Particulate Suspensions as Ultrasonic Contrast Agents for Liver and Spleen

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Ultrasonic backscatter and attenuation coefficients of a medium can be increased by the addition of solid, micron-size inhomogeneities. A potentially useful agent for ultrasonic contrast of liver images has been identified. Iodipamide ethyl ester (IDE) particles can be produced in the form of dense, relatively incompressible solids with high impedance mismatch to water. The chemical, biochemical, andpharmacologic properties of the small, uniform diameter IDE particles permit safe intravenous injection followed by rapid accumulation of reticuloendothelial (RE) cells of the liver and spleen, and later elimination from these organs. Since the particles are phagocytized by RE cells, present in normal liver but not in tumors and many lesions, the selective enhancement of ultrasonic backscatter should improve detectability of lesions that are hypoechoic or isoechoic compared with surrounding tissue. The mechanisms of particle-ultrasound interaction may be described by relative motion attenuation, and scattering from a cloud of dense, incompressible spheres for the case of IDE particles in agar. Thus, values of attenuation and backscatter can be controlled by choice of ultrasound frequency and particle concentration and size. When the particles are accumulated in rat and rabbit livers, additional mechanisms induce attenuation and backscatter in excess of that predicted by IDE in agar. This preliminary work demonstrates that solid, biocompatible particles may be useful as an ultrasonic contrast agent.

Key words: ultrasound; contrast; particles; attentuation; backscatter.

CONVENTIONAL ULTRASONIC imaging is widely accepted for its ability to identify and, to a lesser extent, to characterize focal liver disease. Ultrasound imaging often is the preferred modality for liver evaluation in both staging and follow-up of cancer therapy because of its availability, low cost, and easy patient acceptance.[1] Because neoplastic liver lesions have variable ultrasonographic features which can be quite subtle, a good hepatic ultrasound contrast agent is desirable.

Materials previously investigated for this application include collagen microspheres,[2] aqueous solutions possessing a high speed of sound,[3] perfluorocarbon emulsions,[4] and lipid emulsions.[5]

Iodipamide ethyl ester (IDE) particles developed and evaluated for liver/spleen x-ray computed tomography (CT) image enhancement[6] selectively accumulate only in the normal functioning liver parenchyma and not in liver lesions. Preliminary data reported here indicates this particulate suspension might be useful for hepatic ultrasound image enhancement as well as CT.

Materials and Methods

Sample Preparation

For in vitro studies, saline suspensions of IDE particles prepared as described previously[6] were mixed into 2% agar during cooling. The mixtures were subjected to vacuum while still fluid to remove entrapped air. For ex vivo experiments, sterile saline suspensions of IDE at 100 mgI/cc were injected into the rat tail vein at a rate of 1 cc/min for a total dose of 157 mg/kg. Whole livers were excised from Wistar rats (125 to 175 g body weight) at 2 hours postinjection, placed immediately in chilled degassed saline, and massaged to eliminate air bubbles. Samples of agar or liver were packed under water to 1 to 2 cm thickness then placed in adjustable pill-box-shaped sample holders which use thin plastic wrap covers.

In vivo imaging experiments were conducted using New Zealand White (NEW) rabbits (4 to 5 kg) anesthetized with chlorpromazine (IM 25 mg/kg) and sodium pentobarbital (IV, 15 mg/kg). Sterile, pyrogen-free IDE suspensions at 100 mgI/cc were administered at a rate of 100 mgI/min to a total dose of 300 mg/kg body weight.

