Published in final edited form as:

J Control Release. 2008 November 12; 131(3): 160–166. doi:10.1016/j.jconrel.2008.07.030.

Dynamic microPET imaging of ultrasound contrast agents and lipid delivery

Michaelann S. Tartis, PhD^{1,2}, Dustin E. Kruse, PhD¹, Hairong Zheng, PhD¹, Hua Zhang, PhD¹, Azadeh Kheirloom, PhD¹, Jan Marik, PhD¹, and Katherine W. Ferrara, PhD¹

¹ University of California, Davis, One Shields Ave, Davis, CA 95616

² New Mexico Tech, 801 Leroy Pl, Socorro, NM 87801

Abstract

Interest in ultrasound contrast agents (lipid-shelled microbubbles) as delivery vehicles is increasing; however, the biodistribution of these agents remains uncharacterized, both with and without ultrasound. In this study, an ¹⁸F-labeled lipid ([¹⁸F]fluorodipalmitin), incorporated in microbubble shells, was used as a dynamic microPET probe for quantitative 90-minute biodistribution measurements in male Fischer 344 rats (n = 2). The spleen retained the highest concentration of radioactive lipid at ~2.6 percent-injected dose per cubic centimeter (% ID/cc) and the liver demonstrated the largest total accumulation (~17 % ID). The microbubble pharmacokinetic profile differed from free lipid, which is rapidly cleared from blood, and liposomes, which remain in circulation. Additionally, region of interest (ROI) analysis over 60 minutes post-ultrasound treatment quantified the delivery of lipid by therapeutic ultrasound from microbubbles to kidney tissue (n = 8). The ultrasound sequence consisted of a 200 kPa, 5.3 MHz radiation force pulse followed by a 1.6 MPa, 1.4 MHz fragmentation pulse and was applied to one kidney, while the contralateral kidney served as a control. ROI-estimated activity in treated kidneys was slightly but significantly greater at 0 and 60 minutes than in untreated kidneys (p = 0.0012 and 0.0035, respectively). This effect increased with the number of microbubbles injected (p = 0.006). In summary, [¹⁸F]fluorodipalmitin was used to characterize the biodistribution of contrast microbubble shells and the deposition of lipid was shown to be locally increased after insonation.

Keywords

Ultrasound contrast agents; microPET; targeted drug delivery; [¹⁸F]fluorodipalmitin ([¹⁸F]FDP); therapeutic ultrasound

Introduction

Ultrasound contrast agents are widely used to image perfusion and have been proposed as a carrier for the local delivery of drugs and genes, where therapeutic release from the carrier would be initiated by local insonation [1–2]. Lipid-shelled microbubble contrast agents have a self-assembled shell that provides a flexible, protective membrane around a perfluorocarbon

Corresponding Author Information: Katherine Ferrara, Department of Biomedical Engineering, University of California, Davis, 451 East Health Sciences Dr., Davis, CA 95616, phone: (530) 754-9436 fax: (530) 754-5739, kwferrara@ucdavis.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

gas core. In the clinic, these agents have been successfully used in the measurement of blood volume and flow in cardiology and radiology [13,14]. By incorporating a targeting ligand with a high affinity for intravascular targets into the microbubble shell, local inflammation and angiogenesis have been detected in pre-clinical studies [15,16]. In addition, gene delivery from microbubbles to the vascular endothelium, heart muscle, and pancreas has been demonstrated in pre-clinical studies, in some cases producing a therapeutic level of a protein [2,4,17,18]. However, the microbubble dose required for therapeutic levels of gene delivery was high ($\sim 5 \times 10^9$ bubbles/kg), substantially greater than the human dose used in typical imaging studies ($\sim 5 \times 10^9$ bubbles/30 kg). In addition to ultrasound contrast agent targeting for imaging and delivery of a therapeutic molecule, bioeffects have been observed under various conditions. In the presence of an ultrasound contrast agent, insonation of the kidney with a center frequency equal to or below 1.8 MHz and a peak negative pressure (PNP) of 1.2 MPa has been shown to produce renal damage [19]. Although a variety of ultrasound contrast agent applications have been studied, the delivery of material from the shell to the endothelium has not yet been demonstrated.

Developing strategies for gene and drug delivery with microbubbles requires an improved understanding of the mechanism for delivery and models for the transport of the shell material. Until this time, quantitative data for the biodistribution and pharmacokinetics of the shell material have not been obtained. In this study, a recently-developed ^{18}F -labeled lipid probe for positron emission tomography was used. These labeled lipids can be inserted into vehicles with varied surface architectures [20]. Here, this probe was used to evaluate the biodistribution of the lipid material in the microbubble shell, with and without the application of ultrasound to disrupt the vehicle. A high (therapeutic) dose of contrast agent was injected, as employed in [2], and the fractional injected dose of shell material deposited in the kidney as a result of ultrasound was measured.

