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● *Original Contribution*

PHYSICOCHEMICAL CHARACTERISTICS OF SONAZOID™, A NEW CONTRAST AGENT FOR ULTRASOUND IMAGING

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Abstract—The objective of the current work is to describe the physicochemical characteristics of Sonazoid™, a new ultrasound contrast agent for detection and characterisation of focal liver lesions. It has been demonstrated that Sonazoid™ powder for injection consists of microspheres of perfluorobutane (PFB) stabilised by a monomolecular membrane of hydrogenated egg phosphatidyl serine, embedded in an amorphous sucrose structure. Upon reconstitution with sterile water, stabilised microspheres of PFB are released in a predefined amount and size into a low viscosity, isotonic sucrose solution with a neutral pH. Sonazoid™ reconstituted product contains approximately 8 μl microspheres/ml with volume median diameter of approximately 2.6 μm. The product contains approximately 1.2 billion microspheres/ml of which less than 0.1% are larger than 7 μm. The acoustic properties of Sonazoid™ such as attenuation efficacy, fundamental and second harmonic backscatter efficacy are all well correlated to the microsphere volume concentration. The stability of Sonazoid™ after reconstitution is good, with no significant changes in physicochemical properties 2 h after reconstitution. Pressure stress is well tolerated by both concentrated and diluted Sonazoid™ with no permanent effects of pressures up to 300 mm Hg. The level and consistency of the investigated physicochemical properties demonstrate that Sonazoid™ should be well suited as a contrast agent for medical imaging with ultrasound. (E-mail: per.sontum@ge.com) **© 2008 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound, Contrast agents, Microspheres, Physicochemical properties, Acoustic properties, Perfluorobutane, Phosphatidyl serine.**

INTRODUCTION

Over the past two decades, the pharmaceutical industry has shown a great interest in developing a safe and efficacious ultrasound contrast agent (USCA) for medical imaging. This effort has so far resulted in bringing a few products to the market as well as a number of candidates late in the development pipeline. The principal physical contrast creating mechanism of all these USCAs is the scattering of ultrasound (US) from small envelopes of gas as they undergo volume oscillations in the sound beam. Due to the high compressibility of gases and their ability to resonate when insonated, a population of microspheres is very effective in scattering incident US compared with surrounding blood or tissue. During ultrasound scanning, body cavities and compartments containing microspheres will thus appear white compared with regions without contrast agent.

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The main drive for the development of a safe and efficacious compound has been in producing stable microspheres of a predefined, biologically acceptable, size that enables passage through capillary beds and thus allows for imaging in the entire cardiovascular system. The average diameter of the lung capillaries has been reported to approximately 7 μm with approximately 95% being larger than 4 μm (Hogg 1987). Hence, to optimise for free flowing properties and avoid potential capillary embolism, the size of microspheres in USCAs should preferably be smaller than approximately 4 μm. In addition, the content of larger microspheres should be minimised. To this point, it should be noted that the functional diameter of the capillaries for any given vesicle may be different from the geometric diameter; *e.g.*, surface properties and deformability may influence their retention in the microcirculation.

Various concepts have been explored in developing an USCA with suitable properties. Early products investigated the stabilisation of air bubbles, either by release *in vivo* from micro-porous crystalline sugar structures as with Levovist® (registered trademark of Schering AG)

or by stabilising individual envelops of air by a shell of denatured human serum albumin as with Albunex® (registered trademark of Mallinckrodt Inc.) (Christiansen et al. 1994; Sontum et al. 1997). Both these concepts were successful in bringing microspheres through the capillary bed of the lungs, producing contrast in the bulk volume of the left heart chamber after an i.v. injection. Their stability *in vivo* (*i.e.*, contrast persistency), however, was not sufficient to track contrast into other organs of the body. A primary cause for the insufficient stability of these air-based USCAs is that the small molecules of the air easily diffuse across the stabilising structure. Additionally, as air is highly soluble in the surrounding matrix (*i.e.*, blood), the microspheres simply dissolve too quickly after injection. In trying to improve the stability of the microspheres, a concept of encapsulating air with a more solid, polymer based, shell was investigated (Bjerknes et al. 1997). In this case, however, the thickness/stiffness of the shell lowered the physical, *in vitro* acoustic efficacy of the compound to such a degree that the substance did not deliver the necessary imaging quality (Hoff et al. 2000). In an effort to increase the stability of microspheres without including a rigid stabilising structure, a more slowly diffusing, low solubility gas such as sulphur-hexafluoride or low molecular weight perfluorocarbons may be utilised (Dugstad et al. 1996). These gases, in combination with various flexible stabilising structures, are now used in several products (*e.g.*, Definity®, registered trademark of Bristol-Myers Squibb, SonoVue™, registered trademark of Bracco and Optison™, registered trademark of GE Healthcare.)