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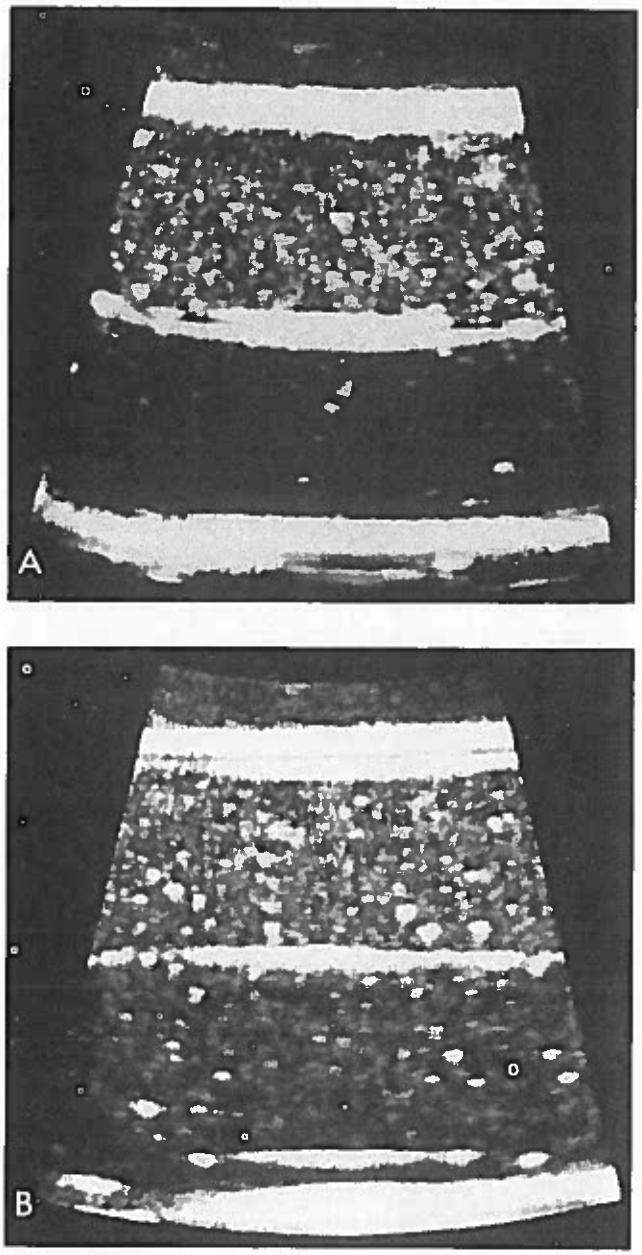

Fig. 1. B-scan images of agar blocks using a 10 MHz sector scanner. In (A) the top medium with bright speckle is 8 mg/cc of 1.0 μm IDE particles in 2% agar. The bottom material, with few echoes, is plain 2% agar. In (B), the top agar block contains 8 mg/cc and the bottom block 3.2 mg/cc of 1.0 μm IDE particles. These demonstrate the increase in backscatter (thus image brightness) with concentration of particles.

Backscatter Imaging

Backscatter images were obtained by scanning specimens with real time mechanical and electronic sector scanners using flat TGC and standard (1 dB/cm/MHz for liver, 0.3 dB/cm/MHz agar) settings at each of 3.5, 5, 7, and 10 MHz.

Results

The effect of particle concentration, in vitro, was evaluated from the overall speckle brightness in 10 MHz B-scan images of agar suspensions of 1.0 μm particles at 8 mg/cc and 3.2 mg/cc. Fig. 1 shows the result, with detectable brightness increase in the top, higher concentration block. Flat TGC curves were used in these scans, but the contrast differences were evident under ramped TGC curves as well. Contrast was also evident at 7 and 5 MHz on other B-scan instruments.

To quantify the backscatter coefficient, comparisons were made against 30% suspension in saline of washed, heparinized, unclotted, dog red blood cells (RBC), which have a reported backscatter coefficient on the order of 10^{-5} $(cm\text{-}sr)^{-1}$, depending on hematocrit value and flow conditions.[7] At 10 and 7 MHz, the IDE suspensions appeared brighter with larger saturated regions of speckle indicating roughly 5 to 15 dB relative increase in the backscatter coefficient of IDE suspensions over 30% RBC in saline.

Ex vivo B-scan images of rat livers at 10 and 7 MHz also demonstrated a detectable brightness increase with livers containing IDE at 1.6 mg/g tissue compared with normal rat livers with no IDE present. Fig. 2 shows 7 MHz cross-section images of packed, excised rat livers containing IDE surrounding a single normal rat liver lobe with no IDE. The normal lobe appears hypoechoic, as would a tumor or lesion in liver which, lacking normal RE cells, would not accumulate IDE particles. Differential contrast is quite evident despite the lower frequency (and resolution) compared to 10 MHz images.

To demonstrate IDE contrast enhancement in vivo at clinically relevant imaging frequencies, NZW rabbits were imaged with a 5 MHz electronic sector scanner before and after IDE administration. Noninjected rabbits imaged concurrently served as controls. At 30 minutes after IDE administration, rabbit livers demonstrated a significantly higher number of echoes compared with noninjected control rabbit livers as shown in Fig. 3.

