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Preliminary Investigation

Ultrasound Contrast for Hepatic Tumors Using IDE Particles

K. J. PARKER,*† R. B. BAGGS,‡ R. M. LERNER,† T. A. TUTHILL,* AND M. R. VIOLANTE†

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Iodipimide ethyl ester (IDE) can be formulated as dense spherical particles with narrow diameter distribution. When IDE particles are injected intravenously, the Kupffer cells of the hepatic sinusoids accumulate particles within 10 to 20 minutes, after which the clearance and excretion of IDE takes place. During the uptake phase, the dense particles act as scattering sites, increasing the echogenicity of normal liver tissue. In comparison, tumors and other lesions remain at pre-injection echogenicity, as they lack Kupffer cells and therefore do not retain particles. This report provides initial studies of contrast enhancement in rabbit livers with implanted VX2 tumors, scanned in vivo and evaluated ex vivo using pulse-echo techniques. The distribution of particles within hepatic lobules may explain why the observed echogenicity is greater than that predicted by single-particle backscatter theory. Directions for future improvements are discussed.

Key words: ultrasound; backscatter; contrast enchancement; liver; metastases; particulate suspensions.

ULTRASOUND CONTRAST AGENTS currently are not in widespread clinical use. Recent developments indicate that agents may soon be available for echocardiology, but solid organ enhancement agents are still in an early stage of development.[1,2] Only a few earlier liver contrast results have been reported using 2-μm collagen microspheres[3] and suspensions of perfluorooctylbromide (PFOB) and FluosolDA (Alpha Therapeutic Corp., Los Angeles, CA).[4,5] Our group has developed a dense particulate formulation that is radiopaque and therefore is useful as an x-ray contrast agent,[6] while also increasing ultrasound backscatter of liver because of the particle impedance mismatch with respect to surrounding tissue.[7,8] Particles of iodipimide ethyl ester (IDE) can be formulated with a mean diameter of 0.05–3.0 μm, with a narrow distribution around the selected mean diameter. The particles can be formulated under sterile conditions, can be stored for over one year at cold temperatures with no aggregation, do not aggregate following intravenous injection and mixing with whole blood, are collected by the Kupffer cells in the liver, and are excreted in a few days.[6,7] The toxicity of IDE in mice is not as high as the parent compound (iodipamide meglumine). Because relatively low doses are required for image enhancement, IDE may have a higher safety index than currently used x-ray contrast agents.[7] Because of these biophysical properties, IDE particles have promise for x-ray and ultrasound contrast. This report presents recent in vivo and ex vivo ultrasound images and quantitative backscatter measurements on rabbit livers with implanted VX2 carcinomas. Tumors initially isoechoic with surrounding liver parenchyma have been visualized as hypoechoic regions following IDE administration. Ex vivo backscatter measurements on freshly excised normal rabbit livers show a significant backscatter increase in IDE treated livers over controls. The distribution of particles within lobules is examined using polarized light microscopy, and the results are related to ultrasound contrast effects.

Methods

IDE particles for these experiments were prepared as described previously[6] and formulated to have diameters in the range of 0.8 to 1.2 μm. A sample particle diameter distribution as measured using a Coulter N4SD laser lightscattering instrument is given in Figure 1. The particles were suspended in a 0.1% polyvinylpyr-

From the *Department of Electrical Engineering, the †Department of Radiology, and the ‡Department of Pathology, Division of Laboratory Animal Medicine, University of Rochester, Rochester, New York.

Reprint requests: K. J. Parker, PhD, Rochester Center for Biomedical Ultrasound, Hopeman Hall, University of Rochester, Rochester, NY 14627.

