Sonicated Echocardiographic Contrast Agents: Reproducibility Studies

(Extract)

Steven B. Feinstein, MD, [1] Mark W. Keller, MD,[2] Richard E. Kerber, MD,[3] Byron Vandenberg, MD,[3] John Hoyte, MA (Cambridge),[4] Chris Kutruff, RN, [5] James Bingel, MD, [6] Theodore D. Fraker, Jr., MD, 6 Richard Chappell, BS, [1] and Alan H. Welsh, PhD, 7 *Chicago, Ill., Charlottesville, Va., Iowa City, Iowa, Indianapolis, Ind., Toledo, Ohio, and Redwood City, Calif.*

This Article describes the production, analysis, and reproducibility of forming microbubbles for contrast ultrasound imaging. The sonication method used to generate microbubbles was tested by four independent observers, and a **subsequent laser particle counter analysis of microbubble size and concentration determined the reproducibility of the method.** The results indicated that the mean bubble size was 3.3 ± 1.2 μm for the entire group, based on three trials of each of the four participants. The characteristics of the bubble size of the microbubbles between observers were assessed with a Poisson distribution with the reproducibility based on the sample mean for each observer's trials. Standardization and calibration of the laser particle counter was accomplished with commercially available latex spheres, sonicated albumin microspheres, and a Coulter counter analysis. Our results indicate that the sonication technique generates small microbubbles with a reproducible uniform size distribution. The method of microbubble production is reproducible and can be widely applied for use in contrast echo-cardiographer perfusion imaging of tissue in a variety of research and clinical studies. (J AM SOC Echo 1989; 2:125-31.)

Ultrasound contrast agents currently employed as intravascular, non-diffusible tracers for the purpose of analyzing tissue perfusion have not been adequately standardized.[1,2] The widespread application of the hand-agitation method for clinical and experimental studies results in rather large and unstable microbubbles. [3-8] Because microbubbles with unstable variable diameters cannot pass through the capillaries, [9,10] quantitative analyses of tissue blood flow perfusion cannot be performed with these agents. By direct microscopic observations, the large and more variable microbubbles transiently occlude capillary circulation. [9,10]

Ultrasonic cavitation or sonication, a technique generally reserved for in vitro tissue disruption has recently been used to create relatively small and stable microbubbles in physiologic solutions, [1,2] The microbubble diameters, decay rates, and ultrasound backscatter properties have been described.[2] Furthermore, for widespread clinical use the sonication technique requires a standardized approach to the production and subsequent analysis of the microbubbles..[11] This study was designed to demonstrate for the first time a standardized and reproducible method of creating and measuring microbubbles for use in contrast ultrasonography.

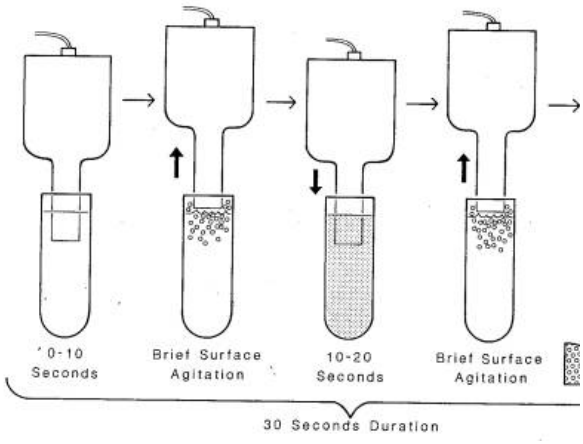

Figure 1 Sonication process for generating microbubbles is illustrated in this schematic representation. With Renografin-76 as the solution, small relatively uniform microbubbles are created by ultrasonic cavitation process.

METHODS

Observers

Individuals from the University of Chicago and three outside campuses participated in this study. The conication principles, analysis methods, and practical application were taught to each participant during his or her visit to the University of Chicago. Between 25 and 50 trials of sonication were necessary for the observers to become proficient at the production of the small and stable microbubbles. After the instruction and practice sessions, each participant performed three trials that were analyzed by the laser counter and served as the basis of this article on reproducibility. All studies were performed in one laboratory at the University of Chicago.

