Stabilized sulfur hexafluoride microbubbles

SF_6 Microbubbles

Kenneth T. Cheng, PhD[1]

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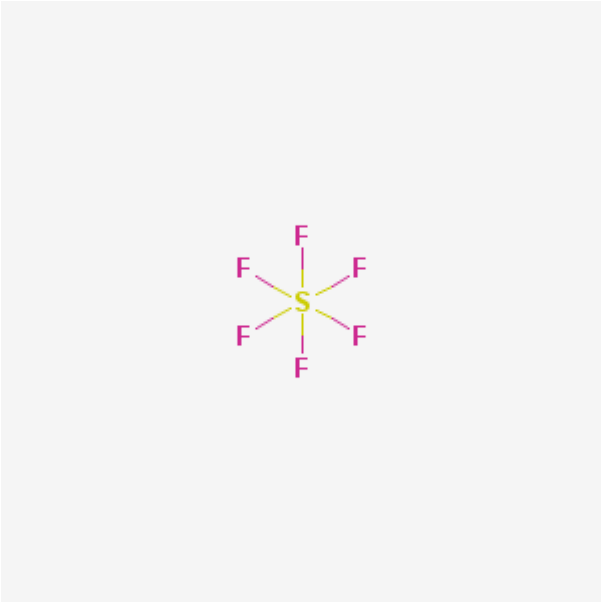
Chemical name:	Stabilized sulfur hexafluoride microbubbles
Abbreviated name:	SF_6 microbubbles
Synonym:	SonoVue®, BR1
Agent Category:	Lipid microbubbles
Target:	Nontarget
Target Category:	Nontarget filling of blood vessels and heart chambers
Method of detection:	Ultrasound (US)
Source of signal:	Microbubbles and sulfur hexafluoride
Activation:	No
Studies:	• *In vitro* • Non-primate non-rodent mammals • Humans



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Background

[PubMed]

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

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Stabilized sulfur hexafluoride microbubbles (SF_6 microbubbles), a preparation of stabilized microbubbles containing sulfur hexafluoride gas were developed as an ultrasound (US) contrast agent to enhance US images (1, 2). The SF_6 microbubbles preparation is used clinically in Europe to enhance US image contrast in echocardiography, Doppler macrovasculature, and Doppler microvasculature (3).

US contrast agents, or echopharmaceuticals, are designed to change the attenuation (absorption, reflection, and refraction) or impedance (resistance to sound propagation) of sound to enhance the differentiation of the signal (echo) of a target organ from that of the surrounding tissue (4-7). Gas-liquid emulsions (microbubbles or gaseous particles) are highly echogenic *invivo* because of the nonlinear rarefaction and compression effects that lead to volume pulsations of microbubbles (5, 8, 9). Human serum albumin, synthetic polymers and phospholipids have been used to construct the membranes of these bubbles. Microbubble preparations of various formulations have been developed, and their clinical usefulness depends greatly on the size and stability of these bubbles *in vivo*. The potential applications of US agents include US contrast enhancement in heart, liver, kidney, breast, blood vessel, pancreas, spleen and gastrointestinal diseases (10-18). The current clinical application of these agents in the United States is primarily in myocardial contrast echocardiography .

Perfluorocarbons (PFCs) are inert, volatile chemicals and can be encapsulated within microbubbles to provide a stabilizing effect. The extremely low water solubility of PFCs sets up an *in vivo* equilibrium in the water-soluble gases that diffuse in and out of the microbubble, but the PFC vapor counterbalances the surface tension and blood pressure forces that push the gases inside the bubble toward dissolution. As a result, the combined properties of the microbubble shell and the PFC gas inside determine the stability and output signal of each microbubble *in vivo*. PFC emulsions were initially studied as oxygen carriers (blood substitutes) (19, 20). Perfluorooctyl bromide (C_8BrF_{17}) was first discovered to possess sufficient lipophilicity to be formulated into stable emulsions, but it was developed as an oral agent for negative magnetic resonance imaging of the gastrointestinal tract (5). Schneider (21) described the development of microbubbles stabilized by phospholipids and a perfluorochemical SF_6 gas. This SF_6 microbubbles agent is commercially available in Europe, and it consists of a lyophilized phospholipids/ poly(ethylene glycol)/palmitic acid powder stored under SF_6 gas. Upon addition of a saline solution, a suspension of microbubbles (2.5 μm mean diameter in the range from 0.7 to 10 μm; 2×10^8 microbubbles/ml) stabilized by a lipidic monolayer is produced (5, 21). Microbubbles <2 μm in diameter do not contribute appreciably to the echogenicity at medical US frequencies, but microbubbles that are >6.2 μm in diameter tend to be trapped in the pulmonary circulation (22).

Serious cardiopulmonary reactions following the administration of ultrasound microbubble contrast agents have been reported (23). In 2007, the US FDA requested that warnings emphasizing the risk for serious cardiopulmonary reactions be added to the labeling of these agents. The uses of these agents are contraindicated in patients with unstable cardiopulmonary status.

