PART III

COMPARATIVE EVALUATION OF STEALTH LIPOSOMES AND MICROBUBBLES IN DIAGNOSIS

Chapter 12

Application of Liposomes and Microbubbles in Diagnosis

12.1 INTRODUCTION (DIAGNOSTIC IMAGING)

This section briefly describes the current diagnostic imaging modalities then gives a more detailed description of ultrasound as a diagnostic imaging tool and the status of ultrasound contrast agents.

12.1.1 Imaging Modalities: Overview

Currently there are five different imaging modalities, x-ray (including CT), optics, nuclear medicine (including PET and SPECT), ultrasound, and MRI that are used for medical diagnostic imaging and therapeutic guidance.

12.1.1.1 X-Ray

This modality uses high-energy ionizing radiation, which has some harmful effects that are dose dependent. However, in most cases, the benefits have been reported to outweigh the risks.

12.1.1.2 CT [computer (assisted) tomography]

This modality, an expansion of the x-ray modality, involves a computerized x-ray technique, which acquires cross-sectional images through the patient's body.

12.1.1.3 Nuclear Medicine

This is an imaging technique wherein small amounts of radioisotopes (radioactive substances) are injected into a patient to help trace a disease. Radiation levels are not greater than those used for routine x-ray examinations. PET and SPECT are two techniques of nuclear medicine.

12.1.1.4 PET (Positron Emission Tomography)

This imaging technique requires the patient to be injected intravenously with a small amount of signal-emitting tracer (radio-isotope) tagged to a simple sugar [Ter-Pogossian, 1995]. The PET scanner records the signals the tracer emits as it travels throughout the body. PET can differentiate between benign and malignant tumors.

12.1.1.5 SPECT (Single Photon Emission Computed Tomography)

This technique acquires tomographic slices through the region of interest. The images acquired are based on the concentration of injected radionuclides. This technique is more complex than x-ray and CT because the source of emission is inside the body cavity. SPECT is inferior to PET because of achievable sensitivity and resolution.

12.1.1.6 Ultrasound

This technique employs sound waves with frequencies from 1 to 15 MHz. Ultrasound imaging will be described in more detail in the next few sections of this chapter.

12.1.1.7 Optical Imaging

This is an imaging technique that uses visible/near infrared (NIR) (non-ionizing) light as a technique for diagnostic imaging. This technique is limited in imaging tissues located at greater depths because of the high scattering that occurs in cross-sections of biological tissue (368).

12.1.1.8 MRI (magnetic resonance imaging)

This imaging technique is based on the absorption and emission of energy in the radio frequency range of the electromagnetic spectrum.

The research objective, as outlined in Chapter 1, is focused on the enhancement of the diagnostic image during sonography and Doppler studies. The limited depth penetration factor eliminated optical imaging as an imaging modality for this application. Because X-Ray and nuclear medicine expose the body to potentially harmful ionizing radiation and injected radioisotopes, respectively, these techniques were also eliminated as potential choices for the imaging modalities used for this project. Ultrasound, unlike MRI, is portable and relatively inexpensive, which made it the more appealing imaging tool for this project.

12.1.2 Ultrasound

Annually in the United States, more than 30 million ultrasound imaging scans of the heart, abdominal organs, and vascular system are performed [Berg, 2001]. While the images produced by ultrasound may be sufficient for diagnosing a variety of conditions; ultrasound is limited in its ability to distinguish between diseased and normal tissue (for example abnormalities in cardiac wall or chambers). Ultrasound waves travel from the exterior of the body, through the body fluid until they hit either a tissue or bone surface. At a boundary of two tissues, part of the ultrasound wave is transmitted through the tissue and part is reflected back. The amount reflected depends on the impedance mismatch of the two tissues. Acoustic impedance (z) is defined as the product of density (r) and speed of sound (c) in a medium, Eq. 2.1 (369).

$$Z = r \times c \quad (2.1)$$

Acoustic impedances for water, air and bone differ from biological tissues, Table 2.1.

Table 2.1: Acoustic Impedance Values

Material	Impedance (Rayl)
Water	1.4
Skull Bone	6.0
Air	0.000415
Blood	1.61
Liver	1.65
Kidney	1.62

Two soft tissue surfaces have similar impedances, and thus the reflection at a soft-tissue boundary will be smaller than a soft-tissue/bone or air interface because of the large impedance difference between bone and air to soft-tissue. This large impedance mismatch can have advantages (increased enhancement of an image), or disadvantages (preventing imaging past bone) depending on the tissue and imaging location. Diseased tissue and healthy tissue have similar acoustic impedances; thus, the reflection from that interface will be small, and detection of diseased tissue will be difficult. This contrast limitation has led to the development of contrast agents.