Materials and Methods

Biodistribution in rats

All animal experiments were conducted under protocols approved by the Institutional Animal Use and Care Committee. Male Fischer 344 rats (Harlan, Indianapolis, IN) served as the animal model. All rats were housed one animal per cage in a 12 hour light cycle environment and allowed access to water and a standard rat chow *ad libitum*. For all procedures, induction of anesthesia was achieved at 3.0–3.5% isoflurane (Halocarbon Laboratory, River Edge, NJ) and maintained at 2.0–2.5%. Respiratory rate and temperature were monitored throughout each procedure to ensure proper levels of anesthesia and comfort. Body temperature was maintained by placing the animals on a heating pad during all procedures and scans, with the exception of catheterization. The rats were catheterized to ensure proper injection of various lipid formulations as well as the euthanasia solution, pentobarbital, in terminal studies. In nonterminal studies, animals were given a 1.0 mL subcutaneous injection of physiologic saline in the scruff of the neck to aid in recovery.

Synthesis of radiolabeled lipid microbubbles

The methods for synthesis of [^{18}F]fluorodipalmitin ([^{18}F]FDP) followed those described in [20]. Freshly prepared [^{18}F]FDP in dichloromethane was transferred to a 2 mL vial, the solvent was evaporated by a stream of nitrogen, and the dry [^{18}F]FDP was combined with 1.5 mL of a lipid mixture which ordinarily produces microbubbles for use as ultrasound contrast agents (1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC); 1,2-Dipalmitoyl-sn-Glycero-3-Phosphate (Monosodium Salt) (DPPA); 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-5000] (Ammonium Salt) (DPPE-PEG5000) in molar ratio 0.82:0.1:0.08)[21]. The solution was sonicated for 15–20 minutes at

55–60 °C to obtain a clear solution then allowed to cool to room temperature. Perfluorobutane gas (30 mL) was bubbled through the solution by inserting a needle and syringe through the vial septum, such that air was purged through a vent and effectively replaced the headspace. The vial was then shaken using Vialmix shaker (Bristol-Myers Squibb Medical Imaging, North Billerica, MA) for 45 seconds. The microbubble suspension was withdrawn into a 3 mL syringe containing phosphate buffered saline and capped to prevent air exposure. The syringe was centrifuged at 1000 rpm for 2 minutes, then the aqueous solution was ejected from the bottom of the syringe and fresh phosphate buffered saline solution was added. This wash procedure was repeated two times for a total of three washes to ensure that unincorporated [^{18}F]FDP molecules were eliminated and that only radiolabeled microbubbles remained in solution (Figure 1). Approximate bubble concentration and size were measured using an Accusizer optical particle counter (Particle Sizing Systems, Santa Barbara, CA) 24 hours post-synthesis (the passing of roughly 12 half-lives) to ensure radioactive decay.

Positron emission tomography (PET)

All PET acquisitions were obtained with the microPET Focus (Siemens Medical Solutions, USA, Inc.). Microbubble solutions were diluted in saline just prior to injection to obtain the desired radioactive content and injected directly into the catheter port. The catheter was removed immediately following the injection to measure residual activity for an accurate calculation of total injected dose. Injected dose was reported as the measured activity in the syringe and needle just prior to injection minus the residual activity within the syringe, needle, catheter, catheter port, and gauze used to halt bleeding after catheter removal. Initial full-body microbubble biodistribution images obtained to evaluate signal to noise ratio used approximately 37 MBq and contained more than 3×10^9 microbubbles per injection. This was reduced to approximately 7.4 MBq and $1\text{--}2 \times 10^9$ microbubbles as the higher activity was not required. Additionally, the $1\text{--}2 \times 10^9$ microbubble injection matched microbubble concentrations reported in other pre-clinical studies of drug and gene delivery. All acquisition and histogram protocols were created and executed with microPET manager software (Siemens Medical Solutions, USA, Inc.) prior to reconstruction. Subsequent image analysis was performed with ASIPro software (Siemens Medical Solutions, USA, Inc.) resulting in time activity curves in units of percent-injected dose per cubic centimeter (% ID/cc). In all cases, quantification of tissue uptake was done blinded to animal and treatment.

Three scanning protocols were used in this study and are described below with designations biodistribution images, quantitative biodistribution and quantification of probe deposition from therapeutic ultrasound. In the biodistribution protocols, the injection was timed and administered over approximately 1 minute. For the quantification of probe deposition from therapeutic ultrasound, a constant rate infusion was used to deliver the solution over 20 minutes while ultrasound was applied.

Biodistribution images—For biodistribution studies, the injection of tracer material was performed on the bed and commenced simultaneously with PET acquisition initiation. The first set of biodistribution acquisitions obtained full-body biodistribution images by using 90-minute continuous bed motion (CBM) scans. A CBM scan allows visualization of the entire rat by moving the bed in and out of the field-of-view (FOV) at a specified rate. Full body image reconstructions were obtained using the maximum a posteriori (MAP) reconstruction algorithm and maximum intensity projections (MIPs) were created using ASIPro software (Siemens Medical Solutions, USA, Inc.) to illustrate these results.