Synthesis

[PubMed]

PFCs are inert organic materials initially developed for handling the extremely corrosive uranium fluorides (19, 20, 24). Some PFCs are derived directly from the manufacturing line that led to Teflon and other diverse industrial surfactants. Two major strategies are commonly used in producing PFCs. One strategy is substituting fluorine atoms for hydrogen atoms in the parent hydrocarbon analog by electrochemical fluorination, fluorination by high-valence metal fluorides, or direct fluorination. Another strategy is combining smaller, reactive fluorinated building blocks by telomerization. SF_6 is a gas at standard conditions and is colorless, odorless, non-toxic, and non-flammable. SF_6 comprises 6 fluorine atoms attached to a central sulfur atom with an octahedral geometry. SF_6 can be prepared from the elements by the combustion of sulfur in fluorine (25),

The European commercial preparation of SF_6 microbubbles is 25 mg of dry, lyophilized powder in an atmosphere of SF_6 contained in a glass vial with elastomeric closure (1, 3). The lyophilysate is composed of a combination of pharmaceutical grade polyethylene glycol 4000 and phospholipids (distearoylphosphatidylcholine and dipalmitoylphosphatidylglycerol). The agent is prepared immediately before use by injecting 5 ml of normal saline through the septum to the content of the vial. The vial is then shaken vigorously for 20 s after which the desired volume of the dispersion can be drawn. One milliliter of the resulting dispersion contains 8 μl SF_6 in the microbubbles, equivalent to 45 μg. The bubble concentration of the suspension is between 100 and 500 million microbubbles/ml. The total volume of SF_6 gas in the bubbles is approximately 5 μl/ml of the reconstituted suspension. The suspension has an osmolarity of 294 mOsm/kg, a pH that is between 4.5 and 7.5, and the viscosity is below 2 mPas.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Schneider (21) described the *in vitro* properties of SF_6 microbubbles after reconstitution with normal saline. When the suspension was left standing for >15–30 min, the bubbles rose to the surface. The suspension could be easily rehomogenized by gently agitating the vial top to bottom. SF_6 microbubbles showed a peak in backscatter coefficient (BSC) at about 3 MHz. When the agent was insonating with a 2.25 MHz transducer, the scattering power increased linearly with the mechanical index (i.e., 10 dB/decade). In second harmonics, the scattering power increased with the square of the mechanical index (i.e., 20 dB/decade). Schneider et al. (1) found no changes in the bubble characteristics or in their echogenicity for at least 8 h after reconstitution. SF_6 microbubbles showed constant echogenic properties over the entire 1–10 MHz frequency range. The BSC was proportional to the bubble concentration over the range 2×10^4 to 3×10^5 bubbles/ml. The critical pressure (Pc; pressure at which absorbance decreased by 50%) was 127 mm

Hg. Microbubble preparations with Pc values<60 mm Hg were not useful to opacify the left ventricle.

Using *in vitro* test phantoms and rabbit liver, Arditi et al. (26) demonstrated that SF_6 microbubbles had promising contrast-enhancing properties when used in differential contrast echography. In an *in vitro* study using a tissue-mimicking phantom, Broillet et al. (27) showed that SF_6 microbubbles were detected in the phantom at 500–700 Hz pulse repetition frequencies (PRFs) when they were circulated at different velocities (1.5–10 cm/s). SF_6 microbubbles showed stronger contrast effects at higher velocities. Moran et al. (28) studied the *in vitro* acoustic characteristics of SF_6 microbubbles at 30 MHz. At concentrations of 0.01–5 million microbubbles/ml, the agent exhibited a linear relationship between log(concentration) and mean backscatter power. At concentrations <0.01 million microbubbles/ml, the mean backscatter levels appeared to reach a plateau.

Rahim et al. (29) used Chinese hamster ovary (CHO) cells to study various factors that affected US/microbubble-mediated gene delivery. β-galactosidase gene delivery was achieved with an efficiency of ~4% to adherent cells at 0.25 MPa acoustic pressure amplitude, 1 kHz PRF and 10 s duration of exposure.

Animal Studies

Rodents

[PubMed]

No publication is currently available.

Other Non-Primate Mammals

[PubMed]

Schneider et al. (1) administered SF_6 microbubbles by i.v.injection in the auricular vein of minipigs to opacify the right and left heart chambers. At doses <0.03 ml/kg, there was a dose-dependent increase of opacification in the left ventricle. No additional increase was found with 0.05–0.2 ml/kg. Extensive acoustic attenuation was observed with doses >0.1 ml/kg. Broillet et al. (27) evaluated SF_6 microbubbles in minipigs by intermittent harmonic-power Doppler imaging and PRFs. At a dose of 0.01 ml/kg, SF_6 microbubbles produced a strong and homogeneous myocardial opacification. Higher doses prolonged the duration of the contrast effect. Varying the PRF appeared to allow the detection of perfusion differences within the myocardium during reversible left anterior descending coronary artery occlusion.

