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Influence of temperature, needle gauge and injection rate on the size distribution, concentration and acoustic responses of ultrasound contrast agents at high frequency

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ABSTRACT

This paper investigated the influence of needle gauge (19G and 27G), injection rate (0.85 ml·min^{-1}, 3 ml·min^{-1}) and temperature (room temperature (RT) and body temperature (BT)) on the mean diameter, concentration, acoustic attenuation, contrast to tissue ratio (CTR) and normalised subharmonic intensity (NSI) of three ultrasound contrast agents (UCAs): Definity, SonoVue and MicroMarker (untargeted). A broadband substitution technique was used to acquire the acoustic properties over the frequency range 17–31 MHz with a preclinical ultrasound scanner Vevo770 (Visualsonics, Canada). Significant differences ($P < 0.001 - P < 0.05$) between typical *in vitro* setting (19G needle, 3 ml·min^{-1} at RT) and typical *in vivo* setting (27G needle, 0.85 ml·min^{-1} at BT) were found for SonoVue and MicroMarker. Moreover we found that the mean volume-based diameter and concentration of both SonoVue and Definity reduced significantly when changing from typical in *vitro* to *in vivo* experimental set-ups, while those for MicroMarker did not significantly change. From our limited measurements of Definity, we found no significant change in attenuation, CTR and NSI with needle gauge. For SonoVue, all the measured acoustic properties (attenuation, CTR and NSI) reduced significantly when changing from typical *in vitro* to *in vivo* experimental conditions, while for MicroMarker, only the NSI reduced, with attenuation and CTR increasing significantly. These differences suggest that changes in physical compression and temperature are likely to alter the shell structure of the UCAs resulting in measureable and significant changes in the physical and high frequency acoustical properties of the contrast agents under typical *in vitro* and preclinical *in vivo* experimental conditions.

1. Introduction

There is an increasing number of applications for the use of UCAs in the preclinical field [1], with an associated increase in the number of published studies utilising UCAs both *in vivo* and *in vitro* [2–5]. However, the differences between the administration of UCAs in an *in vivo* environment at body temperature and an *in vitro* experiment at room temperature need to be considered. The size of needle gauges used for small animal *in vivo* injections are often not cited in the literature but generally range from 24G for rat tail vein injection [6], 27G for mouse tail vein injection [7] and in larger animals – 30G for pig cervical region of nerve [8] and dog lymphatic system [9]. Generally *in vitro* experiments of UCAs are performed at room temperature using 18G – 20G needle gauges – sizes which are widely used in clinical practice and specifically recommended by some contrast agent manufacturers [10]. When bolus injections are undertaken in small animals, although the bolus is considerably smaller (of the order of microlitres), it is generally performed at an injection rate of between 0.5 and 3 ml·min^{-1}, a rate that is commonly used for clinical bolus intravenous injections.

1.1. The influence of temperature

The three commercial UCAs: Definity (Lantheus Medical Imaging, USA), SonoVue (Bracco, Italy) and MicroMarker (untargeted)

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(Visualsonics, Canada) are lipid-coated microbubbles (MBs). The shells of the three UCAs are monolayers but each is formed from a varying combination of different lipids. As a result, it is difficult to determine a specific phase transition temperature for the lipid shells of each and hence the effect of temperature on the lipid-coated MBs is unknown and its resultant influence on microbubble characteristics is unclear. The fluid–gel phase transition temperature of lipids in bilayer states varies from −1 °C to 75 °C [11] and −1 °C to 55 °C [12] depending on the phospholipids acyl chain length. Lipid molecules are limited on the membrane plane in two phases – either fluid: liquid phase; or gel: solid phase, where the liquid phase allows a freer diffusion of molecules than in the solid phase [13]. For this reason, the viscosity, elasticity, free energy and diffusion coefficient of the MB lipid shell can be changed below, at, or above the transition temperature [12]. The lipid shell of Definity consists of three phospholipids (DPPA, DPPC and MPEG 5000 DPPE) [3]. The lipids incorporated into MicroMarker shell are polyethylene glycol, phospholipids (unspecified) and fatty acids (VisualSonics PN11691) [14]. SonoVue possesses an amphiphilic phospholipid shell (a hydrophilic surface outside and a hydrophobic surface inside) [15] and involves two lipids (DSPC and DPPG) [16].

The thermal response of SonoVue has previously been studied over the temperature range 37–43 °C and its lipid transition temperature was inferred to be 40 °C, from observation of changes in its maximal diameter and backscatter intensity (ultrasound frequency: 2.5–8 MHz, low MI: 0.0081–0.113) [17]. For Definity and MicroMarker, the attenuation was measured and found to be higher at 37 °C than at 25 °C over a frequency range from 2 to 25 MHz. Measurements were undertaken in bovine serum albumin and whole blood and no difference in attenuation was found between the two diluents [14]. Individual Definity and SonoVue MBs have previously been measured at room temperature (21 °C) and body temperature (37 °C) using a high speed optical camera and insonated using a 10–80 kPa pulse at 1.7 MHz [18]. Compared with the performance at room temperature, Definity and SonoVue showed an increase in radial expansion and a decrease in onset of acoustic oscillation at body temperature. Extensive temperature dependence studies of SonoVue were completed using 3.5 MHz-ultrasound [16,19]. Increasing the temperature close to the transition temperature resulted in bubbles exhibiting greater radial expansion. Expanding MBs and rapid diffusion was shown to increase the mean diameter, attenuation, backscatter and nonlinear components.