Quantitative biodistribution—A second biodistribution protocol involved 90-minute scans in which the rat torso was continuously in the FOV, facilitating quantification of the biodistribution in major organs. The 90-minute cumulative images were used to draw regions

of interest (ROIs) with ASIPro. To minimize partial volume averaging effects, the regions were drawn conservatively on the interior of each organ border. The dynamic PET data were then dynamically binned into 30 frames: 8×15 sec, 8×1 min, 5×3 min, 5×5 min, and 4×10 min to create time activity curves (TACs) for each organ of interest in the field of view. The mean activity of an organ at each time point was calculated as %ID/cc.

Quantification of probe deposition from therapeutic ultrasound—To quantify changes in the biodistribution due to the ultrasound treatment, a third acquisition protocol included a 60-minute scan of the lower abdomen after a 20-minute ultrasound treatment to one kidney. The treated kidney was randomly chosen with 4 treatments involving the right and 4 involving the left kidney. ROI analysis was performed and reported in the same manner as the 90-minute quantitative scans. In order to obtain a standard for [^{18}F]FDP uptake in various organs, a subset of animals were euthanized directly after the completion of PET scans and organs harvested. The excised organs, or samples thereof, were placed in pre-weighed plastic tubes, weighed for sample mass, and assessed for radioactivity (Wallac Wizard 1470 Automatic gamma counter, Perkin Elmer Life Sciences, Shelton, CT USA). These measurements were acquired in units of percent-injected dose per gram of tissue (% ID/g). For comparison to data obtained by ROI analysis, a density assumption of 1.0 g/cc was made for kidney tissue, to convert activity measured in harvested organs from units of % ID/g to %ID/cc.

Therapeutic ultrasound

Ultrasound was applied to one kidney in each rat such that the contralateral kidney received no ultrasound and served as a control. Using the inner array of the new triple row CoLinear 1–6 ultrasound probe developed by Siemens [22] on the Siemens SONOLINE Antares™ (Siemens Medical Solutions USA, Inc., Ultrasound Division, Mountain View, CA), it was possible to image the kidney and position the probe so that an optimal plane was chosen for the “therapeutic” pulse sequence, referred to in this paper as therapy. The therapeutic sequence was designed as a sequence to maximize delivery of a drug or gene from the microbubble shell to the surrounding endothelium. As the microbubble infusion began, B-mode imaging was used to detect the arrival of microbubbles within the renal vasculature. Once contrast enhancement was visually confirmed, the therapeutic ultrasound pulse sequence was applied using a specific combination of high and low frequency pulses from both the inner array (high frequency) and outer arrays (low frequency) of the transducer for a total of 20 minutes. Color Doppler images were displayed at the end of each pulse sequence, providing a qualitative measure of the amount of contrast left in circulation.

The therapeutic pulse sequence contained a radiation force segment, to translate microbubbles to the endothelial surface, followed by a fragmentation segment, to disrupt the microbubble shell, similar to other drug delivery sequences reported in the literature [1,23]. Radiation force was achieved in Doppler mode with the following parameters: a 20-cycle pulse at a PNP of 200 kPa, a center frequency of 5.3 MHz, and a pulse repetition frequency of 2441 Hz for a two-second duration. Fragmentation was achieved in color Doppler mode with the following parameters: a single frame of a 6-cycle pulse at a PNP of 1.6 MPa and a center frequency of 1.4 MHz. The time between the application of radiation force pulse and the fragmentation pulse was 0.2 seconds, and 10 seconds elapsed between repetitions of the entire radiation force – fragmentation sequence.

The previously described microPET images were acquired immediately following the 20 minutes of therapeutic ultrasound. A total of eight rats over four days were treated to quantify deposition of radiolabeled lipid to the ultrasound-treated kidney. Each day, [^{18}F]FDP-labeled microbubbles were freshly synthesized with sufficient labeled and purified microbubbles produced for two treatments. Due to radioactive decay, roughly twice as many microbubbles

were injected into the second animal compared with the first animal of each day, while injection volume was held constant. The injected radioactivity was closely matched for each image acquisition, providing the opportunity to compare the results with two microbubble concentrations.

Statistical analysis

The sample size used in this experiment was determined by a power analysis based on a paired t-test (Power and sample size calculations program, version 2.1.31; W.D. Du Pont and W.D. Plummer, <http://www.mc.vanderbilt.edu/prevmed/ps/index.htm>). The standard deviation parameter, $\sigma = 0.05$, was obtained from a previous, unpublished study with an ^{18}F probe, showing that the measured difference in activity between left and right kidneys varied by approximately 5%. This information, along with a Type I error probability value, $\alpha = 0.05$, power of 0.8, and mean difference of $\delta = 0.2$, yielded a calculated minimum sample size of 3 pairs. A total of 8 rats were used in the delivery study with one kidney serving as a control, while the other was treated. In addition, concentration effects were studied in 4 pairs. All statistics were performed using Prism 3 (GraphPad Software Inc., San Diego, CA USA). The two-tailed, paired t-test was used to determine statistical significance between mean values, where a $p \leq 0.05$ was considered significant.

