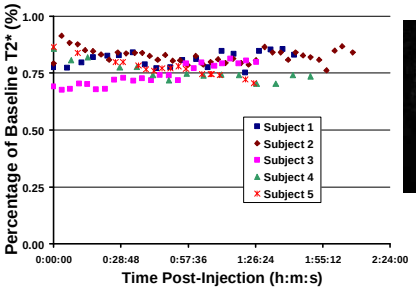

Figure 1: T2* stability measurement

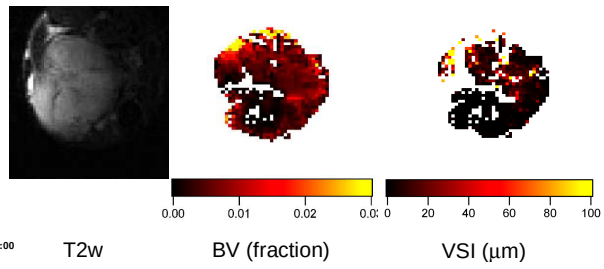

Figure 2: T2-weighted image (pre), BV and VSI map

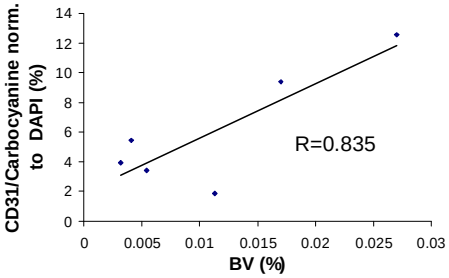

Figure 3: BV vs. Histology marker of perfused vessels

Discussion and Conclusions. T2* measurements indicate that the susceptibility effect of injected ferumoxide is stable for at least 1.5 hours, which allows adequate time for the VSI/BV experiment to be performed. The larger particle size (as compared to ferumoxtran) suggests a better tendency to remain inside the tumour vasculature, although this was not confirmed by histology. The measured $\Delta\chi$ of ferumoxide is 2.4 times higher than the same dose of ferumoxtran (per mg Fe/kg) [1], which increases the accuracy of the BV and VSI value [1]. The utility of ferumoxide in measuring BV was demonstrated in the high correlation between BV and the histology marker for perfused vessels. Different histological methods are needed to provide a better analogue to VSI, such as automatic segmentation of vessel diameters stained with H&E [5] or micro-CT of latex vascular casts [6]. The VSI correlated well with BV, which may suggest that the vessel density remains fairly uniform in the pancreatic cancer model being studied. In summary, we have demonstrated that ferumoxide is a feasible contrast agent for BV and VSI measurements.

Acknowledgments. This work has been supported by the Canadian Institutes of Health Research. **References**: [1] Tropres et al., MRM 2001, 45:397-408. [2] Modo et al., Molecular and Cellular MR Imaging, 2007, p. 66. [3] Cham et al.,AACR 2008, 1122. [4] Carlsson et al., MRI 2006, 24:1179-85. [5] Tropres et al., MRM 2004, 51:533-41. [6] Ungersma et al., ISMRM 2008, 449.