



## A Liposomal Nanoscale Contrast Agent for Preclinical CT in Mice

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**OBJECTIVE.** The goal of this study was to determine if an iodinated, liposomal contrast agent could be used for high-resolution, micro-CT of low-contrast, small-size vessels in a murine model.

**MATERIALS AND METHODS.** A second-generation, liposomal blood pool contrast agent encapsulating a high concentration of iodine (83–105 mg I/mL) was evaluated. A total of five mice weighing between 20 and 28 g were infused with equivalent volume doses (500  $\mu$ L of contrast agent/25 g of mouse weight) and imaged with our micro-CT system for intervals of up to 240 min postinfusion. The animals were anesthetized, mechanically ventilated, and vital signs monitored allowing for simultaneous cardiac and respiratory gating of image acquisition.

**RESULTS.** Initial enhancement of about 900 H in the aorta was obtained, which decreased to a plateau level of approximately 800 H after 2 hr. Excellent contrast discrimination was shown between the myocardium and cardiac blood pool (650–700 H). No significant nephrogram was identified, indicating the absence of renal clearance of the agent.

**CONCLUSION.** The liposomal-based iodinated contrast agent shows long residence time in the blood pool, very high attenuation within submillimeter vessels, and no significant renal clearance rendering it an effective contrast agent for murine vascular imaging using a micro-CT scanner.

The introduction of micro-CT has brought about substantial improvements in anatomic imaging of small animals. However, with the introduction of this technology, new methodologic issues have arisen. Some of the issues faced include that the acquisition time in a typical micro-CT study is around 30 min and can be as long as 1 hr. Such long scan times necessitate a long-residence-time contrast agent unless one resorts to continuous infusion of contrast material or the performance of postmortem studies. Another limitation introduced by imaging small animals on micro-CT scanners is that small animals have correspondingly small anatomic features, and imaging them with acceptable conspicuity requires high relative image contrast (often approximately several hundred Hounsfield units) [1].

Liposomes are spherical vesicles composed of a lipid bilayer envelope, surrounding a central aqueous core (Fig. 1). In a previous study, we showed the use of a novel, long-circulating liposome-based blood pool contrast agent for CT in a rabbit model using a clinical

CT scanner for humans [2]. The agent, which consisted of a conventional, nonionic, iodinated vascular contrast agent that was encapsulated within polyethylene glycol–stabilized (PEGylated) liposomes, showed stable enhancement of the intravascular space for more than 3 hr. Because the usual renal clearance mechanism of the iodinated agent was altered by encapsulation, the agent was primarily cleared via the reticuloendothelial system (RES), consistent with the known mechanism of clearance for stealth liposomes [3].

Although that study proved adequate for imaging moderate-size animals, the smaller anatomic features in the mouse and the poorer noise profile of micro-CT systems in comparison with whole-body systems suggested that the 200-H enhancement of the vascular bed was unlikely to be adequate for imaging the very small (i.e., submillimeter-size) structures in the mice. With this in mind, we have synthesized a second-generation, iodinated liposomal blood pool contrast agent that encapsulates a payload of > 100 mg I/mL for testing in an animal model. We hypothesized that a well-tolerated injection volume (500  $\mu$ L in a

Fig. 1—Schematic diagram of liposome.

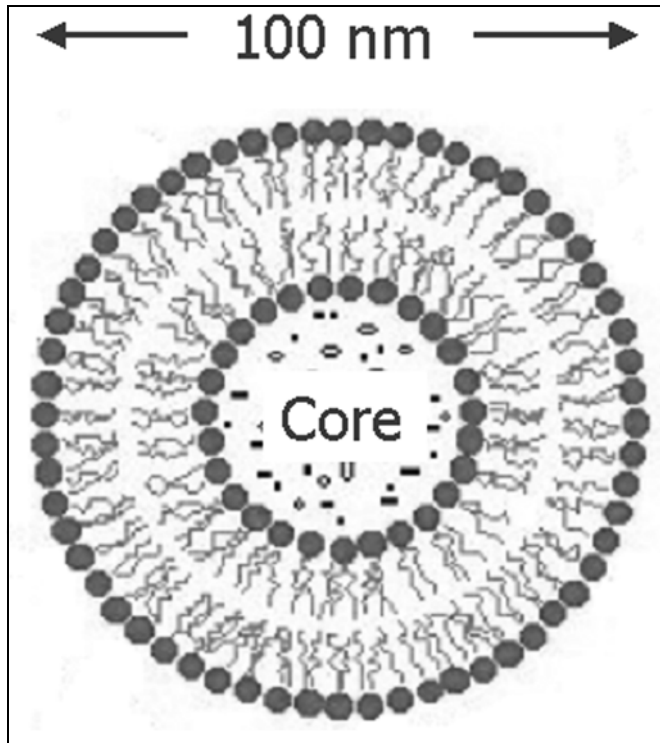
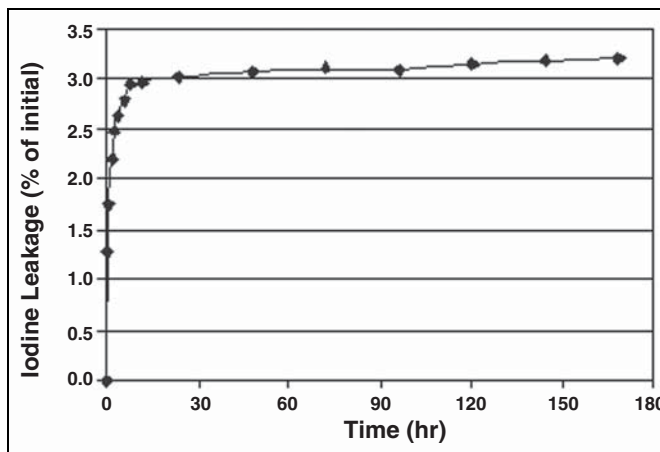