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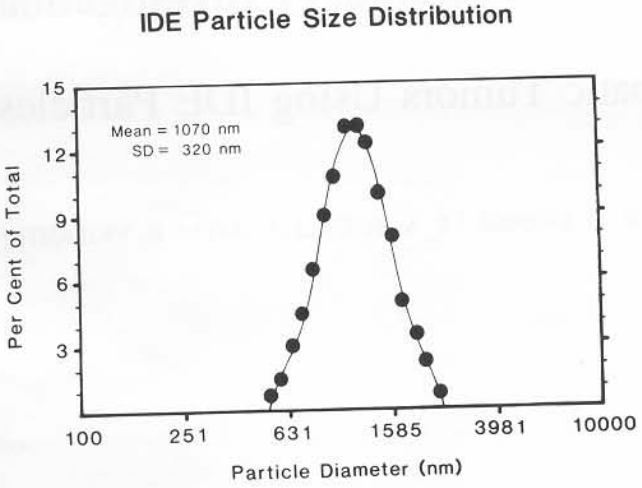

Fig. 1. Histogram of particle diameters in a formulation of IDE particles with mean of 1.7 ± 0.3 μm, as measured by laser light scattering.

rolidine (PVP), 10% lactose solution of distilled water, and injected in concentrations of 60 to 100 mg IDE per cubic centimeter to a dose of 300 mg IDE per kilogram animal weight. The IDE suspensions were infused into the rabbit ear veins after administering short-acting anesthesia consisting of 10 to 20 mg/kg ketamine and 5 mg/kg xylazine injected intramuscularly (IM). Twenty New Zealand White rabbits, 2 kg to 4 kg in weight, received an intrahepatic implant of approximately 2×10^7 viable cells from a VX2 tumor line grown and passaged in a separate group of rabbits. Within 14 to 24 days after the tumor implant, the rabbits developed one or more liver tumors ranging in size from 5 mm to 3 cm, plus frequent masses in the abdominal cavity.

Ultrasound scanning of livers before and after IDE administration was performed using a 7.5 MHz and 5.0 MHz Philips 2500 fixed-focus mechanical sector scanner (Philips, NA, Santa Ana, CA). Interpretation was based on real-time examination and videotape review, and tumors were suspected based on gray scale changes from liver background or mass displacement effects. Criteria for positive identification of an intrahepatic tumor included the following:

1. The suspected region must be identified in scans from two orthogonal planes.
2. The suspected region must move with the liver during respiration and palpation.
3. The suspected region must not be caused by partial volume (out-of-plane) artifacts, as verified by adjacent parallel scan planes.

Suspected tumor locations were identified at the time of scanning with reference to anatomic landmarks such as the free liver edge, the diaphragm, and gall bladder; and the locations were verified after euthanasia by visual examination of the liver and abdominal cavity. The examples reported herein met all criteria listed above and locations were verified postmortem.

Pulse-echo backscatter of eight normal and IDE-treated rabbit livers (without VX2 tumor implants) were performed on fresh excised whole organs. These animals were killed 2 hours after intravenous injection of IDE or the suspending fluid as a control. A Panametrics focused broad-band 10 MHz center frequency transducer was used, and the sample was positioned in the focal plane. Eight to ten lines of independent radiofrequency (RF) waveforms were digitized from echoes returning from 1 mm to 6 mm below the anterior surface of the main lobe of the liver. One rabbit pair was eliminated from the study because of problems in verifying the administered dose of IDE.

Histology sections were obtained from normal and VX2-implanted livers, with and without IDE treatment. Routine 5-μm hematoxylin and eosin (H&E) stained sections were examined with partially crossed Nicol prisms in an Olympus AH-2 photomicroscope.