Sonicator

A Heat-Systems (model 220, Plainsview, New York) with a 1/2 –inch titanium-tipped horn was used as the continuous source of power for the generation of the microbubbles. The power control was set at 7, and the horn was routinely tuned in Renografin-76 solution before each study.

Manufactured latex particles (Duke Scientific, Palo Alto, Califronia) were used for calibration. Serial dilutions of sonicated albumin microspheres of known concentrations based on Coulter counter analysis were used to determine the laser particle concentration measurements.

Laser Analysis

A scanning laser particle counter (Spectrex Corporation, Redwood City, California) was used to determine the in vitro diameters and concentrations of the microbubbles. As the laser beam passed through the solution that contained the microbubbles, a predesigned “sensitive zone” at the center of the container served as the sampling site for the examination. When the laser beam struck a bubble, there was near-angle scatter of deflected light. The magnitude of the laser deflection was directly proportional to the diameter of the bubble. Following a 25-second counting period, the concentration of the bubbles per cubic centimeter was displayed on an electronic readout.

In conjunction with the laser analysis of the absolute particle counts, a Fourier transform of the laser pulse amplitudes measured the diameters of the bubbles within the sensitive zone. Afer a 30-second sampling period, a frequency histogram for the sample was generated. The absolute numbers of the bubbles found in each size channel can be calculated by multiplying the percentages listed in the frequency histogram by the absolute counts determined from the laser scanner.

Withdrawn from the center of the syringe and mixed in a beaker containing 120 ml of Renografin-76. The contents of the beaker were then scanned with the laser counter to determine the in situ diameters and

Light Microscopic Analysis

A stereoscopic light microscope (model CHA, Olympus Corp., Lake Success, New York) with a net eyepiece grid graduated in microns was used to visually assess the measurements performed on the sonication trials.

Sonication Technique

The half-inch titanium horn from a sonicator was placed approximately 0.5 cm beneath the surface of the 8 ml of Renografin-76 contained in a plastic syringe. With the tip of the syringe held firmly by hand, the energy was turned on. After 10 seconds the syringe was lowered enough to permit the tip of the sonicator to briefly contact the surface of the liquid, thus permitting a period of surface agitation. Once the surface agitation occurred, the tip of the sonicator was lowered beneath the surface of the liquid for 10 seconds. The surface agitation process was briefly performed a second time at 20 seconds, followed by the lowering of the horn beneath the surface for the duration of the sonication time. Ultimately, after a total sonication time of 30 seconds, the 8 ml solution of Renografin-76 appeared translucent. A dense white coloration should not be present in the final solution (Figure 1). This white, opaque appearance implies the presence of uncontrolled surface agitation with the development of relatively large and unstable microbubbles.

Laser Sampling Technique

Before each test background counts of particles contained in Renografin-76 were performed. Three separate determinations were recorded by the laser counter. With a predetermined threshold correlation chart provided by the manufacturer, the background counts were considered acceptable if the absolute counts did not exceed 200 counts/ml³. The Soectrex Fourier analysis was not limited by the threshold values, thus the frequency analysis (histogram plots) included the distribution of all background counts.

Immediately after the sonication process was completed, 1 ml of the sonicated solution was

Discussion

concentration of the sonicated microbubbles. The laser counter provided the absolute bubble concentrations within the 1 ml³ region of analysis, and the Fourier analysis listed the microbubble diameter frequency of occurrence within a sampled time period. An example of the frequency histogram is shown in Figure 2. Background particulate counts obtained before the analysis were subtracted to obtain the actual concentration of the sonicated microbubbles. In effect, the background particulate contamination was small (less than 10%) relative to the large numbers of microbubbles measured during the analysis. To minimize coincidence counts with the laser counter, the microbubble concentrations were kept between 700 and 1500, requiring a 1:120 dilution, of sonicated Renografin-76.