Fig. 2—In vitro stability of liposomal iodixanol dialyzed against phosphate-buffered saline at 40°C. Initial iodine concentration in sample is 107.5 mg I/mL. Concentration at 8 hr is 104.3 mg I/mL, and at 7 days it had dropped to 104.1 mg I/mL.



25-g mouse) would provide at least 500-H vascular enhancement and that this degree of enhancement would enable imaging of features in the mouse model.

**Materials and Methods**

*Liposomal Formulations*

Iodixanol (Visipaque 320, GE Healthcare) was concentrated using a FreeZone 4.5-L Benchtop Freeze Dry System (Labconco). Liposomal iodixanol formulations were fabricated by methods similar to those described previously [2]. Briefly, a lipid mixture (200 mmol/L) consisting of 1,2-di-

palmitoyl-sn-glycero-3-phosphocholine (DPPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy (polyethylene glycol)-2000] (DSPE-MPEG 2000) in a 55:40:5 molar ratio was dissolved in ethanol at 70°C. The ethanol solution was then hydrated with concentrated iodixanol (480 mg I/mL) for 2 hr.

Liposomes were extruded with a 10-mL Lipex Thermoline extruder (Northern Lipids) with five passes through a 0.2-µm Nuclepore membrane (Waterman) and seven passes through a 0.1-µm Nuclepore membrane (Waterman). Liposomes were then dialyzed overnight in a 100,000-molecular

weight cutoff (MWCO) dialysis bag against phosphate-buffered saline to remove ethanol and free iodixanol. The resulting liposomal iodixanol formulations (43.8 mg I/mL) were then concentrated using a Pellicon tangential flow filtration cassette and LabScale TFF system (Millipore) to a final concentration of 118.6 mg I/mL and stored in phosphate-buffered saline at pH 7.2.

The size of the resultant liposomal formulations obtained was determined by dynamic light scattering (DLS) using a BI-9000AT Digital Autocorrelator (Brookhaven Instruments), a BI-200SM goniometer (JDS Uniphase), and a Hamamatsu photomultiplier (Brookhaven). Mean size of liposomes as determined by DLS was found to be 112.9 nm with an SE of 1 nm. The polydispersity index for the formulation was 0.107.

*Assessment of In Vitro Stability*

The iodine concentrations of the liposomal formulations were determined by measuring the absorption of ultraviolet (UV) light at 246 nm with a UV-visible light spectrophotometer. The in vitro stability of liposomal iodixanol formulations was determined by measuring the leakage of iodixanol from the liposomes in phosphate-buffered saline at 4°C. In storage, leakage stopped after a few hours due to equilibration of the internal and external phases. To quantitate the rate of equilibration and to show the long-term stability after equilibration, the stored preparation was dialyzed for 1 hr to remove previously leaked iodixanol (about 3–4%) and then tested. In the test, 1 mL of liposomal iodixanol formulation was placed in a 100,000-MWCO dialysis bag and dialyzed against 250 mL of phosphate-buffered saline. At each time point, 1 mL of the dialysate was removed for UV absorption-based iodixanol measurement. After measurement, the samples were added back to the buffer solution to maintain constant volume. The interval of sampling varied over the course of the experiment; frequent sampling was conducted initially, with longer intervals used during the final phases of the experiment (Fig. 2). The experiment was continued until the amount of iodine detected in the external phase reached a constant value.

*In Vivo Studies*

All animal studies were performed on mice weighing between 20 and 28 g under a protocol approved by the Institutional Animal Care and Use Committee at Duke University. A total of five mice were studied. Four of these mice were C57BL/6 mice and the remaining animal (animal 2) was an athymic nude mouse in which a xenograft of the human squamous cell carcinoma (FaDu) could be implanted. This animal was used to assess the viability of implanting this tumor in our animal model for

future studies. Anesthesia was induced with a 50 mg/kg intraperitoneal injection of sodium pentobarbital and 2 mg/kg butorphanol. After endotracheal intubation, anesthesia was maintained with 2–3% isoflurane. A custom-made ventilator was used to deliver the isoflurane [4]. Animals were mechanically ventilated at a rate of 90 breaths/min and at a tidal volume of 0.4 mL. Pancuronium bromide was used to arrest free breathing.