Discussion

The relevant theory for scattering from solid, small (0.1 to 10 μm particles) IDE particles with incident ultrasound in the 1 to 10 MHz band (1.5 to 0.15 mm wave lengths) may be characterized by the long wave length approximations for a cloud of dense, incompressible spheres. Under these conditions the backscattered energy can be shown to be proportional to the sixth power of sphere radius, the fourth power of frequency, the square of the density difference between particles and medium, and the first power of particle concentration.[8] In this regimen, scattering patterns have a directivity peak towards the backscatter (180°). In agar and rat liver experiments presented in previous sections, typical

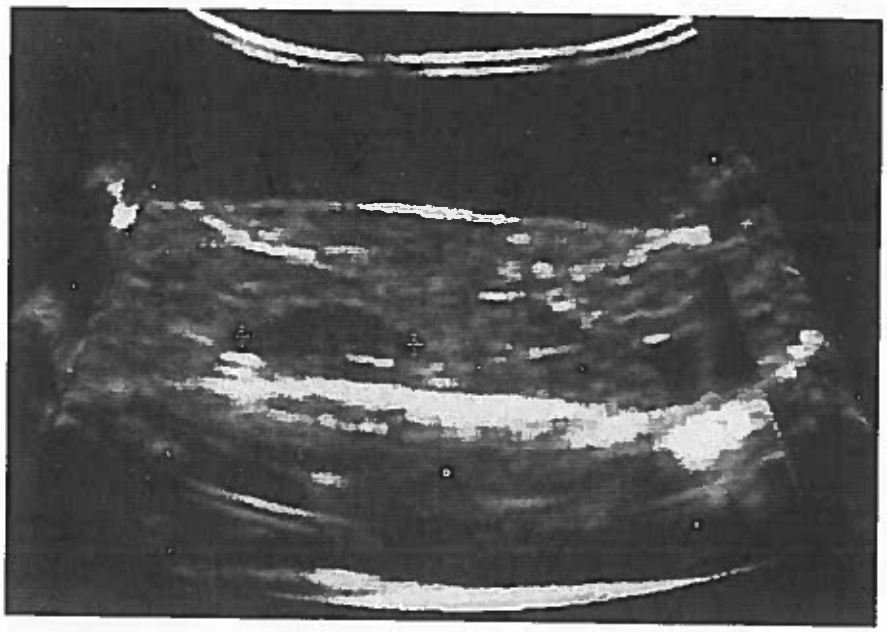

Fig. 2. B-scan images from a 7 MHz sector scanner, of IDE rat livers surrounding a single normal liver. The lobes are packed between thin plastic films, with the normal liver in the lower left quadrant, indicated by cursors. The normal liver appears hypoechoic compared with the livers with 3.2 mg/cc of 1.0 μm particles. This image represents contrast enhancement of an initially isoechoic tumor region in liver. After particle uptake by surrounding normal parenchyma, the "tumor" is clearly defined as a hypoechoic region.

parameter values include a density difference of 2.4 g/cc for particles vs. 1.0 g/cc for H_2O, and IDE concentrations of 10^9 to 10^{10} particles/cc. Calculations using the long wave length approximation indicate backscatter coefficient increases on the order of 10^{-6} to 10^{-5} $(cm\text{-}sr)^{-1}$ (at 10 MHz) when these doses of IDE particles are distributed throughout liver or agar. Images of IDE/agar and 30% suspension of RBCs in saline independently support the case for backscatter coefficient on the order of 10^{-5} $(cm\text{-}sr)^{-1}$ at 10 MHz. However, normal rat liver presumably has a backscatter coefficient much greater than that of blood, thus an increase in backscatter on the order of 10^{-5} $(cm\text{-}sr)^{-1}$ would not necessarily enhance brightness. Nonetheless, images of rat livers with and without particles show unequivocal increase in backscatter with IDE. Thus the IDE contrast enhancement in liver appears to be greater than that occurring in agar/particle suspensions. The active agglomeration of particles by Kupffer cells may influence the backscatter coefficient in vivo.

To be effective as a clinical ultrasound contrast agent the solid IDE particles will require sufficient backscatter enhancement against normal human liver parenchyma, at lower frequencies (3 to 5 MHz) commonly used in abdominal images. The in vivo rabbit liver images at 5 MHz demonstrate that IDE, at a dose of 300 mg/kg, does induce additional echoes compared with unenhanced rabbit livers. Although the increase in echogenicity is not overwhelming at this dose level, careful examination of the images reveals that echo enhancement occurs at the interfaces between liver parenchyma and blood vessels. Because this hyperechogenicity can be attributed to a greater impedance mismatch between IDE/liver and blood, a similar mismatch may occur between normal liver containing IDE particles and lesions such as liver metastases. This edge enhancement or halo effect should improve lesion detectability. This concept will be evaluated in future animal investigations.