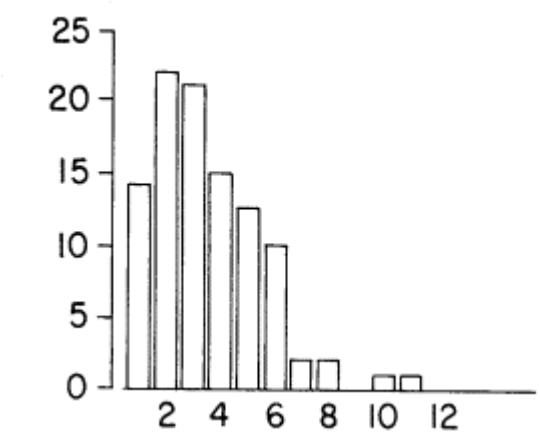

Figure 2 Laser analysis of microbubbles created from Renografin-76 is displayed. Ordinate is size of microbubbles in microns, and abscissa is frequency of occurrence.

Calibration Studies

Particle size diameters were assessed with manufactured solid latex spheres. The size distribution was described as 5.0 ± 0.38 μm. Coulter counter determinations of serial dilutions of the albumin concentrations (spheres per milliliter) were used to check the concentrations recorded from the laser counter.

formation was accomplished. At the end of the sessions, each participant was confident in the production and analysis of the microbubbles

The ability to quantify blood flow by contrast ultrasonography requires that the ultrasound tracer substances (microbubbles) be reproducible and quantifiable in production and analysis. Before the development of the sonication technique, microbubbles for use in contrast ultrasound imaging were created by manual or hand agitation. The microbubbles produced by hand agitation proved to be unstable and difficult to reproduce, [20] and the relatively large diameters prohibited passage through the capillary vasculature, [9, 10] thus presenting a potential threat to safety. In addition, studies have shown that the sonicated microbubble transit times correspond to actual blood flow as measured by electromagnetic flow meters at control and altered physiologic states. [14-16]

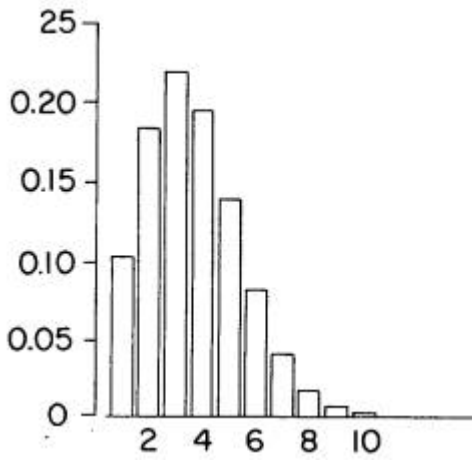

Figure 4 Frequency histogram for expected microbubble distribution characteristics with Poisson model.

Although earlier reports have been published on the sonication method for use in producing contrast ultrasound agents, [1, 2] no analysis of the inter-observer reproducibility of the sonication has been attempted.

This article, for the first time, analyzes the actual production and subsequent analysis method used in preparing sonicated agents for clinical imaging. All four participants independently volunteered for the sonication reproducibility studies. After a 2- to 4- hour instructional period, automated laser analysis of the microbubble created from the sonication technique.

As a measure of quality control, a light microscope was used for visual comparison of bubble sizes as recorded by the laser counter. Although the optics of the two measurement systems differ, the relative diameters could be easily assessed when visualized in the field of the light microscope. From our earlier studies [2] it was observed that light microscope measurements overestimate the diameters of the microbubbles partly because of the compressive effects of the cover slip and the light diffraction created from the refringent surface rim of the bubbles. The relative size distribution of the microbubbles, however, whether measured by laser or light microscopy, were similar in a variety of solutions. [1, 2]

The 12 microbubble size distributions obtained were similar. These distributions were modeled to quantify any differences between them, and it was concluded that any differences were negligible, since similarly shaped distributions with means of 8 to 10 μm would be clinically applicable. Thus the method is reproducible.

This study describes the results of producing and analyzing the sonication method for generating microbubbles. Once instructed in the methods of sonication, the four participants demonstrated the reproducibility of the sonication technique for the production of microbubbles. This technique promises to provide a rapid, economical ultrasound contrast agent that can be widely applied for use in tissue perfusion imaging.