Results

Both in-vivo and ex-vivo liver B-scans demonstrated that small (less than approximately 1 cm in diameter) VX2 tumors are virtually isoechoic with surrounding tissue, with-

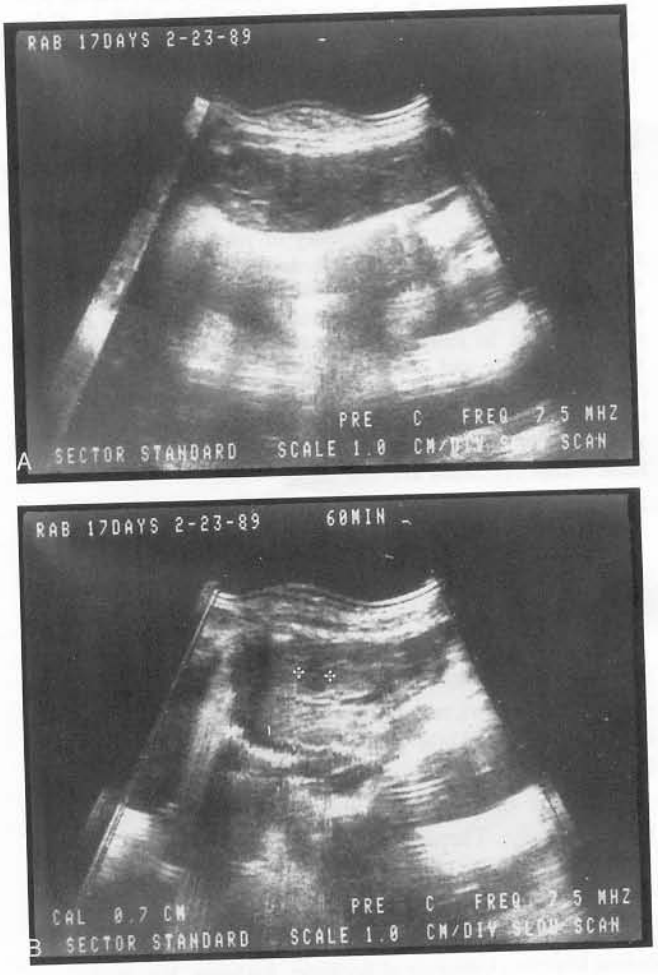

Fig. 2. B-scan images of rabbit liver in vivo (A) before and (B) 60 minutes after IDE administration. The liver parenchymal speckle does not show a 17-day-old VX2 tumor implant in (A), but the tumor is visible as a hypoechoic region (between cursors) in (B).

out noticeable speckle change. An example of an in vivo rabbit liver scan, in sagittal orientation, is shown in Figure 2A. The liver has a 17-day VX2 implant located in the medial right lobe; however, pre-contrast examination fails to detect any conclusive abnormalities. At 60 minutes after IDE administration, the same liver scan (Fig. 2B) shows a hypoechoic mass (cursors) that in real time moved with the liver during respiration and was verified postmortem as an approximately 7-mm diameter tumor. Figure 3 shows the visual anterior surface appearance of a VX2 tumor as a whitish region against the surrounding liver tissue.

Another case, examined 26 days following tumor implantation, is given in Figure 4A, where the abdominal–liver interface shows a hypoechoic mass with a bright rim. This mass remained stationary with respect to whole liver respiratory motion, implying attachment to the abdominal wall, with uncertainty as to the extent, if any, of tumor growth within the liver. Thirty minutes after IDE injection, a hepatic hypoechoic mass located inferior and to the right of the abdominal mass in Figure 4B, is visualized. In another rabbit, a larger (approximately 2.2 cm) tumor is seen at 21 days postimplantation in a sagittal view (Fig. 5A) before IDE treatment. The tumor (cursors) is isoechoic to hyperechoic compared with the surrounding liver tissue. Forty minutes after IDE administration, the same tumor can be seen in Figure 5B, transverse view, as hypoechoic compared with surrounding tissue.