A contrast agent circulates for a limited amount of time (dependent on the properties of the agent). An end to the contrast effect may be due to one or more reasons. The contrast agent may be destroyed in the process of imaging. The pressures imposed upon it by the body whilst in systemic circulation may also destroy it. Alternatively, the encapsulated air/gas may be diffused out of the agent. Natural processes also play a part in agent elimination. There is a rapid uptake of intravenously injected particles by the cells of the reticuloendothelial system (RES), composed mainly of the kupffer cells of the liver and the macrophages of the spleen and bone marrow.

Any ultrasonic scanning equipment consists of a scanner and imaging apparatus. The equipment produces visual images of a predetermined area, in this case, heart region of the rat. Typically, the scanner is placed directly on the skin over the area to be imaged. The scanner houses various electronic components including ultrasonic transducers. The scanner produces ultrasonic waves, which perform a sector scan of the heart region. The ultrasonic waves are reflected by the various portions of the heart region and are received by the generating transducer and processed in accordance with pulse-echo methods. After processing, signals are sent to the imaging apparatus for viewing.

12.2 EXPERIMENTAL

12.2.1 Selection of animals

Wistar-Albino Rats of both sex weighing 200-300 gm obtained from Zydus Research Center, Gujarat, India. The animals were housed in departmental animal house under natural light conditions, fed a standard pellet diet and water ad libidium. Their care and handling were in accordance with the provisions of Social Justice and Empowerment Committee as recognized and adopted by the Ministry of Government of India, New Delhi. Institute's Animal Ethics Committee approved the project. No diet restriction was enforced prior to studies. Three rats were taken for the study in each group.

12.2.2 Materials

Egg Phosphatidylcholine (PC), Hydrogenated Soya PC (HSPC), methoxy polyethylene glycol (M.Wt-2000), phosphatidylethanolamine (PE), Disteroyl Phosphoglycerol (DSPG) were purchased from Sigma Chemical Co., St.Louis, M.O. Poly (D, L-lactide-co-glycolide), Coumarine 6 dye (model dye). All other chemicals and reagents were of analytical grade.

12.2.3 Apparatus

Power Doppler with HDI 5000 Scanner (Philips ATL, Bothell, WA) at a center frequency of 1.67 MHz, Ultrasonography Machine (JUST Vision 200, Toshiba, Japan)

12.2.4 Preparation of ultrasound contrast agents

12.2.4.1Dye solution

Stock solution of Coumarine 6 (100 µg/ml) was prepared by dissolving 5 mg of Coumarine 6 in 0.5 ml of methanol in to 50 ml of volumetric flask and volume was made up with water for injection.

12.2.4.2 Liposome based ultrasound contrast agents

As described in Chapter 5, Preparation of liposomes, Section 5.2.3

12.2.4.3 Microbubble based ultrasound contrast agents

As described in Chapter 5, Preparation of Microbubbles, Method 3: Mixing cum sonication technique, Section 5.6.2.3.3. (AALs)

12.2.5 Sonography and Doppler studies

The animals were treated with either liposomes or AALs and compared with the free dye solution. The different formulations were injected through the tail vein at the dye dose of 120 µg/kg in rats. The animals were anaesthetized and upper and

lower limbs were tied on wooden plate. The gel was applied to remove hair from the skin of animals. The probe was kept at the position of heart, imaged through the ribs and lungs and real time clips and images were taken using sonographic and Doppler instruments.

12.3 Results and Discussion

12.3.1 Sonography studies

In this method, after the animal was anaesthetized, the scanner was on the place and the contrast agents were injected. The contrast agents may flow through a vein to the right venous side of the heart, through the main pulmonary artery leading to the lungs, across the lungs, through the capillaries, in to the pulmonary vein, and finally in to the left atrium and the left ventricular cavity of the heart. It was observed that it would not easy to detect and image rat heart, when dye solution or liposomes were administered (Figure 12.1 and 12.2). With administration of microbubbles, it was found that it would be sufficient to detect and image rat heart and produced images having vividly contrast areas. They simply enhanced the backscatter signal, which is demonstrated by an increase in gray-scale level (Fig. 12.3). The enhanced scattering may be visualized as dark echogenic areas. The chambers of the heart were also detected after administration of microbubbles, while no clear area of heart would found after administration of dye solution or liposomes. The microbubbles produced noticeably clearer and more detailed images of the heart as compared with dye solution or liposomes. The reason would be that the microbubbles are intense sound wave reflectors because of the acoustic differences between the liquid and the gas. No significance difference was observed in heart rate or blood pressure after administration of microbubbles (Figure 12.4).

