



Effects of Dose, Sex, and Species Variations on Imaging Efficacy of a Hepatocyte-Selective Contrast Agent in Normal Rodents

GN Ton¹, WC Dow², DA Bakan², M Melchior², JP Weichert¹

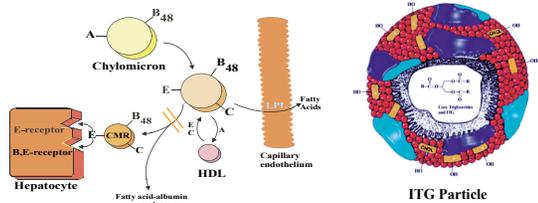
¹University of Wisconsin-Madison, WI and ²Alerion Biomedical Inc., San Diego, CA



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.^{2,3} However, relatively long acquisition times preclude the use of conventional water-soluble contrast media for imaging pathological conditions in soft tissues. Exploiting the endogenous lipid metabolism pathways that occur naturally in the body, we have developed a hepatocyte-selective CT contrast agent consisting of a polyiodinated triglyceride (ITG) in a lipid emulsion delivery system. The ITG vehicle mimics chylomicron remnants (CMR), CMR particles represent a class of naturally-occurring plasma lipoproteins that selectively shuttle lipids to hepatocytes in the liver (Fig 1). The contrast agent has been evaluated for its ability to provide excellent hepatic opacification for prolonged periods of time after injections in both normal and diseased model animals.^{4,5}

Fig 1. Hepatic uptake of Chylomicron Remnants and macromolecular structure of ITG particle



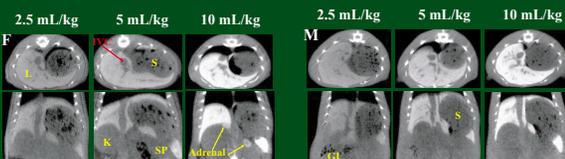
The aim of this project is to examine the effects of administration dose, sex, and species variations on the in vivo microCT imaging efficacy of the liver contrast agent in normal mice and rats.

Materials and Methods

The liver contrast agent, which is now commercially available as FenestraTM LC, from ART Advanced Research Technologies (Montreal, Canada) provided a final iodine concentration and mean particle size of 50 mg I/mL and 200 nm, respectively. Fenestra LC was administered as a single i.v. bolus dose via tail vein injection. Three doses (2.5, 5, and 10 mL/kg) were given to both female and male Sprague-Dawley rats (n=2-3/group), and two doses (10 and 15 mL/kg) were injected to male FVB mice (n=3). Anesthetized (2% isoflurane) animals were scanned using a GE eXplore Locus microCT system (80 kVp, 450 μ A, 200 msec/view, 5 frames/view, 400 views/scan and 93 μ m³ resolution) prior to and at predetermined time intervals following administration of the hepatocyte-selective agent.

Images were reconstructed 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 Units (HU). Normalized signal intensity (HU/voxel) was obtained from the difference of enhanced SI and the baseline value.

Fig 2. Liver-enhanced microCT images of female (left) and male SD rats (right) 4 hours following IV administration of Fenestra LC at various doses.



Excellent liver contrast enhancement and soft tissue identification were observed in both female and male rats 4 hours following injection of Fenestra LC (10 mL/kg).

Results and Discussion

Fig 3. MicroCT body kinetics of Fenestra LC in an anesthetized male rat following administration of a single 10 mL/kg dose

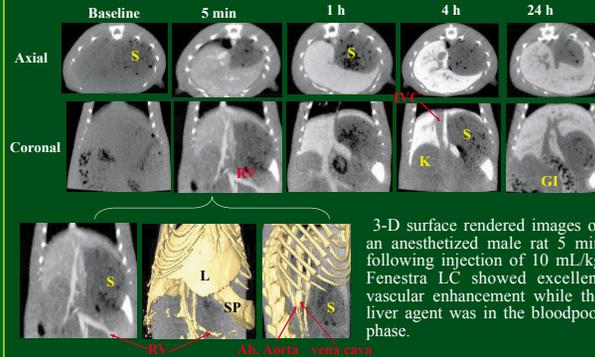
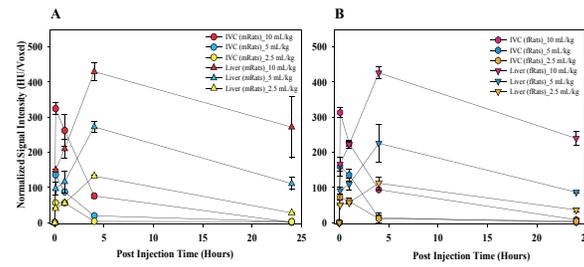


