In Vivo MicroCT Imaging Characteristics of a Long-Acting Blood-Pool Agent in Normal and Tumor-Bearing Mice

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Introduction

High-resolution microCT systems capable of sub 20-micron isotropic spatial resolution provide an attractive new approach for noninvasively studying models of human diseases in live small animals.[1,2] However, relatively long acquisition times preclude the use of conventional water-soluble contrast media for imaging pathological conditions associated with vascular abnormalities. Utilizing the ability of PEG chains to modify surfaces of biological macromolecules, we have developed a long-acting blood-pool contrast agent (BP) based on our hepatocyte-selective CT contrast agent platform for vascular microCT imaging applications.[3,4] Macromolecular structures of BP and ITG particles are shown in Fig 1. Incorporation of PEG moieties into the phospholipid monolayer shell of the ITG vehicle interferes with the association of Apo-E, which is believed to be essential for hepatocyte recognition. Delaying the sequestration of ITG by liver cells results in prolonged vascular residence time. The liver (ITG) and vascular (BP)contrast agents are now known as Fenestra™ LC and VC, respectively.

The overall goal of this project is to evaluate the in vivo imaging efficacy of Fenestra VC as a macromolecular blood pool-selective and microCT compatible contrast agent to potentially monitor tumor angiogenesis in tumor-bearing mice. In addition, tissue intensity-time profiles of the agent were examined in normal mice for direct comparison.

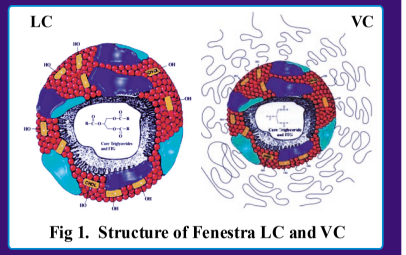


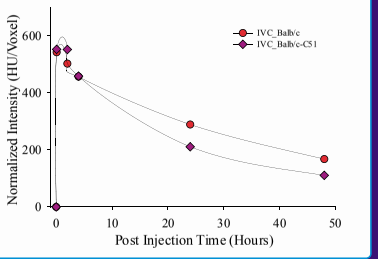
Fig 1. Structure of Fenestra LC and VC

Materials and Methods

The blood-pool contrast agent (Fenestra VC) was obtained from ART Advanced Research Technologies Inc. (Montreal, Canada) . The finale iodine concentration and mean particle size of this lipid emulsion were 50 mg I/mL and 180 nm, respectively. Mice were placed on a soft diet at least 1 day prior to the initiation of the study to minimize streak artifacts produced by rodent chow. Fenestra VC was administered as a single IV (tail vein) bolus dose (120 mL/kg bw) into normal or tumor-bearing mice. Anesthetized mice were scanned using a Siemens microCAT II system (70 kVp, 500 µA, 200 msec, 720 steps) prior to and within 10 minutes of injection.

Images were reconstructed (Feldkamp filtered back projection algorithms in real-time) and subsequently displayed and analyzed using Amira 3-D visualization software (V3.1). CT values of volumetric ROIs in the inferior vena cava (IVC) and liver were normalized to Hounsfield Units (HU). Normalized signal intensity (HU/voxel) was obtained from the difference of enhanced SI and the baseline value.

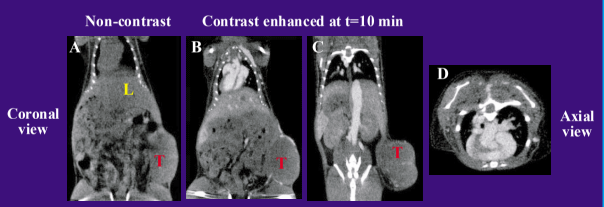
Fig 2. Normalized Vascular Intensity-Time Profiles of Fenestra VC in normal and C51-bearing mice



Imaging data from both normal and C51 tumor-bearing mice exhibited comparable vascular contrast enhancement profiles (Fig 2). Signal intensities in the IVC remained above 400 HU after 4 hours. Unlike water-soluble contrast agents which rapidly diffuse into extravascular spaces, the blood-pool contrast agent remained in the circulation for several hours, affording susbtained vascular contrast enhancement.