The changes in post-IDE echogenicity described above were observed in 6 of 20 rabbits with VX2 implants. In most of the remaining cases, two technical problems degraded the diagnostic quality of the B-scan images. The first was the growth of some tumors at the extreme inferior edge of the right lobe. In these cases the proximity to the bowel and lack of surrounding normal parenchyma prevented an

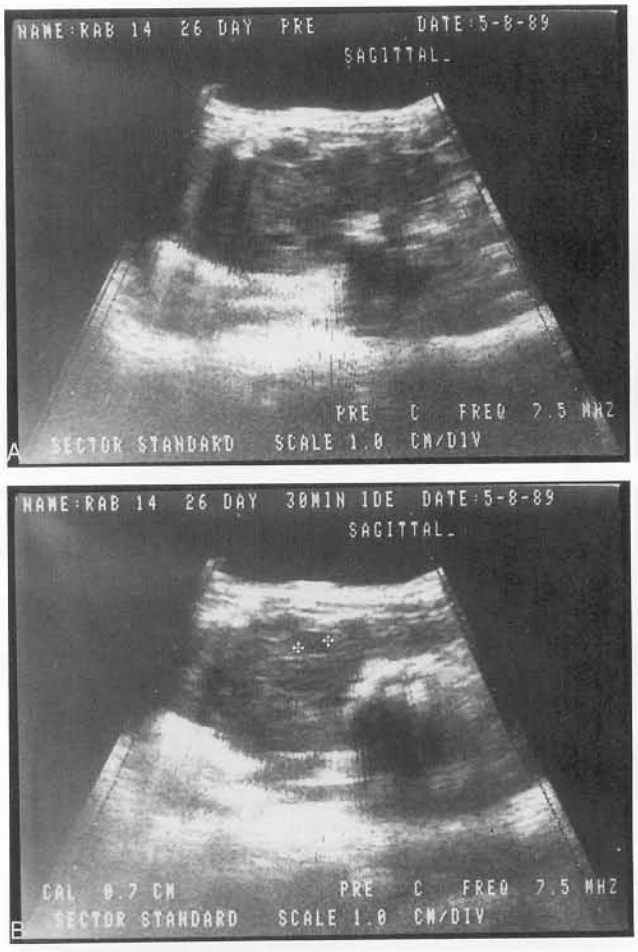

Fig. 4. B-scan images of a rabbit liver in vivo 26 days after implantation of VX2 carcinoma. (A) There is a hypoechoic mass found to be attached to the abdominal wall. (B) The same liver 30 minutes after IDE injection shows an additional hypoechoic region (between cursors), which is intrahepatic.

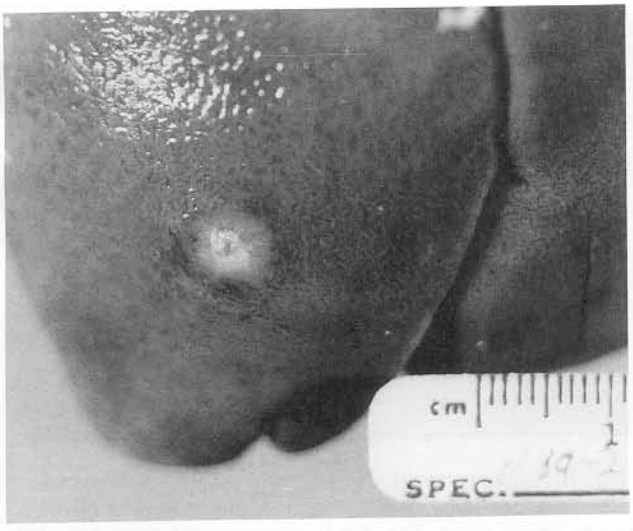

Fig. 3. Excised liver showing 20-day-old implant in the right lobe. The anterior surface is seen, with a whitish discoloration at the surface of the VX2 tumor.

assessment of echogenicity. In other cases, a poor acoustic window into the liver resulted from the presence of large abdominal masses combined with bowel and ribs. Because of the limited numbers and these technical problems, no attempt is made to derive true positive fraction and related statistics at this stage.