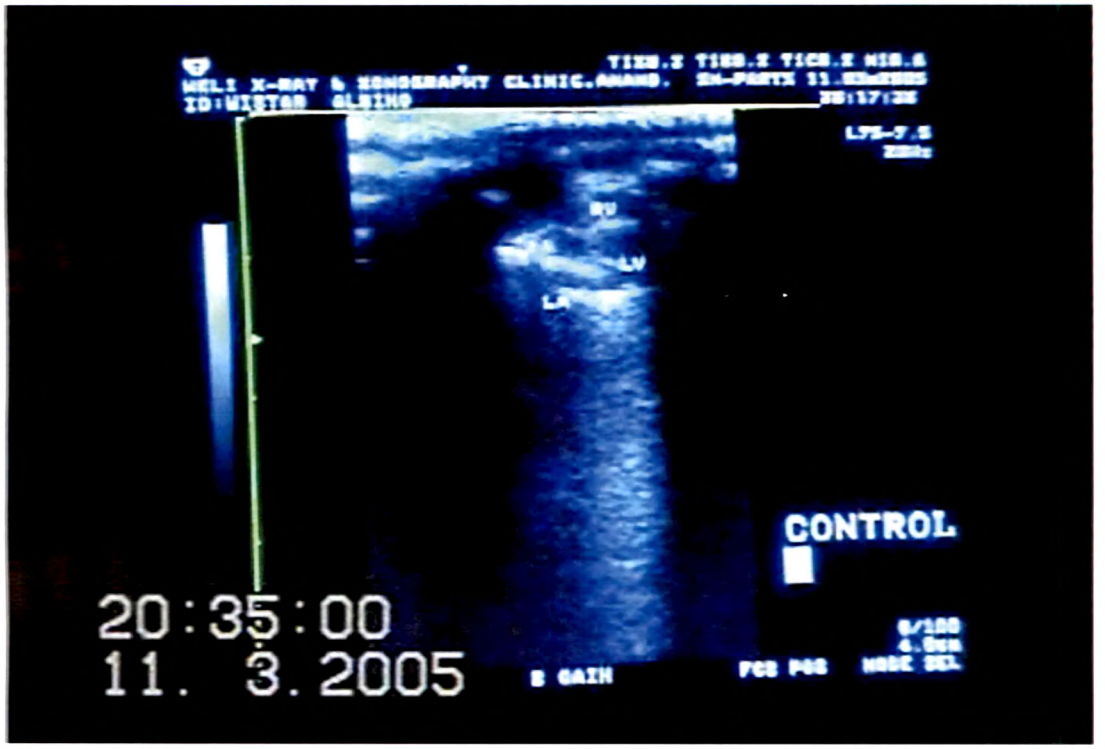

Figure 12.1: A Cardiac image showing a view of the heart in B-mode imaging (sonography) after administration of dye solution

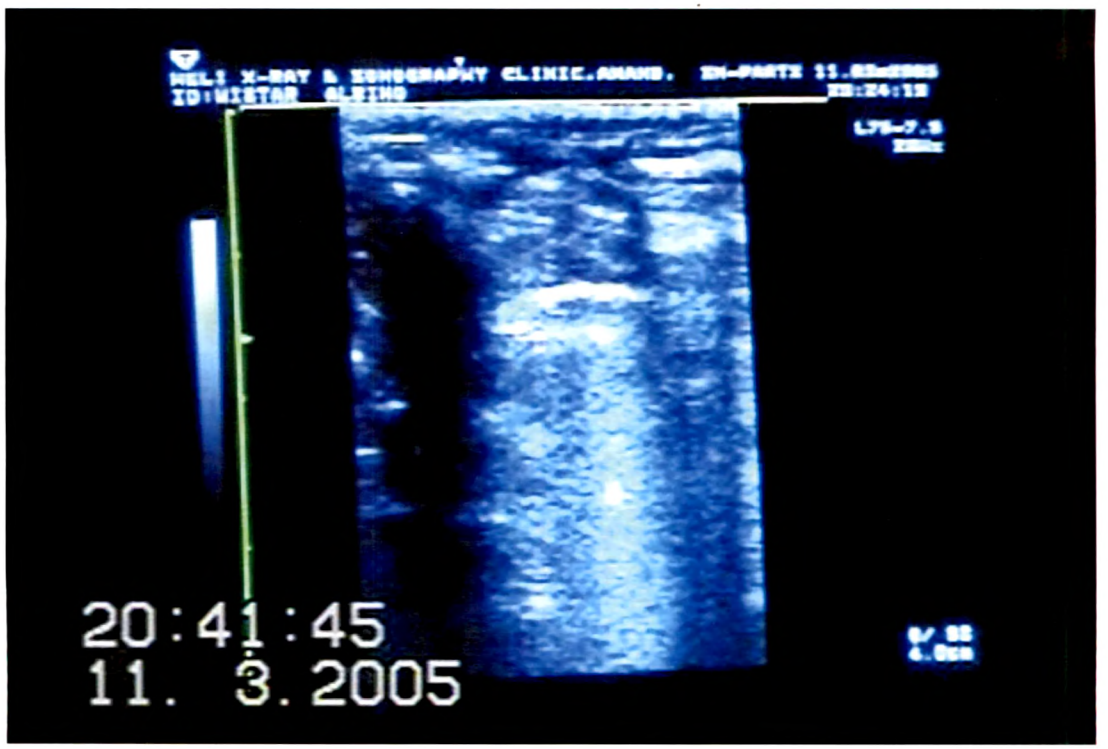

Figure 12.2: A Cardiac image showing a view of the heart in B-mode imageing (sonography) after administration of liposomes