Iodinated liposomes were infused via a tail vein cannula at a volume dose of 0.5 mL/25 g of mouse weight. This was chosen as a standard volume for infusion because it was well tolerated by the animals. A total of five mice were independently studied on different dates. The formulations used were all designed to show large degrees of contrast enhancement but varied slightly due to differences in the fabrication process (83–105 mg I/mL). A total imaging time of 2 hr was planned for all experiments. All animals were imaged at 60 min. In addition, four animals were also imaged immediately after infusion (designated time 0), four animals were imaged at 30 min, three animals were imaged at 90 min, and three animals were imaged at 120 min (Table 1). One animal (animal 5) died 60 min after imaging began, likely due to complications from anesthesia. For each animal, we recorded attenuation coefficients over time in the following locations: descending aorta, myocardium within the interventricular septum, within the blood-filled chamber of the left ventricle, kidney, liver, and spleen.

Animal monitoring consisted of an airway pressure tracing monitored with a solid-state pressure transducer on the breathing valve and ECG recording measured by electrodes taped to the footpads. Body temperature was recorded using a rectal (or peroral) thermistor that was also used to control heat lamps that maintained the core body temperature at 36.5°C. The animals were placed in the cradle in a vertical position with flexible tubes and wires carrying anesthesia gas and physiologic signals suspended from above to allow free rotation. All physiologic signals were displayed on a computer using a LabVIEW application (National Instruments). After the study, the animals were sacrificed with an overdose of pentobarbital sodium (Nembutal, Abbott Laboratories).

**TABLE 1: Experimental Parameters**

Animal No.	Animal Type	Animal Weight (g)	Dose (mg I/kg)	Liposomal Formulation (mg I/mL)	Total Scanning Time (hr)	Scanning Time Points (min)
1	C57BL/6	25	1,660	83	2	0, 30, 60, 90, 120
2	Athymic nude	20	2,100	105	1.5	0, 30, 60, 90
3	C57BL/6	28	2,054	100	2	0, 30, 60, 90, 120
4	C57BL/6	25	2,000	100	4	0, 60, 120
5	C57BL/6	25	1,800	90	1	30, 60

## CT

**Micro-CT system**—The micro-CT system used in this work has previously been described [5, 6]. Animals were positioned vertically in a rotating cradle placed immediately in front of a stationary detector. A high-flux rotating anode X-ray tube (SRO 09 50, Philips Medical Systems) with a dual 0.3/1.0 mm focal spot was used, which is sufficient to support exposures as short as 9 msec required to limit cardiac motion blur. A high-resolution detector with 50 × 50 μm pixels covering an image matrix of 2,048 × 2,048 (Microphotonics X-ray Image Star camera, Photonics Science) was used over an active area input of 106 × 106 mm. We used a hardware feature that combined pixels to a 2 × 2 array that reduced the effective detector pitch to 100 μm.

**Image acquisition**—Imaging was performed using the following parameters: 80 kVp, 170 mA, and 9-msec exposure. Projections were acquired over a circular orbit of 189° (i.e., 180° + fan angle) with a step angle of 0.75° using a total of 252 projections. Acquisition time of each projection set was approximately 8–10 min; therefore a 12-phase cardiac scan took approximately 120 min total to acquire. Scanning was done with the animal placed at a source-to-object distance of 480 mm, an object-to-detector distance of 40 mm, and a source-to-detector distance of 520 mm resulting in a geometric blur of the focal spot that matched the Nyquist sample at the detector [5]. This resulted in measured exposure for each image set of 16 R ( $4.13 \times 10^{-3}$  C/kg).

**Image reconstruction**—Projection images were used to reconstruct tomograms with a Feldkamp algorithm using Parker weighting [7]. Cobra EXXIM software package (EXXIM Computing) was used. Data were reconstructed as isotropic 1,024 × 1,024 × 1,024 arrays with effective digital sampling in the image plane of 92 μm because the magnification factor for the geometry used was 1.08. All data sets were acquired with ventilatory synchronization (on end-expiration) and cardiac gating on different points of the ECG cycle. Both temperature ( $36.5 \pm 1^\circ\text{C}$ ) and heart rate (R-R interval = 90–100 msec) were relatively stable during imaging.

**Blood pool imaging**—Contrast enhancement was measured at regions of interest in the descending aorta, myocardium within the interventricular

septum, within the blood-filled chamber of the left ventricle, kidney, liver, and spleen in Hounsfield units (H). The relative enhancement, defined as the mean difference in attenuation postinjection and preinjection of the contrast agent was measured. Results were plotted as time–attenuation curves.