As apparent from above, for USCAs the physical state of the active ingredient may be more important to product performance than its content or chemical composition. To facilitate a sufficient understanding of a product and to assure consistent performance both regarding efficacy and safety, comprehensive physicochemical characterisation and control is imperative. The objective of the current work is to give a thorough description of important characteristics of Sonazoid™ (registered trademark of GE Healthcare), a new USCA recently approved in Japan for detection and characterisation of focal liver lesions. During clinical use of Sonazoid™, imaging is performed in two stages; vascular-phase imaging, while the contrast is predominantly in the blood-pool and delayed-phase imaging, when microspheres have been taken up or trapped by Kupffer cells. Vascular phase imaging is performed shortly after administration and arterial-phase information can be obtained by monitoring the first passage of contrast into the liver. Because of the increased arterial vasculature surrounding malignant lesions, these become "bright rimmed" and easily detectable from normal tissue. The dynamics and nature of vascular contrast enhancement can be used to distinguish between different kinds of malignant lesions, such as hepatocellular carcinoma and metastasis, and different types of benign lesions, such as hemangioma and focal nodular hyperplasia, *etc.* Kupffer-phase imaging is performed after typically 10 min when the hepatic parenchyma will appear homogeneously bright (*i.e.*, filled with contrast) whereas malignant lesions will appear dark; the entire liver can be easily imaged in this phase and scanned for the presence of suspicious lesions. The mechanism behind the contrast enhancement observed during Kupffer-phase imaging has been shown to be due to the trapping of microspheres by the Kupffer cells present in the hepatic parenchyma (Miyahara et al. 2006; Watanabe et al. 2007). These cells are not present in malignant lesions which hence will appear as clear contrast defects in the image. The safety and clinical utility of Sonazoid™ have been demonstrated (Gordon 2006; Moriyasu 2006).

Sonazoid™ consists of perfluorobutane gas (PFB) microspheres stabilised by a membrane of hydrogenated egg phosphatidyl serine (HEPS). The product is formulated and presented as a lyophilised powder for injection that before use is reconstituted with sterile water to release stabilised microspheres of PFB in a predefined concentration and size distribution in an isotonic sucrose solution.

MATERIALS AND METHODS

Materials

Sonazoid™ is formulated as a powder for injection consisting of lyophilised sucrose entrapping HEPS stabilised PFB microspheres under a PFB headspace. Sonazoid™ is aseptically produced by continuous homogenisation of PFB in an aqueous dispersion of HEPS. After the initial microsphere generation, the concentration and size distribution of microspheres is adjusted through a series of controlled separation steps. The final dispersion, targeted to yield 8 μl microspheres per ml in the reconstituted product, is made isotonic by addition of sucrose. Two ml of the dispersion is filled into 10 ml glass vials and lyophilised. After lyophilisation, the vial head space is back-filled with PFB before stoppering. Before use, the product is reconstituted by addition of 2 ml of water through a supplied vented filter (5 μm) spike (Codan Chemoprotect® Spike, Codan GmbH & Co., Germany) followed by manual mixing for 1 min. After reconstitution, the product appears as a milky white, homogeneous dispersion. As the dispersion is nontransparent, visual inspection for extraneous particles is difficult. To ensure the absence of such particles, the product is withdrawn through the filter spike into the syringe before administration. After reconstitution, if left nonagitated the microspheres will start to segregate by flotation and form a