Results

MicroPET biodistribution

With permission of Marik *et. al.* [20], biodistribution data ($n = 3$) and representative images for free lipid and liposomes were plotted together with the findings in this study for the purpose of comparison. PET images and biodistribution data ($n = 2$) suggest that microbubble shell material has a different pharmacokinetic profile from that of freely-injected lipid and liposomal formulations (Figure 2, Figure 3, Figure 4). The mean microbubble diameter was $1.33 \mu\text{m} \pm 1.20 \mu\text{m}$, measured after a 24-hour decay period, for the studies in which microbubble biodistribution data were recorded (Figure 4C and 4D). For this microbubble distribution, the highest density of the microbubble shell material localized in the spleen, peaking in roughly 20 minutes at 2.6 % ID/cc. The activity level within the spleen remained more than twice that of kidneys, liver, lung and blood levels for the duration of the scan (Figure 4). The peak activity level within the lungs was roughly 1.3 % ID/cc immediately upon injection, decreasing to 0.8 % ID/cc at 90 minutes. The peak activity levels within the kidneys and liver were roughly 0.5 % ID/cc. The activity in the blood pool peaked immediately after injection at 1.2 % ID/cc and was reduced to roughly half of this peak value at 90 minutes. In scans where the bladder was in the field of view, elevated activity levels were observed at approximately 5 minutes (data not shown). Videos illustrating the biodistribution over the 90-minute scans for each formulation can be found in the online journal article (Supplementary Video 1, Supplementary Video 2, Supplementary Video 3). Images obtained from $\sim 37 \text{ MBq}$ injected dose (with the microbubble dose of 3×10^9 microbubbles per injection) revealed a buildup of shell material in the vena cava (Figure 2); this accumulation was not observed with the lower dose.

Ultrasound mediated delivery

Therapeutic ultrasound pulses were applied over 20 minutes (Figure 5), with the color image within the region of interest presenting the color flash associated with microbubble destruction. The intensity and spatial extent of the color flash observed on the ultrasound system decreased but were not completely eliminated during the 20-minute duration of insonation. Static microPET images were then obtained from the insonated region and facilitated quantification of the activity within each kidney over 60 minutes (Figure 5). Results from the ROI analysis were compared with activity levels in kidneys harvested immediately after the 60-minute scan, $n = 8$ (Figure 6).

Without attenuation or scatter fraction correction, ROI analysis underestimated activity within all kidneys, as compared with the harvested organ analysis. The linear regression between the ROI measurements and the harvested measurements deviates both in slope and offset from $y = x$ ($y = 0.56x + 0.05$).

A change in the intensity of the insonified kidney was not apparent on the microPET images; however, analysis demonstrated a significant difference. On average and at all time points, the increase in activity in the treated kidney relative to the untreated kidney was ~15 – 20 % in both the harvested and ROI analyses (Figure 6), where the activity as detected by PET was significantly greater at the 0 and 60 minute time points ($p = 0.0012$ and 0.0035 , respectively). The absolute difference between treated and untreated kidney activity was typically less than 0.1 %ID/cc. This difference fluctuated by less than 0.05 %ID/cc over the 60 minute scan. The estimated amount of lipid deposited within the treated kidney was 0.204 ± 0.166 μg , calculated using harvested activity measurements. This measurement represents an average over both concentration groups, which had significantly different amounts of material deposited (Figure 7).

Approximately $1\text{--}2 \times 10^9$ microbubbles were injected into a blood volume of ~12 ml or 2×10^5 microbubbles/ μl (as compared with a typical human bolus dose of 2×10^3 microbubbles/ μl). Although the number of bubbles injected was high compared to the clinical dose in all cases, the % ID/cc of the ^{18}F probe deposited within the kidney, as estimated using PET, was significantly greater when the total number of bubbles injected was doubled ($p = 0.006$) (Figure 7).

As noted earlier, this study was conducted with ultrasound parameters known to produce hematuria in small animals in the presence of ultrasound contrast agents. In this study, red cell extravasation was noted during examination of kidney tissue that was in the insonified volume in all 8 treated kidneys.