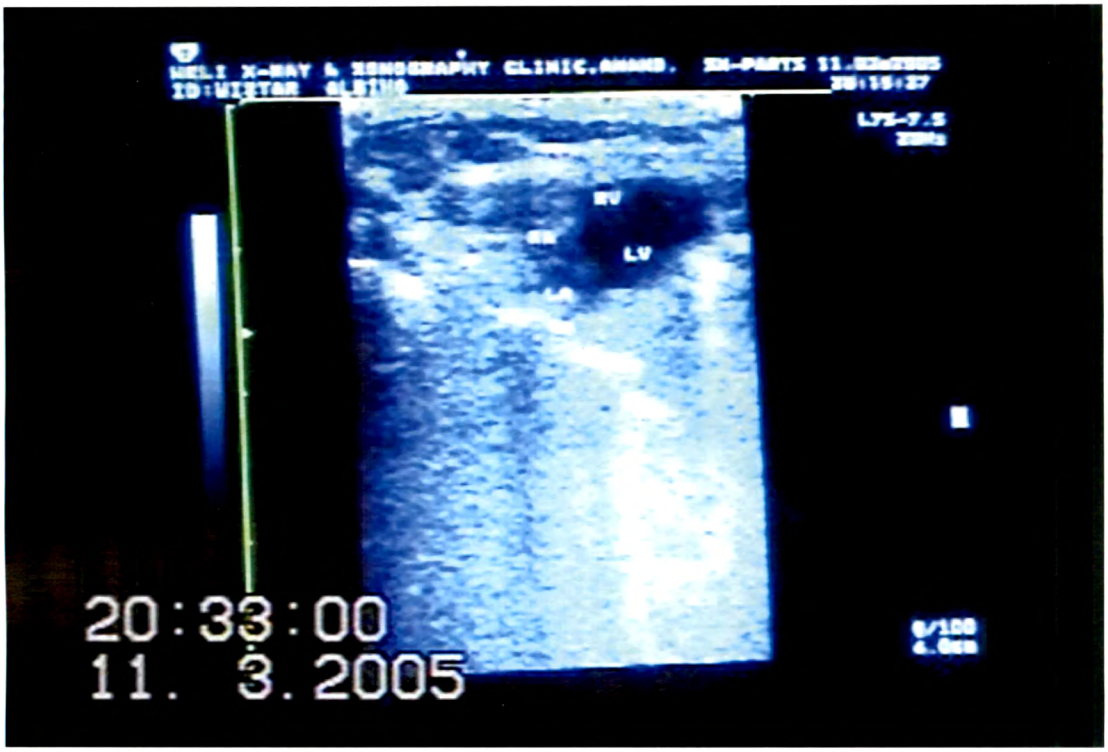

Figure 12.3: A Cardiac image showing a view of the heart in B-mode imaging (sonography) after administration of microbubbles