## Results

### In Vitro Stability

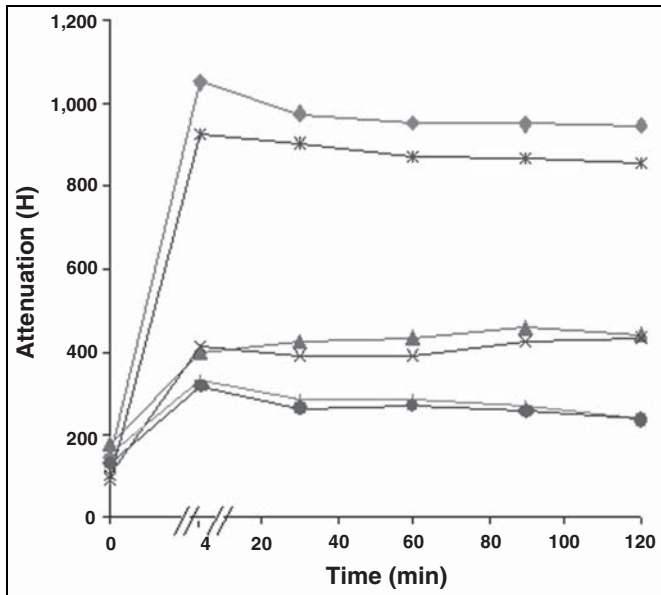
A decrease in the iodine concentration of the liposomal iodixanol formulation after the 1-hr dialysis cleaning step to 107.5 mg I/mL from the initial 118.6 mg I/mL was observed. The 107.5 mg I/mL formulation was then used to perform the in vitro stability study. Cumulative leakage of approximately 3.2% of the total iodine content, which is a small amount that is acceptable for storage purposes, was seen (Fig. 2). The leakage began to stabilize at approximately 8 hr and eventually reached a steady state at the end of day 7. Based on these data, the in vivo sample was dialyzed for 8 hr before use, achieving a final concentration of 105 mg I/mL.

### Blood Pool Imaging

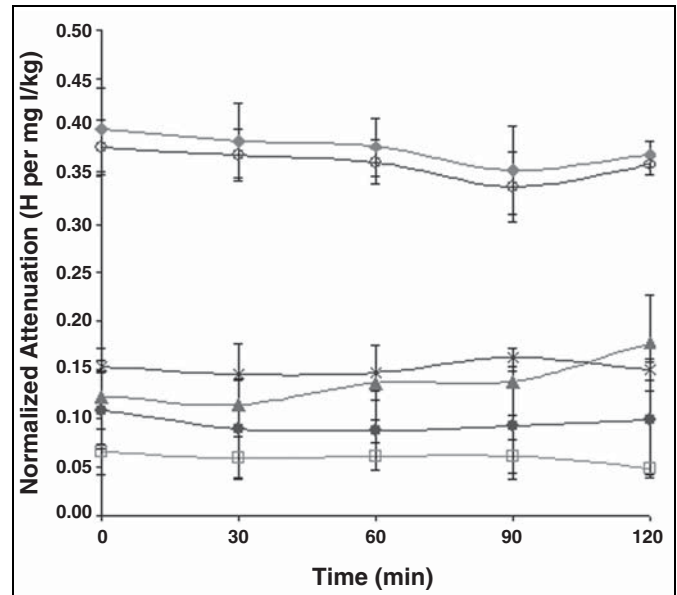
Time–attenuation curves in the regions of interest over time showed an initial enhancement of about 900 H in the aorta, indicating a high blood pool iodine concentration (Fig. 3). CT density slightly decreased over time to a level of approximately 800 H after 2 hr. Excellent contrast discrimination was seen between the myocardium and blood in the left ventricle (650–700 H). Enhancement of renal arteries and veins was seen, but no significant nephrogram was identified, indicating the absence of renal clearance of the agent. Liver and spleen enhancement was substantially lower than vascular enhancement and remained relatively stable over 120 min, consistent with the expected delayed clearance of the liposomal iodixanol formulation via the RES due to the PEGylated formulation (Fig. 4).

Because the agent could produce high opacity for several hours, a number of anatomic structures were clearly visible. The volume-rendered image of the cardiac and pulmonary vascular trees acquired after injection of the liposomal contrast agent shows the right and left ventricles, aorta, pulmonary trunk, and inferior vena cava (Fig. 5). The long residence time at stable, high opacity enables simultaneous respiratory- and cardiac-gated image acquisition, thus limiting motion artifacts to less than 100 μm [8] and enabling the extremely high resolution of these images (100-μm isotropic voxels) and, in turn, the

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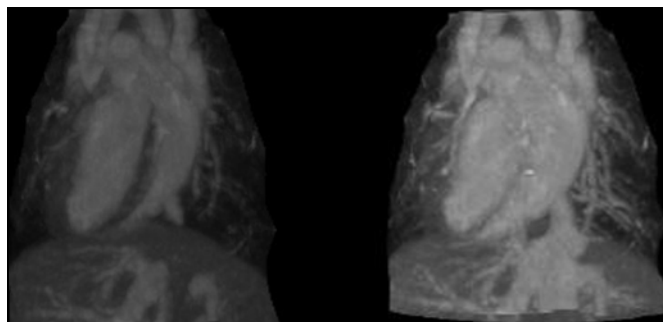