Discussion

Dynamic PET imaging demonstrated substantial differences in the biodistribution of lipid-shelled microbubbles as compared to liposomes or free lipid under the same experimental conditions. Following the injection of free [^{18}F]FDP in a diluent, activity accumulated in the liver (~ 5.75% ID/cc peak) and spleen (~ 4.5% ID/cc peak) within the first 10 minutes and cleared to levels equivalent to blood, lung, and kidneys (< 1% ID/cc) by the end of a 90-minute scan [20]. Full body images indicated that the ^{18}F label is eliminated through the urine and it was hypothesized that the [^{18}F]FDP was metabolized within the liver freeing the [^{18}F] fluoropropane-2,3-diol. Similarly, Marik et al showed that a liposomal formulation, composed of a variety of lipids, remained in circulation at a stable level (~ 2.5% ID/cc) for more than 90-minutes, with blood activity levels twice the levels observed in liver, kidneys and spleen [20]. Here, the circulation time of the lipid contained within the microbubbles shell was less than the circulation time of a PEGylated liposome but greater than the freely-injected lipid.

The lipid shell material was traced in this study and we recognize that the pharmacokinetics of the gas core of the particle are not expected to be identical to the shell. When using microbubbles for imaging it is the kinetics of the gas that are important; however, for delivery of drugs or genes attached to the shell, the kinetics of the shell become important. Studies of microbubble echogenicity within the kidney obtained with similar microbubbles to those used here demonstrated a blood half-life of 4 minutes for the echogenic particle [24]. In this study, the ultrasound images demonstrated that a fraction of the injected dose of microbubbles continued to circulate throughout a 20-minute insonation, possibly as a result of the high concentration that was injected.

The accumulation of the lipid shell material within the spleen observed after the injection of microbubbles was also expected, since the diameter of microbubbles, around 1 – 10 μ , exceeds the 200–500 nm diameter of channels within the spleen [25,26]. The concentration of shell material in the spleen remained nearly constant throughout the 90-minute duration of these studies. Harvested organ data show that 7.1 ± 2.57 % of the total injected dose was found in the spleen, as compared with more than 14.3 ± 4.83 % of the total dose in the liver (estimating liver mass from body weight [27]). It is possible that the small percentage of large microbubbles within the injected microbubble population may be responsible for the high accumulation in the spleen. A monodisperse and smaller microbubble population may reduce the accumulation.

Unfortunately, practical constraints precluded immediate size measurement of radiolabeled microbubbles. This measurement took place only after a 24-hour decay period in which the microbubble solution was placed behind shielding at room temperature. During this period the size distribution may have shifted, with larger microbubbles likely persisting longer. Additionally, it has been demonstrated that accumulation of particles in the spleen is different from species to species due to different capture mechanisms [28].

Dynamic PET imaging was shown to be capable of quantifying lipid material deposited as the result of ultrasound pulses. The overall volume of material delivered to the insonified kidney was relatively small (μ g/plane of ultrasound) in comparison to what is desired for drug delivery techniques [29], particularly given the large dose of the administered contrast agent. The protocol used here is similar to those shown to produce successful gene transfection in other studies of the pancreas [2] thus the small amount of material deposited in such a protocol may have a significant impact if the deposited material has been transported across the endothelium as in [30]. In the future, several mechanisms may greatly improve the efficiency of this drug delivery method. *In vitro*, delivery of materials from the microbubble surface into cells is greatly increased by the presence of liposomes/nanoparticles on the bubble surface [31]. Attachment of particles with a small radius of curvature to the microbubble shell may greatly increase the fusogenicity of the drug-carrying lipid vehicle [31–33]. The shell material employed in this study was equivalent to that of a standard contrast agent, with no attempt to increase specific or nonspecific adhesion. The addition of targeting ligands to the microbubble surface can also increase adhesion of the lipid material to the endothelium. Finally, this was a feasibility study to determine whether localization of the shell material could be detected with PET. The ultrasound parameters used in this study were optimized for *in vitro* transfer of material but have not yet been optimized for insonation of microbubbles *in vivo*. Decreasing the pressure during the radiation force portion of the pulse may reduce the number of microbubbles destroyed before reaching a vessel wall, which in turn may increase the amount of deposited material. In addition, three-dimensional scanning of the ultrasound probe to cover a larger region of the kidney may increase deposition. Here, the pulse sequence was applied only to one plane.

Images obtained after the injection of a high concentration of microbubbles show the challenges of repeatable systemic administration of large concentrations of buoyant agents through the tail vein. A 1-minute bolus administration of microbubbles containing roughly 37 MBq of activity, used to acquire the dynamic data required for initial studies, resulted in a dense plug of labeled lipid within the inferior vena cava. In order to avoid this effect, the bolus must be delivered over a longer time period or at a lower concentration, as used in all subsequent studies. While the effect of buoyancy in sequestering microbubbles in veins is less likely to impact delivery in human studies, some investigators have reported that massage of the area near the site of injection can free additional microbubbles in human studies [34]. One out of the eight treated animals receiving a combination of microbubbles and ultrasound died after insonation and while under anesthesia of an unknown cause. In this case, there was no evidence of a build up of material in the vena cava or elsewhere in the region of the acquired microPET scan.