Another area of research concerns the optimization of the mechanical and chemical properties of the compound, to increase backscatter coefficients in vivo while maintaining the highest possible margin of safety. Because backscatter increases as the square of density difference, compounds with density greater than IDE, produced as particulate suspensions, should be better contrast agents. Similarly, increased particle compressibility could be achieved by increasing the lipid component of the molecules. Thus, while IDE particles show promise for x-ray and ultrasound contrast enhancement, other compounds with improved ultrasonic and safety properties will be investigated.

The results from the current investigation clearly demonstrate that suspensions of solid particles, which have higher density and lower compressibility than water, could be useful for liver ultrasound image enhancement.

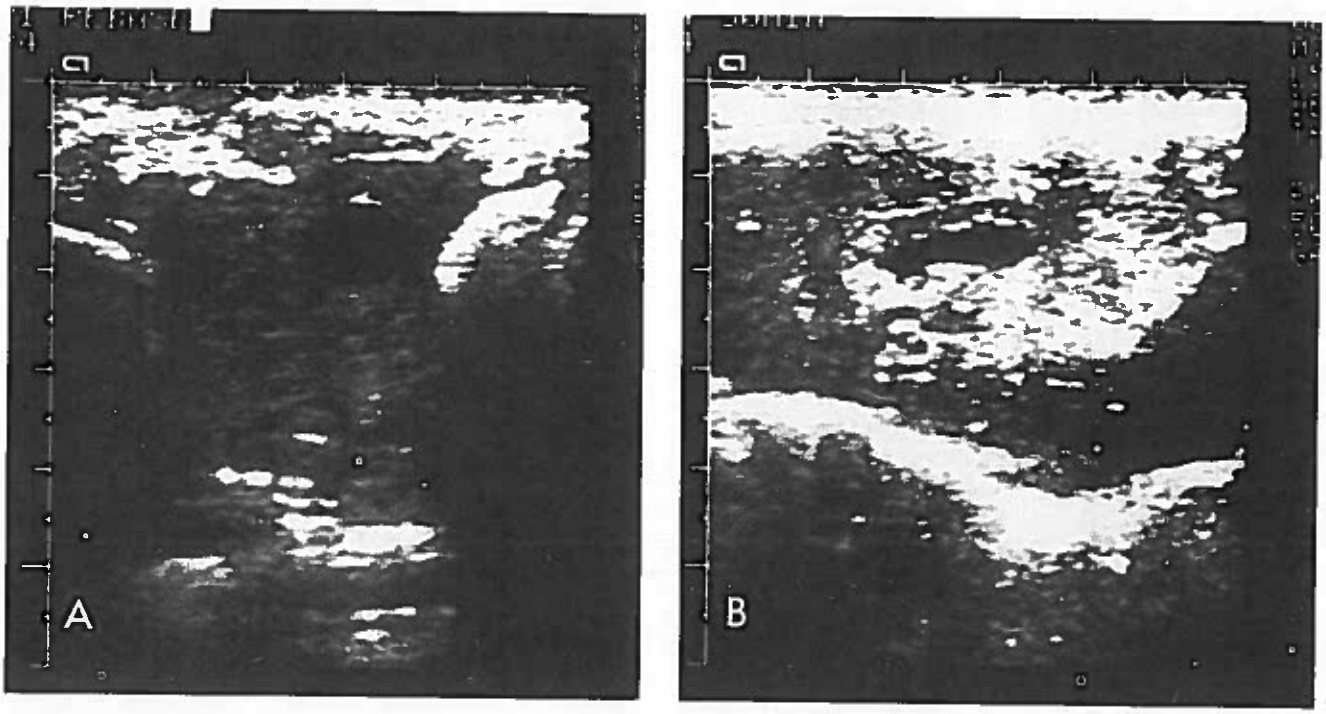

Fig. 3. B-scan images from a 5 MHz electronic sector scanner of rabbit livers (A) with no IDE; and (B) at 30 minutes following intravenous administration of IDE at 300 mg/kg. The increased echogenicity of the IDE liver appears to be caused by an enhanced impedance mismatch between the IDE/liver parenchyma and blood.

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