Effects of Administration Dose, Species and Strain Variations on MicroCT Imaging Characteristics of a Long-Acting Blood-Pool Agent in Normal Rodents

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Introduction

Recently introduced microCT scanners[1-3] capable of sub 20-micron spatial resolution afford the potential to more effectively monitor disease model conditions in small animals. Despite being able to provide this exceedingly high spatial resolution, the widespread adoption of this imaging method in living systems has been hindered by the relative lack of soft tissue contrast associated with taking such thin tomographic slices and comparably long acquisition times that preclude the use of conventional water-soluble contrast agents. In order to achieve a comparatively prolonged and stable circulatory half-life, a novel long-acting vascular CT contrast agent has been developed and evaluated for its ability to visualize and noninvasivetly assess vascular characteristics in normal and disease model conditions.[4] The vascular contrast agent was recently commercialized and is now known as Fenestra™ VC.

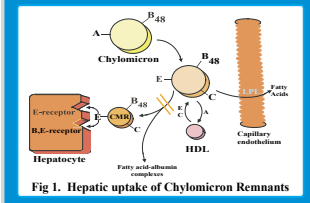


Fig 1. Hepatic uptake of Chylomicron Remnants

Fenestra VC is a refined, surface modified version of our hepatocyte-selective contrast agent ITG (Fenestra LC). This agent mimics chylomicron remnants and thereby selectively delivers the contrast-producing moiety into the hepatic parenchyma following administration Association of circulatory Apo-E with the ITG particles is believed to be essential for hepatocyte recognition. Thus, interfering with its Apo-E interaction is excpected to retard hepatocyte recognition (Fig. 1).

The macromolecular structure of the blood-pool contrast agent, Fenestra VC (BP), is shown in Fig. 2. Attachment of PEG chains to the surface of the ITG vehicle prevents Apo-E association and decreases its uptake by hepatocytes resulting in prolonged circulatory retention. The goal of this study is to evaluate the in vivo CT imaging characteristics of the blood-pool agent in various strains of mice and rats as a function of administration dose.

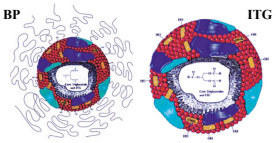


Fig 2. Structure of BP and ITG Particles

Materials and Methods

The blood-pool contrast agent (Fenestra VC) was obtained from ART Advanced Research Technologies Inc. (Montreal, Canada). The final iodine concentration and mean particle size of this lipid emulsion were 50 mg I/mL and 180 nm, respectively. Fenestra VC was administered as a single i.v. bolus dose via tail vein injection. Three doses (7.5, 10, and 15 mL/kg) were given to female Sprague-Dawley rats (n=2-3/group) and two doses (10 and 15 mL/kg) were injected to female C57Bl/6 (n=3) mice. Other strains of mice (Balb/c, FVB, Nude, and Scid) were administered with 15 mL/kg (n=3). Anesthetized animals were scanned using a GE eXplore Locus microCT system (80 kVp, 450 µA, 200 msec/view, 5 frames/view, 400 views/scan and 93 µm resolution) prior to and at predetermined time intervals following administration of the blood-pool agent.

Images were recontructed with the EVSBeam software (GE Medical System) and subsequently displayed and analyzed using both Amira 3-D visualization software (V3.1) and an open-source MicroView program (http://Microview.sf.net). CT values of volumetric ROIs in the inferior vena cava (IVC) and liver were normalized to Hounsfield Unit (HU). Normalized signal intensity (HU/voxel) was obtained from the difference of enhanced SI and the baseline value.

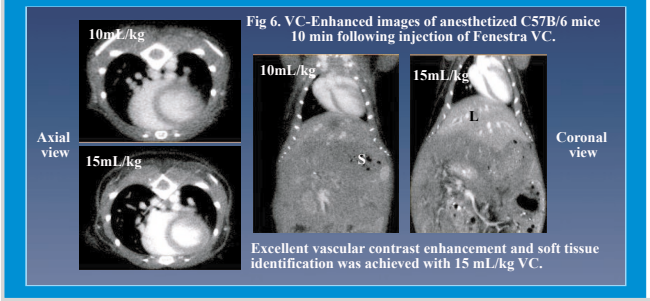


Fig 6. VC-Enhanced images of anesthetized C57Bl/6 mice 10 min following injection of Fenestra VC.

Excellent vascular contrast enhancement and soft tissue identification was achieved with 15 mL/kg VC.

Results and Discussion

Fig 3. VC-Enhanced MicroCT Vascular Images of Anesthetized Rats at T=10 min

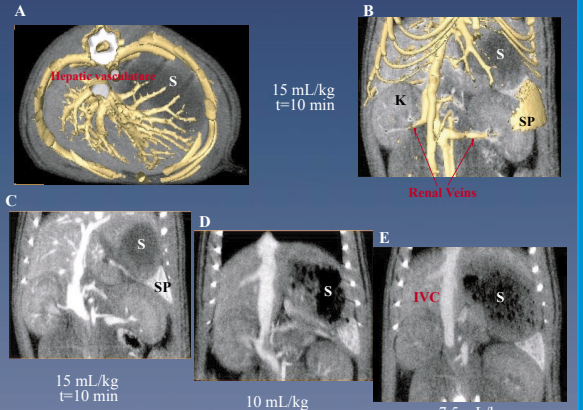
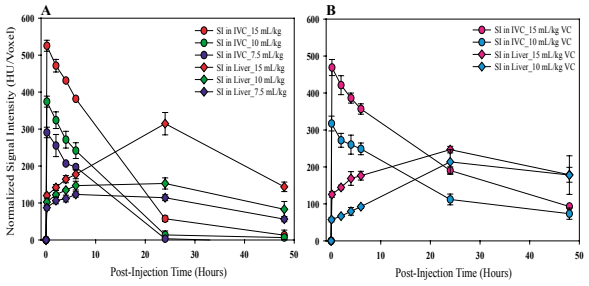