A systematic difference between the %ID/cc estimated by PET and % ID/g estimated by gamma counts was noted. Due to the size of the sample tubes, the kidney was bisected prior to gamma count measurement. If fluid was lost in this process, the volume of the kidney may be reduced and the effective % ID/g increased. Alternatively, corrections for attenuation and scatter were not applied to the PET data leading to a reduced measured activity of centrally-located organs [35–37]. In the past these corrections have generally been assumed to be minimal given the size of small animals such as rats [38]. However, simulations and phantom studies have indicated that attenuation and scatter correction can improve resolution by 0.2 mm, uniformity by 12% and contrast by 3–5% [38]. Subsequent studies have shown that attenuation correction (using a global scaling factor, emission segmentation, transmission data, CT data, or other techniques [37]) is useful for quantitative studies in small animals [36]. In the future, transmission scans will be acquired for attenuation correction.

The ultrasound parameters used here were anticipated to produce local damage to the endothelium as shown in [19,30]. Red cell extravasation, as observed, may limit the use of such parameters in the clinic or it may be transient. Direct correlation of the PET measurements with histology was not possible as the samples were used for gamma counts and thus not immediately preserved. Future studies should characterize and quantify these effects.

In conclusion, this is the first time that microbubble biodistribution has been demonstrated on a full body scale *in vivo*. Microbubbles have a unique pharmacokinetic profile and knowledge of this profile can serve as a starting point for drug delivery applications. In this rat model, the density of accumulation of microbubble shell material was greatest within the spleen (~ 2.7 % ID). Without a targeting ligand, the volume of shell material detected within the insonified region was small (<1% ID) but significant. This dynamic microPET technique may be valuable in future studies where radiolabeled therapeutic molecules are used in conjunction with ultrasound and microbubbles to validate spatially localized delivery *in vivo*, in this case local deposition of lipid by ultrasound in renal tissue is confirmed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the following grants: NIH CA 103828 and EB002952. The authors would like to thank the Center for Molecular and Genomic Imaging and the individuals who have contributed to this work: Jennifer Fung for technical assistance during scanning and figure preparation, Dave Kukis for cyclotron operation, Dr. Doug Rowland for helpful discussions.

References

1. Tartis MS, McCallan J, Lum AF, Labell R, Stieger SM, Matsunaga TO, Ferrara KW. Therapeutic effects of paclitaxel-containing ultrasound contrast agents. *Ultrasound Med Biol* 2006;32(11):1771–1780. [PubMed: 17112963]
2. Chen SY, Ding JH, Bekerredjian R, Yang BZ, Shohet RV, Johnston SA, Hohmeier HE, Newgard CB, Grayburn PA. Efficient gene delivery to pancreatic islets with ultrasonic microbubble destruction technology. *P Natl Acad Sci USA* 2006;103(22):8469–8474.
3. Kimmel E. Cavitation bioeffects. *Crit Rev Biomed Eng* 2006;34(2):105–161. [PubMed: 16749890]
4. Kipshidze NN, Porter TR, Dangas G, Yazdi H, Tio F, Xie F, Hellinga D, Wolfram R, Seabron R, Waksman R, Abizaid A, Roubin G, Iyer S, Colombo A, Leon MB, Moses JW, Iversen P. Novel site-specific systemic delivery of Rapamycin with perfluorobutane gas microbubble carrier reduced neointimal formation in a porcine coronary restenosis model. *Catheter Cardio Inte* 2005;64(3):389–394.