**Fig. 3**—Time-attenuation curves in specific regions of interest in mouse model using liposomal contrast agent (100 mg I/mL). On *x*-axis, time (*t*) = 0 is attenuation measured at precontrast injection. Remaining time points represent attenuation measured postinjection of liposomal contrast agent. Curves (from highest to lowest on *y*-axis) show data for blood (left ventricle) (◆), aorta (\*), liver (▲), spleen (×), muscle (⊥), and kidney (●).



**Fig. 4**—Normalized attenuation is defined as relative enhancement (i.e., attenuation postinjection minus attenuation preinjection) per milligram iodine dosed per kilogram of body weight. On *x*-axis, time (*t*) = 0 is defined as first set of measurements postinjection, taken 5 min after administration of liposomal contrast agent. Number of animals at each time point was *n* = 4 for *t* = 0 and 30 min, *n* = 5 for *t* = 60 min, and *n* = 3 for *t* = 90 and 120 min. Curves (from highest to lowest on *y*-axis) show data for blood (left ventricle) (◆), aorta (○), spleen (×), liver (▲), kidney (●), and muscle (□).

**Fig. 5**—Maximum intensity projections of cardiac and pulmonary vascular trees in mouse acquired after injection of liposomal contrast agent.



visualization of submillimeter features in the vasculature.

The ability to perform gated 9-msec acquisitions of data based on both cardiac and respiratory telemetry allowed for the acquisition of 4D (3D spatial + time) cardiac images of the beating heart and the display of the images as a cine series (Fig. 6). This technique was described by our group in an earlier study [9]. Both the left and right ventricles are clearly visible. The descending aorta and the inferior vena cava are also clearly seen in the axial slice. By performing serial integrations over the extent of the chambers (base to apex of the heart) at end-systole and end-diastole, it is possible to calculate important cardiac pa-

rameters such as the end-systolic volume, end-diastolic volume, and ejection fractions.