The influence of Doppler system settings on the clearance kinetics of SF_6 microbubbles was reported by Seidel et al. (30). Six dogs were investigated with a transcranial Doppler system. Each dog received 0.03 ml/kg SF_6 microbubbles by i.v. injection at a rate of 1 ml/s. The Doppler time intensity curve showed a two-phase decrease with a very short

distribution phase α and an elimination phase β. Altering the system settings appeared to have a significant effect on the mean peak Doppler intensity.

Kaps et al. (31) studied the pharmacokinetics of SF_6 microbubbles in dogs. Five dogs received two i.v. infusion rates (35 or 70 ml/h for 7 min) of SF_6 microbubbles and were imaged by transcranial Doppler. The study demonstrated a dose-dependent level of increased Doppler mean intensity within the brain circulation. The results showed that only the 70-ml/h infusion rate produced a stable level of increased Doppler mean intensity and reached 24 to 26 dB over baseline values. The 35-ml/h infusion rate did not produce a stable level of increased Doppler mean intensity. After infusion termination, time-intensity curves revealed a linear decrease (±0.066 dB/s for 70 ml/h infusion rate) that was not related to the infusion rate.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

Schneider (21) studied the fate of SF_6 in human volunteers after a single i.v. administration of 0.3 ml/kg. The blood distribution $t_{½}$ was about 1 min, and the elimination $t_{½}$ was approximately 6 min. More than 80% of SF_6 gas was excreted through exhalation after 11 min. Morel et al. (32) evaluated the blood kinetics and pulmonary elimination of SF_6 microbubbles in 12 healthy subjects. Each subject received two i.v. bolus doses of 0.03 and 0.3 ml/kg with 3–14 days between doses. For both doses, the maximum blood concentration was reached within 1–2 min and then rapidly declined. For the lower dose, it was not possible to define the distribution and elimination phases. For the higher dose, the mean distribution $t_{½}$ times were 0.97 min (men) and 1.23 min (women), and the median elimination $t_{½}$ times were 8.25 min (men) and 6.93 min (women). The route of SF_6 elimination was via the lungs in the exhaled air with approximately 40–50% of the dose being eliminated within the first minute and with >75% of the dose eliminated by 11 min (33). In a study of 12 healthy volunteers, Kaps et al. (34) found that SF_6 microbubbles were safe, well tolerable, and provided a long-lasting US contrast enhancement in transcranial Doppler sonography.

The safety and tolerability of SF_6 microbubbles were evaluated in 66 healthy volunteers (doses of 0.003–0.12 ml/kg) during two placebo-controlled phase I studies and 12 patients with chronic obstructive pulmonary disease (COPD; dose of 0.057 ml/kg) during a phase II placebo-controlled study (35). For both phase I and phase II studies, no serious adverse events (AEs) occurred. There were seven nonserious AEs judged as possibly or definitely related to SF_6 microbubbles in the healthy volunteers, and there were two nonserious AEs reported in the COPD patients. There was one report of local heat at the injection from

the healthy volunteers. Torzilli (36) reviewed the adverse effects of SF_6 microbubbles in 3,212 subjects and reported that the overall incidence of AEs was 7.7% (possible, probably, or unknown relationship to the use of SF_6 microbubbles), and the incidence of serious AEs was <0.0002%. Rare serious AEs were predominantly hypersensitivity reactions. In 2004, a precautionary and temporary suspension of SF_6 microbubble use in cardiac studies was issued in Europe because of three case reports of fatal outcomes in patients with a high underlying risk for major cardiac complications. Piscaglia and Bolondi (37) performed a retrospective analysis of 23,188 studies in abdominal examination from 28 Italian centers. No fatal event was reported. The overall rate of all AEs was 0.125%, and the rate of serious AEs was 0.0086%.

In a multicenter evaluation of SF_6 microbubbles for improved endocardial border delineation, Nanda et al. (12) studied 138 patients with highly suspected cardiac disease. Patients received four bolus injections of 0.5, 1, 2, and 4 ml of SF_6 microbubbles. Scores of left ventricle opacification (LVO) were significantly higher than the control group with saline injection. Across all doses, 73-93% of patients had moderate to complete LVO. The mean duration of useful contrast effect ranged from 0.8–4.1 min. Opacification was negligible in all patients who received saline injection. Although SF_6 microbubbles have not been approved by the United States Food and Drug Administration, the safety and efficacy of SF_6 microbubbles for US contrast enhancement have been studied for the diseases of the heart (176 patients) (12, 33, 38), vascular structures (566 patients) (10, 13, 34), liver (478 patients) (11, 39-43), breast (196 patients) (14), spleen (46 patients), pancreas (cystic pancreatic masses with a different vascularization pattern) (31 patients) (44), and gastrointestinal tract (Crohn's disease with a thickened bowel wall) (15 patients) (18).

Supplemental Information

[Disclaimers]

The European Agency for the Evaluation of Medicinal Products May 2004

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