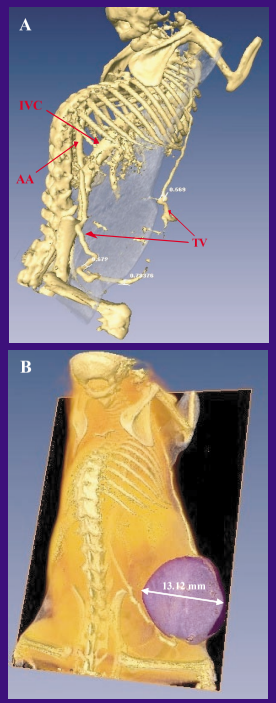
Results and Discussion

Fig 4. Fenestra VC-enhanced microCT vascular images obtained following iv injection (20 mL/kg) into an anesthetized Balb/c mouse bearing a colon carcinoma C51 xenograft.



Poor soft tissue contrast is clearly shown in the non-contrast coronal image (A). The superior quality of vascular contrast enhancement achieved after 10 min post injection of Fenestra VC is readily apparent in Fig 4(B-D).

Fig 5. Fenestra VC-enhanced microCT high density surface-rendered 3-D images obtained in an anesthetized Balb/c mouse bearing C51 xenograft following a single iv dose of 20 mL/kg.



Combined 3-D surface rendering and coronal slice image display excellent abdominal vascular rendering including the inferior vena cava (IVC), abdominal aorta (AA), hepatic vessels as well as tumor (T) feeder vessels (TV). Fenestra VC-induced contrast enhancement (Fig 5A) permitted the quantitative characterization of the tumor vascular network. Enlarged and well-developed extratumoral feeder vessels are evident as a direct result of angiogenesis. The diameters of the major tumor feeder vessels shown in Fig 5A could be measured accurately and were in the range of 0.60-0.79 mm. It should be noted that the image was acquired at 62x62x73 µm³ voxel resolution. Thus Fenestra VC is clearly capable of providing superior anatomic vascular imaging at or in excess of the limits of spatial resolution of the microCT II. A microCT segmentation image of the same mouse (shown in Fig 5A) allows tumor volume to be measured accurately in real time, providing substantial flexibility to monitor tumor development as well as response to therapy. The exceptional tolerance profile of Fenestra VC, permits the administration of a second dose (20 mL/kg bw) within 2 weeks to noninvasively monitor progression of tumor growth with respect to tumor volume and tumor vascular characterization in the same mouse. MicroCT images obtained 10 min following injection of the second dose is shown in Fig 6.

Liver uptake, as measured by an increase in the signal intensity, was faster in C51-bearing mice than in normal Balb/c mice at 24 hours post injection (Fig3). Kinetic profiles of the two groups clearly demonstrated a direct correlation between the blood clearance and liver uptake of Fenestra VC. The contrast agent was well tolerated in the tested mice when given as a single iv injection of 20 mL/kg bw, followed by microCT imaging at various time intervals according the specified protocol.

Fig 3. Normalized Liver Intensity-Time Profiles of Fenestra VC in normal and C51-bearing mice

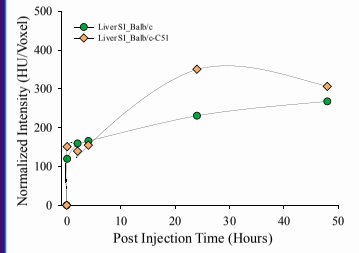
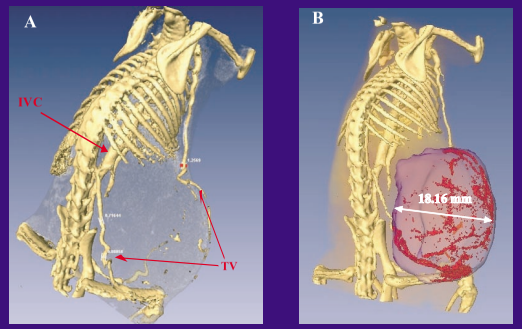


Fig 6. Fenestra VC-enhanced microCT high density surface-rendered 3-D images obtained from the same mouse (shown in Fig 5) 10 min following a 2nd iv injection of VC (20 mL/kg) 2 weeks after the 1st dose.



Combined 3-D surface rendering and coronal slice demonstrate the major tumor feeder vessels (TV) visible in Fig 6. grew significantly longer and larger in diameter in comparison to the measurements obtained following the 1st injection of Fenestra VC. Tumor size increased substantially within the 2-week period. Utilizing the superior vascular enhancement capacity of Fenestra VC in conjunction with microCT, quantitative characterization of tumor progession associated with angiogenesis in the same tumor-bearing mouse was possible.

Conclusions

Owing to its macromolecular nature, Fenestra VC offers the capability of providing superior vascular imaging which may prove extremely useful for assessing progression of tumor growth and monitoring tumor response to therapy at the vascular level including anti-angiogenic agents.

References

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