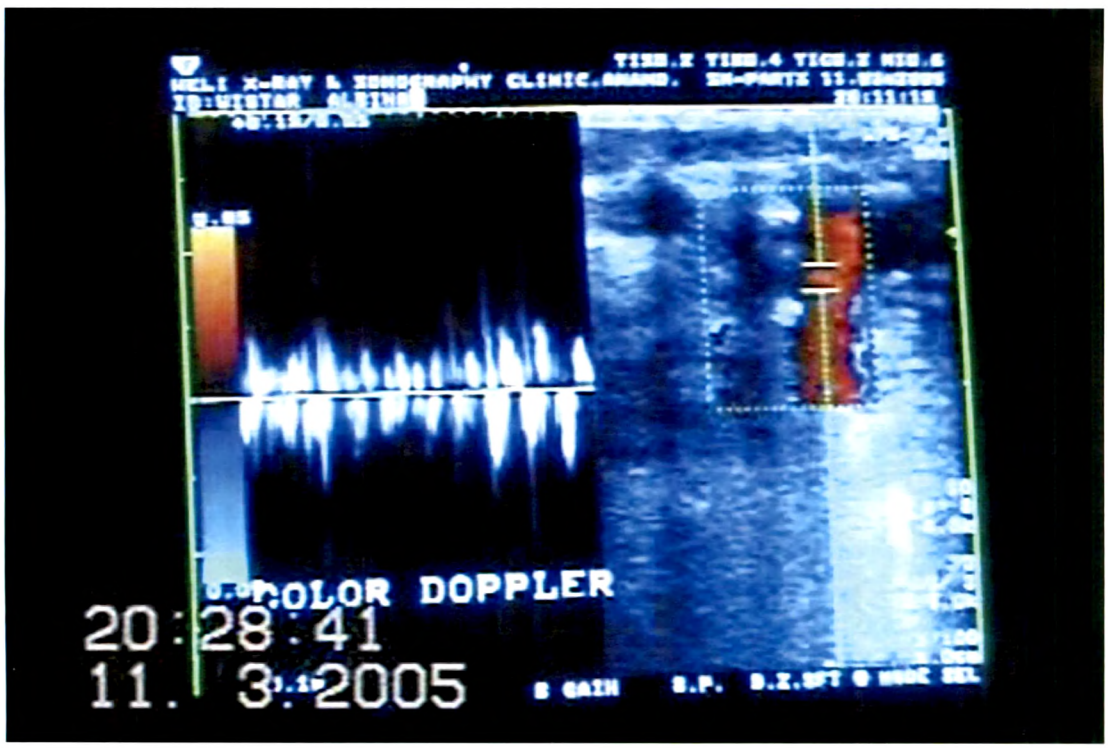

Figure 12.4: A Cardiac image showing heart rate in conventional imaging (Color Doppler) after administration of microbubbles

12.3.2 Doppler studies

Most of the Doppler techniques are multipulse techniques (i.e., a packet of pulses is transmitted in the same line of sight); they are susceptible to tissue motion that is not eminent in B mode imaging (sonography). Microbubbles produced marked augmentation of the ultrasound signal for several minutes after an intravenous administration (Figure 12.6), in Doppler studies. The signals received from the heart containing microbubbles enhanced and the detectability of flow from small vessels present in the tissue (heart) was increased, which was not found that much enhanced in case of dye solution or liposomes (Figure 12.5, 12.7). Images were noisy and showed repeated signals throughout its depth after administration of dye solution or liposomes. Tissue (heart) motion generated Doppler signals (clutter) that were even stronger than the contrast-enhanced signals from blood. The contrast-enhanced signals from blood result in a flash signals, which is a severe problem in conventional Doppler applications.

The flash signals were here reduced by the combination with second harmonic filtering (power Doppler) (Figure 12.9). During administration of microbubbles (Fig. 12.9), a clear opacification of the left ventricle (LV) and a part of the right ventricle (RV) was obtained. Since the acoustic beam has to fit between the ribs and avoid the lungs, part of the beam may actually be obstructed and reflected back. These reflections are often reflected back in the body again after reflecting from the transducer and they register as coming from a deeper region. As seen in Figure 12.8 and 12.10, the clutter (flash signal) was making the heart very difficult to assess. Harmonic imaging (Power Doppler) is used and the flash signal level is dramatically reduced producing a clearer image of the heart muscle. Apparently, the harmonic beam suffers less than the fundamental from the passage through the acoustic window.

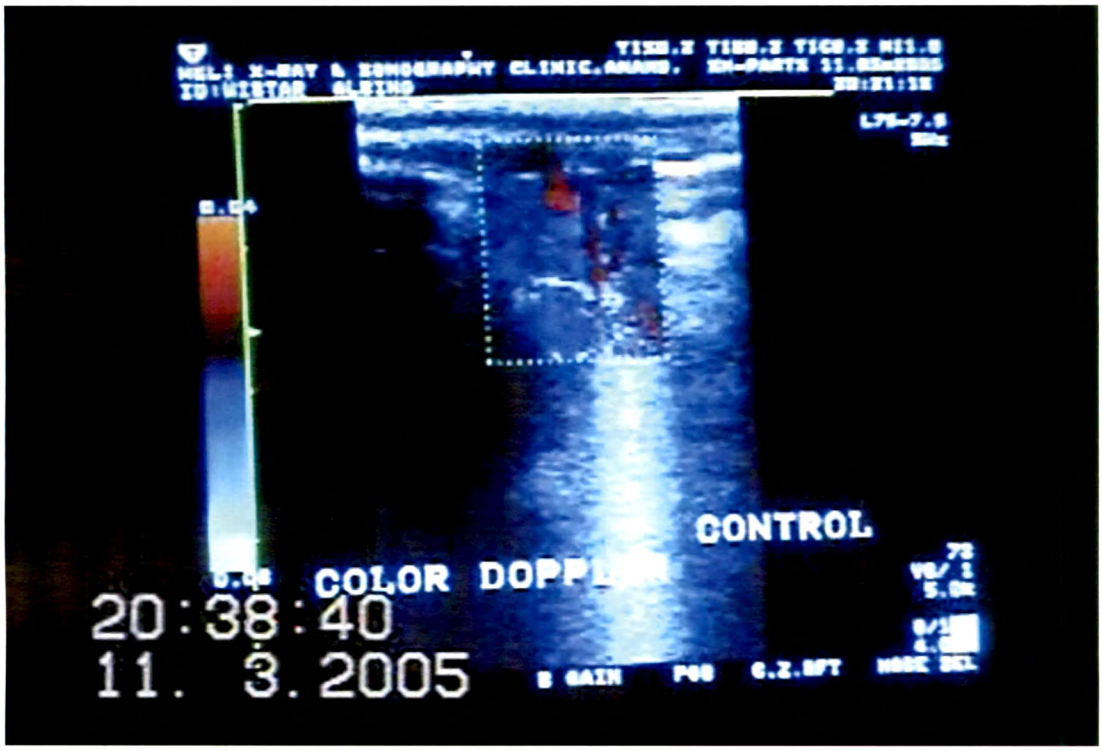

Figure 12.5: A cardiac image showing doppler signals received (red color) in conventional imaging (Color Doppler) after administration of dye