1.2. The effect of needle size and injection rate

Talu et al. [20] measured the variation in concentration and mean diameter of one in-house, lipid-encapsulated UCA at 3 concentrations (1, 5, 10×10^8 MBs·ml^{-1}) using 3 needle gauges (23G, 27G and 30G) at 5 injection rates (0.01, 0.03, 0.1, 0.3 and 0.5 ml·sec^{-1}). With increasing needle gauge (decreasing inner needle diameter), the measured concentration of microbubbles was shown to decrease suggesting destruction of the MBs. In addition, a reduction in mean diameter was observed and attributed to an overall decrease in number of MBs in the population. It was suggested that this was due to diffusion by the forced compression and possible preferential destruction of large MBs. A similar study investigating the influence of administration variables (2 needle gauges: 18G and 25G; 2 syringes: 5 ml and 10 ml; 5 discrete flow rates from 2 to 3.3 ml·min^{-1}; 2 suspending fluids: distilled water and 95% volume-glycerol) was performed using another in-house lipid MB [21]. The results from this study showed that in addition to the hydrostatic pressure, shear stress due to the increasing pressure and velocity gradient played a key role in destroying MBs. However, this study found that an increasing volume flow rate (i.e., injection rate) reduced the destruction of MBs, which is not in agreement with the study of Talu et al. [20]. The discrepancy was attributed to the difference in initial diameter, size distribution, concentration and composition of MBs. An *in vivo* experiment to improve plasmid transfection using SonoVue MBs-mediated gene transfection tested the effects of using 3 needle gauges (25G, 27G and 29G) and showed that increasing needle gauge increased MB destruction and reduced MB diameter [22].

From the above studies, temperature, needle gauge and injection rate have been shown to have significant effects on the size distribution and acoustic properties of UCAs. However, the implications of these results for preclinical studies remain unclear. This is due to several factors. Firstly, the previous studies are limited to the ultrasound frequencies relevant for clinical applications and little research has been performed at high frequencies applicable for preclinical applications. Secondly, the effect of temperature, needle gauge and injection rate have been discussed separately but the potential interactions between them remain indistinct. Thirdly, a wide range of in-house MBs have been studied while commercial UCAs (both clinical and preclinical) are the products that are most commonly used for preclinical studies and the influence of these parameters on these commercial agents has not been fully investigated at high frequencies.

The aim of this paper is to investigate the influence of needle gauge, injection rate and temperature on the mean diameter, concentration, acoustic attenuation, contrast to tissue ratio (CTR) and normalised subharmonic intensity (NSI) of solutions of Definity, SonoVue and MicroMarker (untargeted) over the frequency range of 17–31 MHz. In particular, the changes observed in these measurements for SonoVue and MicroMarker, the most commonly used contrast agents for preclinical studies, under typical *in vivo* and *in vitro* experimental conditions will be discussed.

2. Method and materials

2.1. UCAs preparation

UCAs were reconstituted based on the manufacturers' guidelines and were ready for experimental use after standing at room temperature for 20 mins. The information of the three UCAs is listed in Table 1. Based on the maximum concentration from the manufacturer's published literature, MBs were diluted in air saturated distilled water to reach a concentration of 0.8×10^6 MBs·ml^{-1}. At this concentration, the occurrence of multiple scattering and shadowing of Definity MBs was previously shown to be avoided [23].

2.2. The control of needle gauge and injection rate

In this study, two needles (19G and 27G) (Becton, Dickinson and Company (BD), USA) are selected: a 19G (internal diameter (I.D.) = 0.686 mm) needle is generally used in human intravenous injection and also in some *in vitro* experiments while a 27G (I.D. = 0.21 mm) needle is commonly used for mouse-tail injections and mouse intra-cardiac injections. Two injection rates are studied: 0.85 ml·min^{-1} and 3 ml·min^{-1}, where 0.85 ml·min^{-1} is a typical injection rate used in bolus injections into a mouse tail vein during preclinical studies and 3 ml·min^{-1} a typical injection rate applied in *in vitro* experiments. The steady and reproducible injection rate was controlled using a syringe pump (Aladdin, World Precision Instruments Inc., USA) by connecting the test needle with 1 ml-syringe (I.D. = 4.78 mm) to reach an injection rate of 0.85 ml·min^{-1} and 2 ml-syringe (I.D. = 8.66 mm) to reach a 3 ml·min^{-1} injection rate.