These echogenicity changes have been visualized on the nonquantitative B-scan images, where absolute backscatter measurements cannot be inferred. However, mathematical considerations of fully developed speckle models indicate that a ratio of approximately 1.5 to 2 in backscattered pressure (intensity factor, 2.3 to 4) is needed to visualize and identify by statistical models, distinct regions in this 5 mm to 2 cm diameter scale.[9]

Ex vivo backscatter measurements using 1.2-μm diameter IDE particles substantiate the premise that a significant backscatter increase may be produced by IDE uptake in the liver. Power spectra (intensity vs. frequency of the backscattered echoes) are shown in Figure 6, where the average

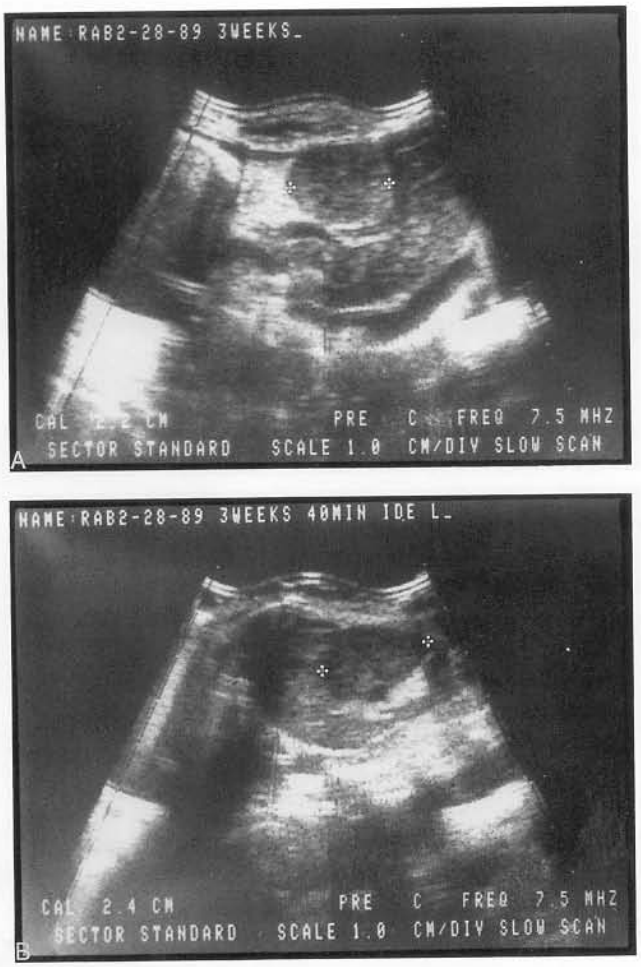

Fig. 5. B-scan images of rabbit liver in vivo 21 days after implantation of a VX2 carcinoma. (A) A 2.2-cm tumor is visualized between cursors. The tumor is isoechoic to hyperechoic with respect to the surrounding liver parenchyma (right and below) in this precontrast scan. (B) 40 minutes after IDE administration, the tumor (between cursors) is now visualized as a hypoechoic mass compared to the normal parenchyma.

of the three normal and three IDE liver echo spectra are given. The increased signal is significant over the 2–12 MHz bandwidth. In terms of the corresponding time domain echoes, the average root mean square (rms) (amplitude) backscatter for normal livers was 1.46 ± 0.27 mV, as compared with 2.03 ± 0.13 mV for IDE livers. Thus, an increased backscatter amplitude of approximately 40% is observed in the IDE livers.