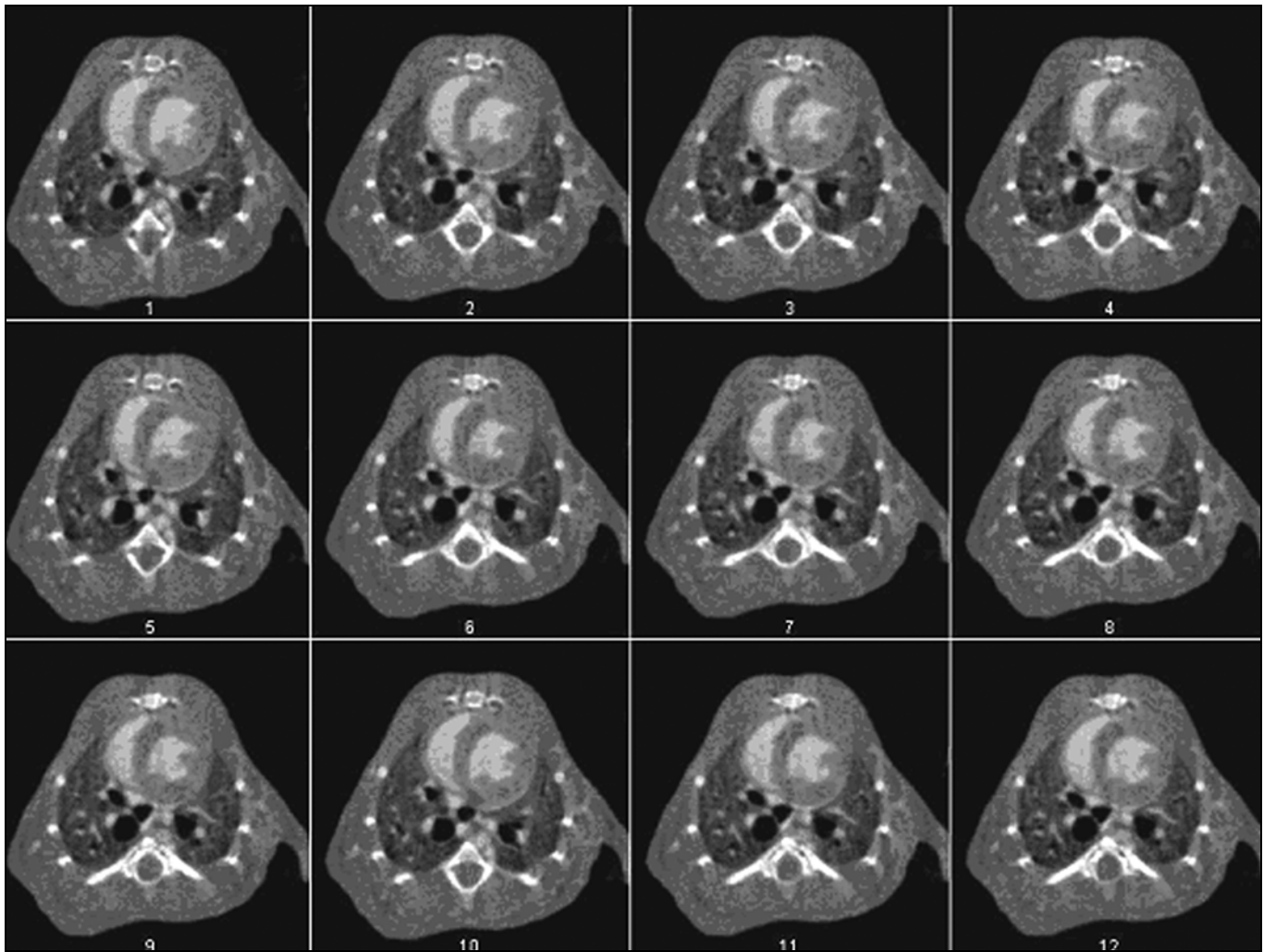
### Discussion

With the current emphasis on imaging in both drug development and therapeutic monitoring, there is an enhanced need for facile preclinical imaging in small animal models. The goal of this study was to determine if the use of liposomal contrast agents for high-resolution micro-CT of low-contrast, small-size vessels in murine models was feasible. We showed that liposomal formulations encapsulating high concentrations of iodinated contrast agents, which have long residence times and produce stable, high opacification of the

blood pool, can produce much greater conspicuity of anatomic features of small size than previously described low-concentration agents. These properties provide a potential means for micro-CT to fulfill the imaging requirements of modern preclinical studies.

In a previous study, we showed that a liposomal formulation containing a relatively low iodine concentration of 37 mg I/mL could produce enhancement levels of 150 to 200 H in an anesthetized rabbit (rather than mouse) model on a clinical whole-body scanner (rather than on micro-CT as in the present study) [2]. In addition, that study did not use cardiac and respiratory gating as we did in the present study. The previous study was adequate to illustrate the concept of iodinated liposomes as a blood pool contrast agent with a long circulation half-life. Large vessels were readily depicted, but we were unable to resolve very small (< 1 mm) features because the contrast conspicuity at the beam energies and intensities used in the whole-body CT scanner were limited.

Although the low-concentration agent may be adequate for imaging of anatomic features of greater size than present in a mouse on a whole-body scanner, the relatively low iodine concentration would necessitate unacceptably high injected volumes to adequately



A

**Fig. 6**—Images through thorax obtained at 12 distinct intervals during the cardiac cycle.  
**A**, Single transverse axial section of heart.  
**(Fig. 6 continues on next page)**

opacify the mouse microvasculature for detection with micro-CT. These limitations were overcome in the present study by using a high-iodine-concentration contrast agent capable of creating adequate contrast resolution to opacify the structure and a micro-CT system with spatial resolution capable of resolving tiny murine anatomic features.