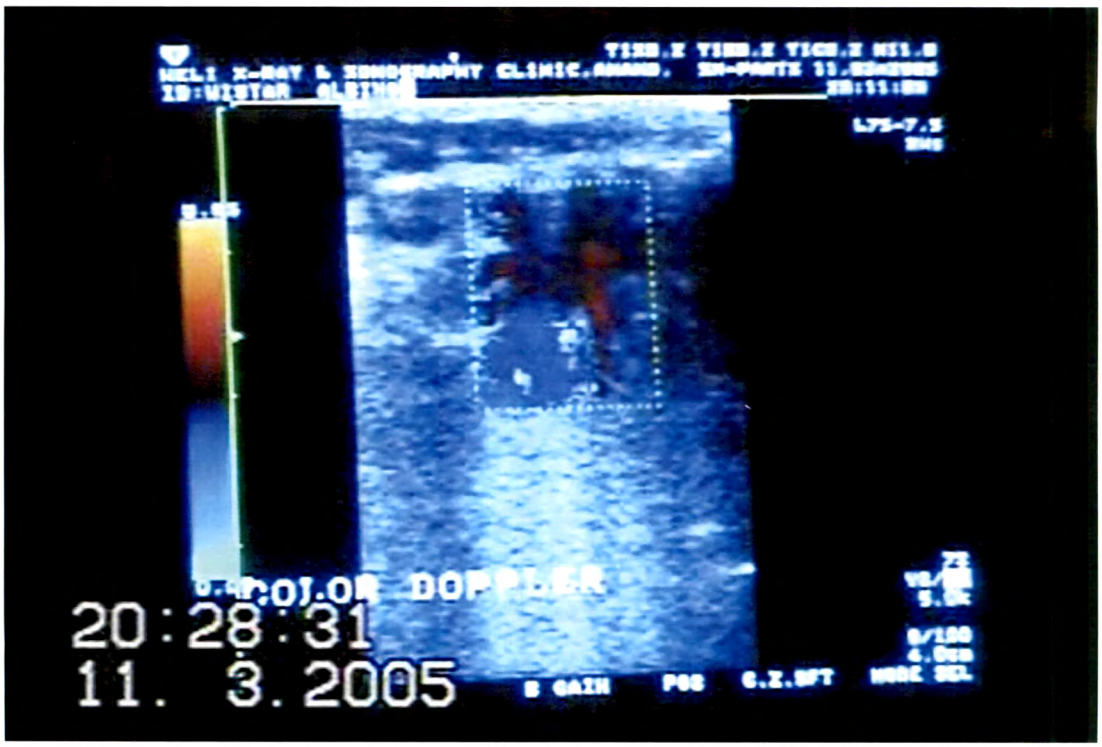

Figure 12.5: A cardiac image showing doppler signals received (red color)in conventional imaging (Color Doppler) after administration of microbubbles