Discussion

According to the theory of simple scattering from randomly positioned, small particles,[2,7] the backscatter coefficient should increase with frequency to the fourth power. The results shown in Figure 6 are not consistent with this simple model in terms of the frequency dependence of backscatter, as the ratio of the two curves is relatively constant over 2 to 12 MHz. We postulate that this discrepancy in-

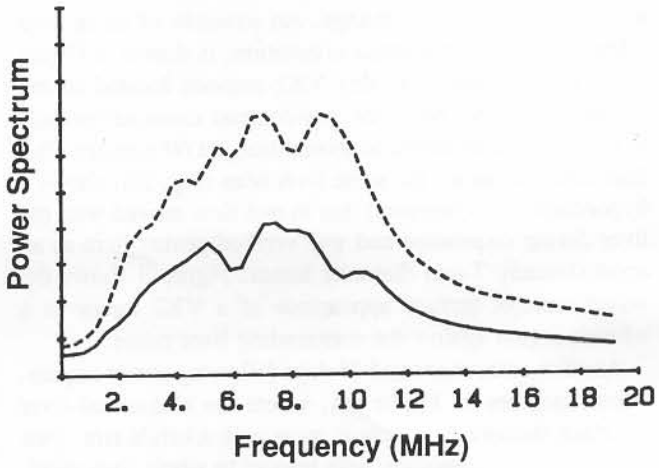

Fig. 6. Ex vivo backscatter measurements using a 10 MHz center frequency, broad band, pulse-echo system. Echoes are taken from the anterior quadrant of the liver right lobe. Three normal livers and three livers at 2 hours following IDE administration are given. The average power spectrum (echo intensity vs. frequency) indicates a significant increase in backscatter over a broad band of frequencies. Vertical scale: linear, arbitrary units; horizontal scale: frequency in MHz.

volves the biodistribution of particles. Instead of being randomly positioned, these IDE particles accumulate in Kupffer cells, where as many as 30 particles per Kupffer cell have been counted in electron microscopy sections.[6] Thus, the "effective" scattering diameter may be significantly larger than the micron diameter of the injected particles.

Furthermore, we have discovered an additional lobular pattern of IDE particle deposition. Figure 7 shows a histologic section of liver excised 2 hours after introduction of IDE. The polarized field shows IDE particles (or groups of

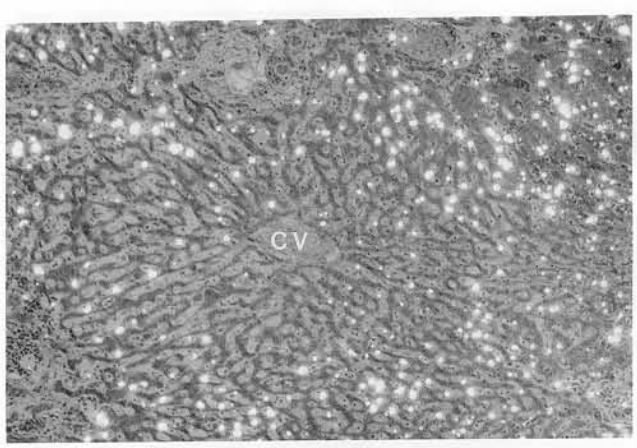

Fig. 7. Histology section of normal liver with IDE particles. Under polarized light the IDE agglomerates in Kupffer cells appear as bright spots. The IDE is concentrated away from the central vein (cv) and toward the portal traids. The lobular pattern of distribution may influence the ultrasound backscatter properties of IDE in liver.

particles) as bright spots. The lobular pattern of central vein (center) surrounded by portal triads (peripherally) also can be seen. The IDE clusters tend to be in the periphery of the lobules, not uniformly distributed as are the Kupffer cells within the sinusoids. This zonal pattern of IDE deposition can be observed by visual inspection of the whole liver. A regular mottled pattern of white (high IDE concentration) is always observed around centrilobular pink or purple colored regions on the exterior surfaces of the liver. Thus, the biodistribution of IDE produces a concentration of particles on two different physical scales: first, the particles are concentrated by Kupffer cells, and second, the distribution of Kupffer cells containing IDE particles is peripheri-lobular and not uniformly distributed. These factors modify the backscatter such that simple theories of randomly distributed particles are incomplete.

Acknowledgments

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