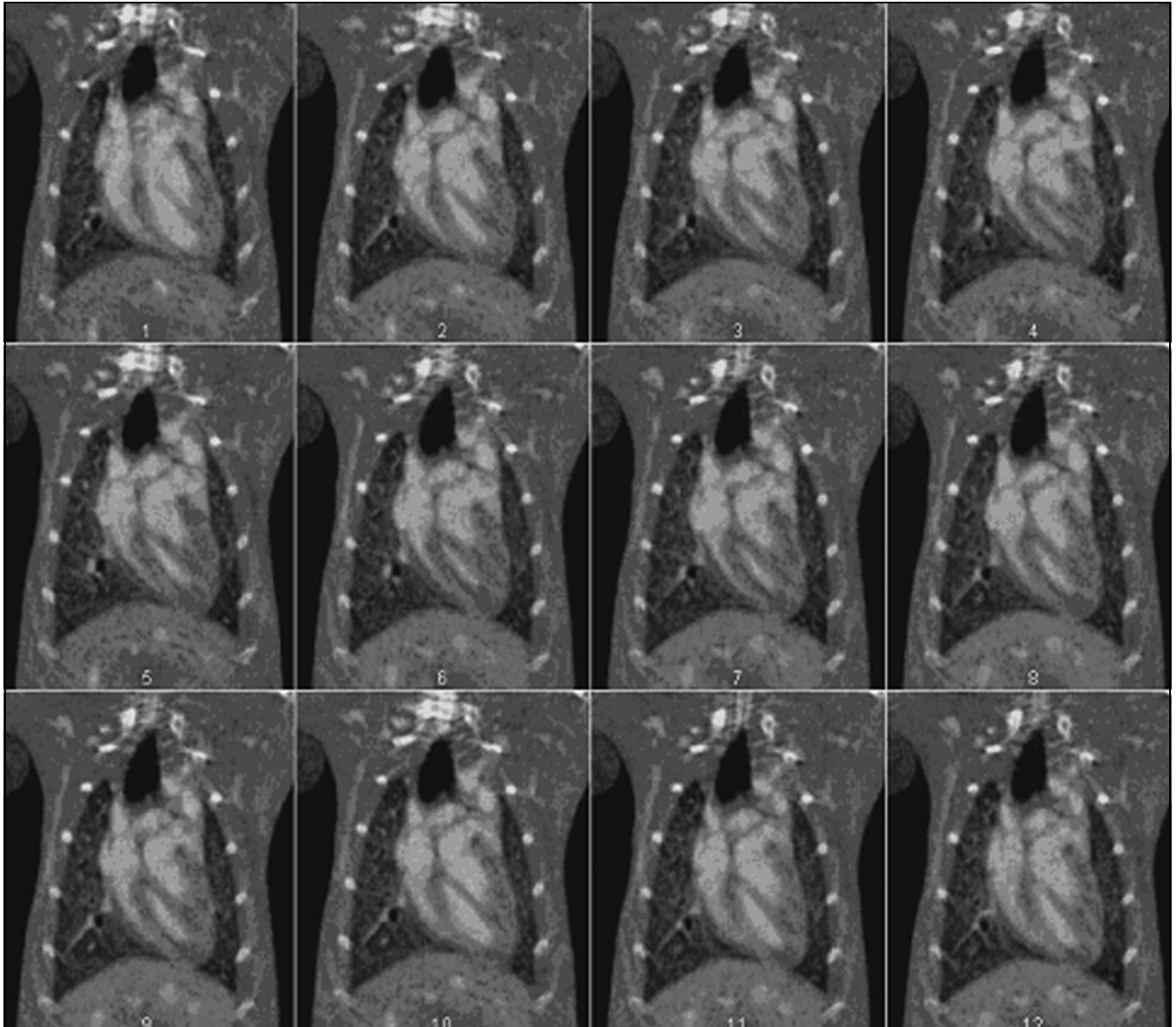
Assuming adequate spatial resolution to resolve an anatomic feature, the conspicuity of the feature on a CT scan is dependent on two primary factors: the contrast resolution and the noise level of the scanner. The contrast-to-noise ratio, which is defined as the ratio between contrast difference and noise, is the measurement used to define contrast resolution,

whereas noise is usually defined as the SD of the measured CT density in a region of uniform attenuation (usually water). Therefore, in order for a feature to be clearly visible, its opacity needs to be greater than both the contrast resolution limit and the noise limit. Also, it is important to note that physiologic motion such as cardiac and respiratory motion usually reduces the effective spatial resolution [8].

Noise limits are primarily dependent on the scanner used. Typical whole-body scanners have a noise limit between 1–3 H (0.1–0.3%) and typical micro-CT scanners have noise limits of 50–100 H [1]. For a human interpreter, the performance sensitivity is known to be defined by the Rose condition, which

states that to reliably discriminate between two densities, the contrast difference should be 3 to 5 times greater than the noise [10]. Therefore, for a clinical scanner for humans, differences of 5 to 10 H are frequently detectable. On our micro-CT system, we would require two structures to have a mean attenuation difference of approximately 150 H to distinguish between them.

Using our liposomal preparation from the previous study, containing an iodine concentration of 37 mg I/mL [2], it is likely that an infusion volume of approximately 60% of total blood volume would be needed to achieve opacification sufficient for visualization of the smaller features in mice im-



**B**

**Fig. 6 (continued)**—Images through thorax obtained at 12 distinct intervals during the cardiac cycle.  
**B.** Coronal section of heart.

aged on a micro-CT instrument. Therefore, the goal of this study was to determine if the use of liposomal contrast agents at acceptable infusion volumes (i.e., < 30% of total blood volume) for high-resolution micro-CT of low-contrast, small-size vessels in murine models was feasible. The data in the present study show that the infused dose (0.5 mL/25 g) of the approximately 100 mg I/mL liposomal preparation elicited nearly 800-H relative enhancement, which proved sufficient for the much greater conspicuity of anatomic

features of small size and higher resolution images needed for micro-CT.

Torchilin et al. [11] investigated the use of micelle-encapsulated iodinated contrast agents to take advantage of the intrinsic long circulating time of these particles. They speculated that the monolayer boundary or envelope of the micelles was more efficient than the bilayer composition of liposomes. They synthesized maleimide-polyethylene glycol-iodine-polylysine micelles with an iodine content of 34%, which resulted in a low overall concentration of 22 mg

I/mL that required large injection volumes of approximately several milliliters in rats. Although these investigators found a threefold enhancement in the aorta over a 3-hr period (i.e., approximately 250 H at 170 mg I/kg dose), the intrinsic inability of these particles to hold large concentrations of iodine and the resultant necessarily large infusion volumes have resulted in low enthusiasm for this approach.