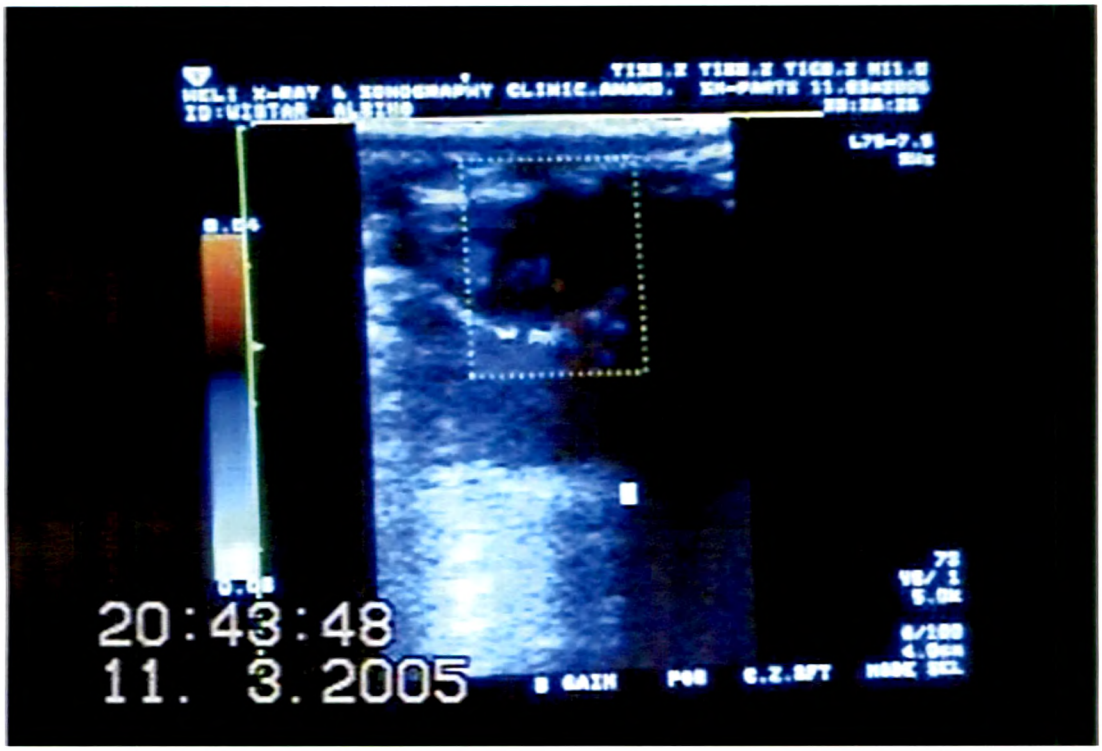

Figure 12.7: A Caridac image showing doppler signals received (red color)in conventional imaging (Color Doppler) after administration of liposomes

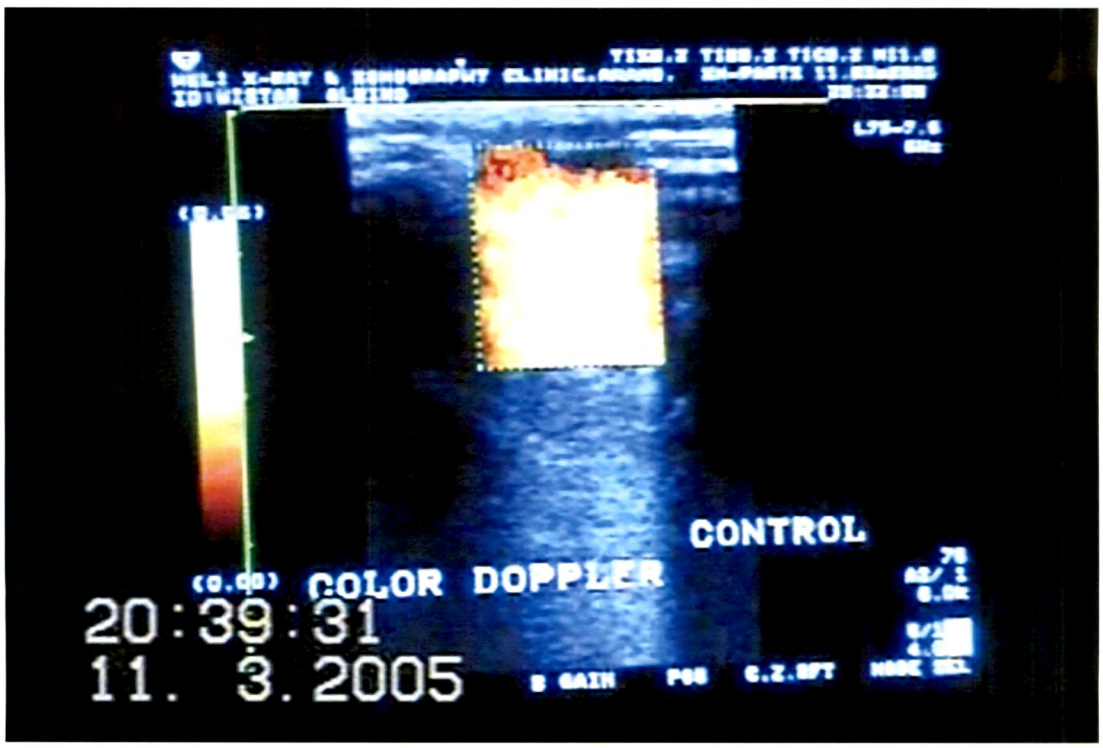

Figure 12.8: A Caridac image showing doppler signals received (red color)in harmonic imaging (Power Doppler) after administration of dye solution