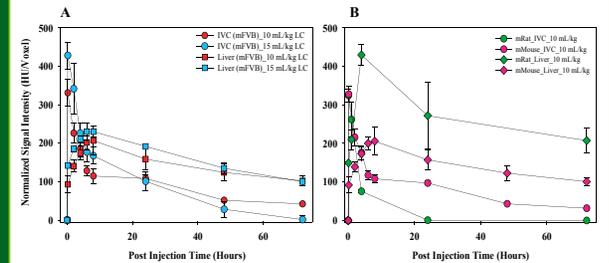
Fig 3. shows time sequential Fenestra LC-enhanced 2-D and 3-D microCT images of an anesthetized rat following intravenous administration of a single 10 mL/kg dose. Owing to the liver-selective capability and prolonged in vivo residence time, real-time body kinetics of Fenestra LC in rats can be monitored. Non-contrast axial and coronal images of the rat showed poor soft tissue contrast in the thoracic and abdominal cavities. Axial and coronal microCT images of the rat obtained at indicated post injection time intervals revealed the liver signal intensity peaks after 3 hours following an iv injection. The IVC and hepatic vascular network became visible after 4 hours due to signal void as a result of the removal of Fenestra LC from the circulation and its localization in hepatocytes. Quantitative characterization of the vascular network is possible with the use of combined 3D surface rendering and 2D microCT images of the rat obtained during the blood-pool phase of Fenestra LC (=5 min post injection).

Fig 4. Normalized Signal Intensity-Time Profiles of Fenestra LC in Male (A) and Female Sprague-Dawley Rats (B)



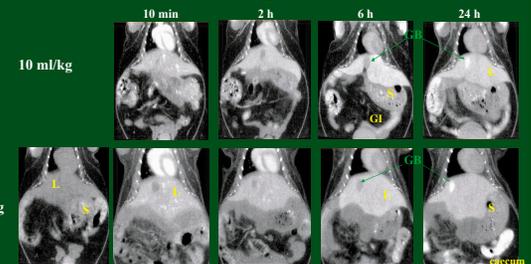
Normalized contrast enhancement profiles of Fenestra LC in the blood and liver tissues following various administration doses in female and male SD rats are shown in Fig 4. Imaging data demonstrated that tissue contrast intensity in both rats and mice was dose dependent. Vascular signal intensity in rats decreased rapidly after 4 hours and returned to background level after 24 hours following intravenous administration of a single bolus dose. Liver signal intensity peaked after 3 hours followed by a gradual exponential decay owing to hepatobiliary elimination. Both female and male rat groups exhibited comparable tissue enhancement profiles.

Fig 5. Normalized Signal Intensity-Time Profiles of Fenestra LC in (A) Male FVB Mice and (B) Male SD Rats vs FVB Mice (10 mL/kg)



The effect of species variation on the liver opacification activity of Fenestra LC is clearly shown in Fig 5B. Liver uptake, as measured by an increase in the signal intensity, was significantly faster in male SD rats than in male FVB mice following administration of an iv bolus dose. The rate of the contrast agent was removed from the vasculature in rats was significantly faster than in mice. Pharmacokinetic profiles of the two groups clearly demonstrated a direct correlation between the blood clearance and liver uptake of Fenestra LC.

Fig 6. MicroCT body kinetics of Fenestra LC in anesthetized male FVB mice following injections of 10 or 15 mL/kg dose



Excellent vascular contrast enhancement was achieved initially as the liver contrast agent was introduced directly to the blood stream via tail vein injection, followed by an increase in the degree of liver contrast enhancement as the hepatocyte recognition and localization initiated thereafter. Elimination via hepatobiliary system was evident after 24 hours as indicated by opacification in the gall bladder and GI tract.

Conclusions

In vivo imaging results demonstrate that Fenestra LC provided excellent hepatic contrast enhancement in the rodent models tested. Species variation strongly influenced the in vivo microCT imaging characteristics, while the effect of gender variant on the enhancement profiles was minimal. Due to its biomimetic nature, Fenestra LC provides both superior anatomic and functional capabilities.

References

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