5. Korpany G, Chen S, Shohet RV, Ding J, Yang B, Frenkel PA, Grayburn PA. Targeting of VEGF-mediated angiogenesis to rat myocardium using ultrasonic destruction of microbubbles. *Gene Ther* 2005;12(17):1305–1312. [PubMed: 15829992]
6. Unger EC, Hersh E, Vannan M, Matsunaga TO, McCreery T. Local drug and gene delivery through microbubbles. *Prog Cardiovasc Dis* 2001;44(1):45–54. [PubMed: 11533926]
7. Kheirrolomoom A, Dayton PA, Lum AFH, Little E, Paoli EE, Zheng HR, Ferrara KW. Acoustically-active microbubbles conjugated to liposomes: Characterization of a proposed drug delivery vehicle. *J Control Release* 2007;118(3):275–284. [PubMed: 17300849]
8. Lee LY, Wang CH, Smith KA. Supercritical antisolvent production of biodegradable micro- and nanoparticles for controlled delivery of paclitaxel. *J Control Release* 2008;125:96–106. [PubMed: 18054107]
9. Suzaki R, Takizawa T, Negishi Y, Utoguchi N, Sawamura K, Tanaka K, Namai E, Oda Y, Matsumura Y, Maruyama K. Tumor specific ultrasound enhanced gene transfer in vivo with novel liposomal bubbles. *J Control Release* 2008;125:137–144. [PubMed: 18035442]
10. Suzuki R, Takizawa T, Negishi Y, Hagsawa K, Tanaka K, Sawamura K, Utoguchi N, Nishioka T, Maruyama K. Gene delivery by combination of novel liposomal bubbles with perfluoropropane and ultrasound. *J Control Release* 2007;117(1):130–136. [PubMed: 17113176]
11. Takahashi M, Kido K, Aoi A, Furukawa H, Ono M, Kodama T. Spinal gene transfer using ultrasound and microbubbles. *J Control Release* 2007;117(2):267–272. [PubMed: 17166615]
12. Vandenbroucke RE, Lentacker I, Demeester J, De Smedt SC, Sanders NN. Ultrasound assisted siRNA delivery using PEG-siPlex loaded microbubbles. *J Control Release* 2008;126(3):265–273. [PubMed: 18237813]
13. Leong-Poi H, Song J, Rim SJ, Christiansen J, Kaul S, Lindner JR. Influence of microbubble shell properties on ultrasound signal: Implications for low-power perfusion imaging. *J Am Soc Echocardiogr* 2002;15(10):1269–1276.
14. Brannigan M, Burns PN, Wilson SR. Blood flow patterns in focal liver lesions at microbubble-enhanced US. *Radiographics* 2004;24(4):921–935. [PubMed: 15256618]
15. Rychak JJ, Lindner JR, Ley K, Klivanov AL. Deformable gas-filled microbubbles targeted to P-selectin. *J Control Release* 2006;114(3):288–299. [PubMed: 16887229]
16. Ellegala DB, Leong-Poi H, Carpenter JE, Klivanov AL, Kaul S, Shaffrey ME, Sklenar J, Lindner JR. Imaging tumor angiogenesis with contrast ultrasound and microbubbles targeted to $\alpha(v)\beta3$. *Circulation* 2003;108(3):336–341. [PubMed: 12835208]
17. Xie F, Tsutsui JM, Lof J, Unger EC, Johanning J, Culp WC, Matsunaga T, Porter TR. Effectiveness of lipid microbubbles and ultrasound in de clotting thrombosis. *Ultrasound Med Biol* 2005;31(7):979–985. [PubMed: 15972204]
18. Bekereditian R, Chen SY, Grayburn PA, Shohet RV. Augmentation of cardiac protein delivery using ultrasound targeted microbubble destruction. *Ultrasound Med Biol* 2005;31(5):687–691. [PubMed: 15866418]
19. Dalecki D. WFUMB Safety Symposium on Echo-Contrast Agents: bioeffects of ultrasound contrast agents in vivo. *Ultrasound Med Biol* 2007;33(2):205–213. [PubMed: 17239521]
20. Marik J, Tartis MS, Zhang H, Fung JY, Kheirrolomoom A, Sutcliffe JL, Ferrara KW. Long-circulating liposomes radiolabeled with [^{18}F]fluorodipalmitin ([^{18}F]FDP). *Nucl Med Biol* 2007;34(2):165–171. [PubMed: 17307124]
21. <http://www.definityimaging.com/pdf/prescribinginfo.pdf>
22. Stephens, LXD.N.; Proulx, T.; Walters, W.; Dayton, P.; Tartis, M.; Kruse, DE.; Lum, AF.; Kitano, TK.; Stieger, SM.; Ferrara, KW. Multi-Frequency Array Development for Drug Delivery Therapies. Conf. Proc. IEEE Ultrason. Ferr; 2006. Session 5A-1
23. Shortencarier MJ, Dayton PA, Bloch SH, Schumann PA, Matsunaga TO, Ferrara KW. A method for radiation-force localized drug delivery using gas-filled lipospheres. *IEEE T Ultrason Ferr* 2004;51(7):822–831.
24. Borden MA, Sarantos MR, Stieger SM, Simon SI, Ferrara KW, Dayton PA. Ultrasound Radiation Force Modulates Ligand Availability on Targeted Contrast Agents. *Mol Imaging* 2006;5(3):139–147. [PubMed: 16954028]

25. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol Rev* 2001;53(2):283–318. [PubMed: 11356986]
26. Oku N. Delivery of contrast agents for positron emission tomography imaging by liposomes. *Adv Drug Deliver Rev* 1999;37(1–3):53–61.
27. Schoeffner DJ, Warren DA, Muralidara S, Bruckner JV, Simmons JE. Organ weights and fat volume in rats as a function of strain and age. *J Toxicol Env Health* 1999;56(7):449–462.
28. Demoy M, Andreux JP, Weingarten C, Gouritin B, Guilloux V, Couvreur P. Spleen capture of nanoparticles: influence of animal species and surface characteristics. *Pharm Res* 1999;16(1):37–41. [PubMed: 9950276]
29. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science* 2004;303(5665):1818–1822. [PubMed: 15031496]
30. Stieger CCSM, Qin S, Adamson RH, Curry RE, Wisner ER, Ferrara KW. Enhancement of vascular permeability with low frequency ultrasound. *Radiology* 2007;243(1):112–121. [PubMed: 17392250]
31. Kheirrolomoom A, Dayton PA, Lum AF, Little E, Paoli EE, Zheng H, Ferrara KW. Acoustically-active microbubbles conjugated to liposomes: Characterization of a proposed drug delivery vehicle. *J Control Release* 2007;118(3):275–284. [PubMed: 17300849]
32. Kheirrolomoom A, Satpathy GR, Torok Z, Banerjee M, Bali R, Novaes RC, Little E, Manning DM, Dwyre DM, Tablin F, Crowe JH, Tsvetkova NM. Phospholipid vesicles increase the survival of freeze-dried human red blood cells. *Cryobiology* 2005;51(3):290–305. [PubMed: 16185682]
33. Bentz J, Duzgunes N. Fusogenic capacities of divalent cations and effect of liposome size. *Biochemistry* 1985;24(20):5436–5443. [PubMed: 4074706]
34. Lindner J. Oral Personal Communication. 2007
35. Tai YC, Ruangma A, Rowland D, Siegel S, Newport DF, Chow PL, Laforest R. Performance evaluation of the microPET focus: a third-generation microPET scanner dedicated to animal imaging. *J Nucl Med* 2005;46(3):455–463. [PubMed: 15750159]
36. Fahey FH, Gage HD, Buchheimer N, Smith HC, Harkness BA, Williams RC, Bounds MC, Mercier J, Robbins ME, Payne RM, Morton KA, Mach RH. Evaluation of the quantitative capability of a high-resolution positron emission tomography scanner for small animal imaging. *J Comput Assist Tomo* 2004;28(6):842–848.
37. Chow PL, Rannou FR, Chatziioannou AF. Attenuation correction for small animal PET tomographs. *Phys Med Biol* 2005;50(8):1837–1850. [PubMed: 15815099]
38. Yao R, Seidel J, Johnson CA, Daube-Witherspoon ME, Green MV, Carson RE. Performance characteristics of the 3-D OSEM algorithm in the reconstruction of small animal PET images. Ordered-subsets expectation-maximization. *IEEE T Med Imaging* 2000;19(8):798–804.

Figure 1.
Schematic of formulations. A) [^{18}F]FDP microbubbles B) [^{18}F]FDP in ethanol and C) liposomal [^{18}F]FDP.

Figure 2.

Full body maximum intensity projections (MIPs) following injection of radiolabeled formulations. A) Injection of [^{18}F]FDP microbubbles produces accumulated activity in the lung, liver, and spleen B) Injection of [^{18}F]FDP microbubbles at a higher concentration produces accumulated activity in lungs, spleen, and a large vessel C) Injection of [^{18}F]FDP in ethanol results in accumulated activity in the liver, spleen, and bladder and D) Following injection of liposomal [^{18}F]FDP activity is long-circulating. Free and liposomal [^{18}F]FDP images were obtained from Marik et. al. for comparison.

Figure 3.

Torso maximum intensity projections (MIPs) used for ROI analysis. A representative image from each formulation is shown, A) [^{18}F]FDP microbubbles, B) [^{18}F]FDP in a small amount of ethanol for solubility, C) liposomal [^{18}F]FDP. Free and liposomal [^{18}F]FDP images were obtained from Marik et. al. for comparison.

Figure 4.

Time activity curves (TAC) following the injection of three radiolabeled formulations illustrating distinct pharmacokinetics. A) free [^{18}F]FDP TAC and B) liposomal [^{18}F]FDP TAC were obtained from Marik et. al. for comparison. C) [^{18}F]FDP-microbubble TAC for blood, kidneys and spleen and D) [^{18}F]FDP-microbubble TAC for lung and liver.

Figure 5. Details of ultrasound-PET study A) ultrasound color image during the first 5 minutes of therapy showing microbubble collapse (vertical lines indicating each ultrasonic line of sight) within a region of the kidney B) color ultrasound image during the last 5 minutes of therapy C) the apparatus for ultrasound treatment followed by PET image acquisition D) microPET image (MIP) of abdomen acquired after treatment.

Figure 6. Dynamic microPET data obtained from ROI analysis of control (top) and treated (bottom) kidneys. Time zero in these graphs is immediately following 20-minute insonation. Numbers in the legend indicate animal identity. The activity in each treated kidney (right) is higher than its corresponding control (left).

Microbubble number effect on lipid deposition

Figure 7.

The percent difference between the PET estimates of % ID/cc in control and treated kidneys normalized by the % ID/cc in the control kidney, for two injected microbubble concentrations. A two tailed, paired t-test indicates that the 2X concentration results in a significantly larger percent difference of [^{18}F]FDP deposited in control and treated kidneys, $p = 0.006$.