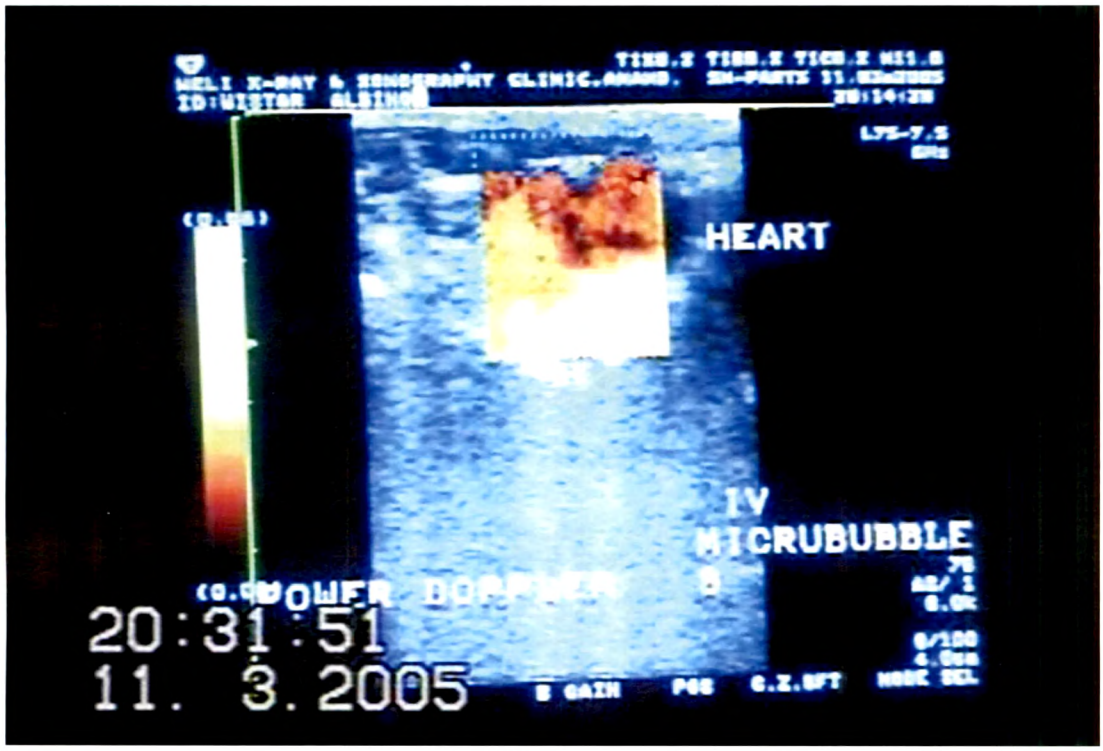

Figure 12.9: A caridac image showing doppler signals received (red color) in harmonic imaging (Power Doppler) after administration of microbubbles

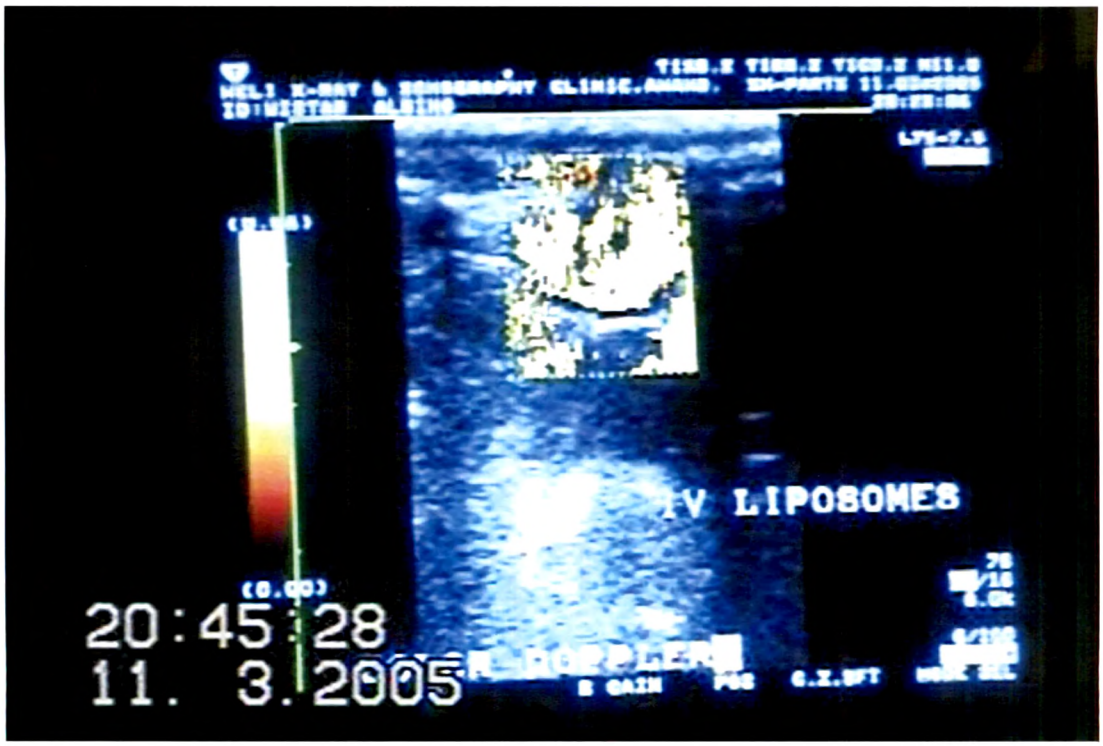

Figure 12.10: A caridac image showing doppler signals received (red color) in harmonic imaging (Power Doppler) after administration of liposomes

12.4 Conclusion

Microbubble-enhanced ultrasound improves visualization of the chambers of the heart. However, in the absence of contrast agent, a significant proportion of examinations do not allow accurate diagnosis of heart diseases. Harmonic power Doppler may be an effective tool for the detection of flow in the small vessels of organs, which may be moving with cardiac pulsation. It may be currently considered as one of the most sensitive techniques available in terms of agent-to-tissue ratio.