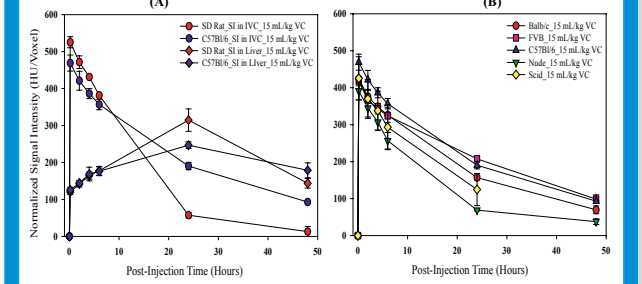
Fig 3. shows Fenestra VC-enhanced 3D microCT images of an anesthetized rat 10 minutes following intravenous administration of a single 15 mL/kg dose. Combined 3D surface rendering and an axial slice image (Fig. 3A) shows enhanced hepatic vascular network. The renal cortex and renal medulla of the left kidney (K) are clearly visualized in Fig 6B. Effect of administration dose on the in vivo imaging efficacy of Fenestra VC is shown in Fig 3(C-E). Coronal CT images (C-E) of anesthetized rats obtained 10 minutes after injection of the blood pool at three different doses show excellent vascular enhancement was achieved at 15 mL/kg dose. At the lowest test dose (7.5 mL/kg), adequate vascular intensity and soft tissue identification could be obtained at 10 minutes post injection. Due to the relatively large size of the rats, only a portion of the body could be imaged at a time.

Fig 4. Normalized Signal Intensity-Time Profiles of Fenestra VC in SD Rats (A) and C57Bl/6 Mice (B)



Normalized contrast enhancement profiles of the blood pool agent in the blood and liver tissues following various administration doses in female SD rats and C57Bl/6 mice are shown in Fig 4. Imaging data demonstrated that the degree of tissue contrast intensity in both rats and mice was dose dependent. Vascular signal intensity in rats and mice decreased to approximately 73% and 77%, respectively, after 6 hours following intravenous administration of a single bolus dose. Unlike water-soluble contrast agents which rapidly diffuse into extravascular space, the blood pool contrast agent remained in the circulation for several hours, producingly susbtained vascular contrast enhancement.

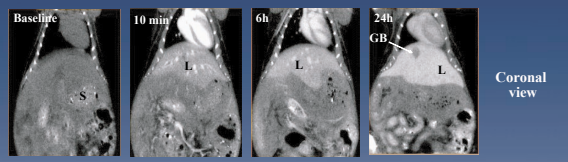
Fig 5. Normalized Signal Intensity-Time Profiles of Fenestra VC (A) in SD Rats vs C57Bl/6 Mice and (B) in Various Strains of Mice (B)



The effect of species variation on the blood pool activity of Fenestra VC is clearly shown in Fig 5A. Blood clearance, as measured by a decrease in vascular signal intensity, was significantly faster in SD rats than in C57Bl/6 mice, affording a higher level of liver localization in rats 24 hours post injection of Fenestra VC. Vascular contrast enhancement profiles of the blood pool agent in five different strains of mice following intravenous administration of 15 mL/kg dose is shown in Fig 5. Among the female mice, the FVB group displayed a slower rate of vascular clearance, while the other strains all exhibited comparable rates albeit somewhat faster than that observed for the FVB. Results from the imaging study demonstrated that effects of species variation on the in vivo imaging charactertistics of Fenestra VC were more significant than that of strain variation.

All the tested mice and rats remained active and displayed normal social behaviors prior to their sacrifice within 1-4 weeks after injection. Intravenous administration of Fenestra VC at a dose of 15 mL/kg followed by a serial CT scanning according to the specified protocol was well tolerated in both rats and mice.

Fig 7. Time sequential VC-Enhanced images of anesthetized C57B/6 mice following injetion of Fenestra VC (15 mL/kg).



Inherent poor soft tissue contrast was observed with the baseline scan. Fenestra VC visibly remained in the vasculature for several hours, producing excellent vascular contrast enhancement. Elimination via hepatobiliary system was evident after 24 hours.

Conclusions

In vivo imaging results demonstrate that the vascular contrast agent provided sustained vascular contrast enhancement for several hours in a variety of rodent models. Both administration dose and species variations had significant effects on the in vivo imaging characteristics of Fenestra VC, however slight and manageble differences in the vascular signal intensity were observed between various strains of mice. Fenestra VC with its superior blood pool activity and acceptable injection tolerance profiles provides substantial flexibility in the type and scope of studies that can be performed.

References

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