Desser et al. [12] attempted to produce a liposomal CT contrast agent by preparing large liposomes (550 nm in diameter compared

with the 100-nm liposomes used in our study) that encapsulated iodixanol. Although the preparation contained about 200 mg I/mL, only 40% of it was encapsulated, providing a liposomal concentration of 80 mg I/mL but resulting in an unacceptably large unencapsulated fraction. Furthermore, at this large size, vesicles were cleared rapidly by the RES despite a hydrophilic (PEGylated) coating, and the agent had poor circulation time. In their study, a dose of 150 mg I/kg produced initial enhancements of about 360 H, which rapidly dropped below 80 H in less than 5 min because the unencapsulated iodine cleared via renal excretion whereas the encapsulated portion cleared via the RES. This preparation therefore suffered from potential renal toxicity problems based on the unencapsulated iodine fraction and also failed to enhance circulation time. Thus, it provided no practical advantage over conventional contrast agents.

Using another approach, Sachse et al. [13] incorporated iopromide into 150-nm stealth (PEG-coated) liposomes. However, at this size, the clearance rate of liposomes allowed only 71-H enhancement after 45 min despite a relatively high dose of 250 mg I/kg. Leander [14] developed liposome-encapsulated iodixanol particles with a particle size of 350 nm and an iodine content of 200 mg I/mL. However, the formulation consisted of 40% of the total iodine within the liposomal interior and the remaining 60% in the external phase. A human phase I clinical study of the formulation indicated rapid clearance of the agent from blood circulation due to the large particle size of the nanoparticles [15]. This resulted in a maximum initial blood pool enhancement of about 150 H, which dropped below 80 H in less than 10 min.

In another study, Bakan et al. [16] tested a lipid emulsion containing polyiodinated triglycerides for hepatocyte-specific delivery. Although these emulsions had a nominal particle size of 100 nm, they were sequestered in the liver quite rapidly, possibly due to the lack of a hydrophilic coating. Also, with a maximum iodine content of 50 mg I/mL, they exhibited relatively low, transient blood pool enhancement and a maximum hepatic enhancement of only approximately 120 H. The same investigators have since tested a PEGylated version of the lipid emulsion with longer circulation time and higher iodine concentrations [17]. The circulation time is still modest, approximately 60 min, and the blood pool enhancement is modest. However, the particles appear to localize quite well in inflammatory lymph nodes, thought by

the authors to be due to the chylomicron-remnant-like structure of the lipid molecule. This preparation was used to depict lymph nodes in a dog model and exhibited about 200-H enhancement in some nodes. Based on the research, a blood pool contrast agent is commercially available as Fenestra VC (Alerion Biomedical) containing iodine in a concentration of 50 mg/mL. This contrast agent was recently used for a cardiac micro-CT study by Badea et al. [9] and provided a relatively constant enhancement over 3 hr, with maximum enhancement of approximately 620 H (aorta) and 90 H (kidney cortex). The maximum enhancement difference between blood and myocardium in the heart was approximately 500 H.

Measurement of the degree of iodine leakage in our formulation was important to assess the risk of excessive exposure of the subject to free iodine and the attendant risks of renal toxicity as well as anaphylaxis in sensitive individuals. Our in vitro stability study showed that the formulation is stable within liposomes and hence has a shelf life of at least 7 days in phosphate buffer solution at 4°C. Leakage of only approximately 3% of the total iodine was found, and that leakage was likely due to the osmolarity or concentration difference between the interior of liposomes and the external environment. This degree of leakage is sufficiently low to practically eliminate renal toxicity as a realistic concern. Thus, in clinical use, agents such as ours would not likely be contraindicated in patients with renal insufficiency, as is the situation with conventional iodinated agents.

At equivalent doses of iodine, conventional agents would expose the renal medulla to more than 30 times the iodine dose associated with the use of our encapsulated agents. Thus, the liposomal encapsulation process greatly reduces overall iodine exposure. In fact, it is possible that these agents may be administered preferentially in individuals with renal insufficiency. Although it is also possible that the liposomes described here may confer some protection against anaphylaxis compared with standard iodinated contrast agents, further work along this line of investigation is needed before definitive claims can be made.

In summary, our study shows that a liposomal formulation encapsulating a high concentration of iodine molecules has many properties that optimize its use as a contrast agent for small animal imaging on a micro-CT scanner, including a long residence time and a high degree of opacification of very small blood vessels. The long residence time eliminates the

need for bolus infusion and rapid scanning, which are presently substantial limitations for dynamic contrast-enhanced imaging on micro-CT scanners imposed by the long scan times. Many features of our liposomal contrast agent formulation, including the low level of free iodine (thereby minimizing risk of renal toxicity) and long residence time (making them ideal blood pool contrast agents), render them potentially highly useful imaging agents and deserving of consideration for experimental trials in humans.

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