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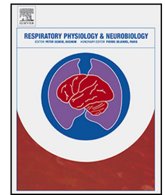

Expansion of bubbles under a pulsatile flow regime in decompressed ovine blood vessels

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ABSTRACT

After decompression of ovine large blood vessels, bubbles nucleate and expand at active hydrophobic spots on their luminal aspect. These bubbles will be in the path of the blood flow within the vessel, which might replenish the supply of gas-supersaturated plasma in their vicinity and thus, in contrast with our previous estimations, enhance their growth. We used the data from our previous study on the effect of pulsatile flow in ovine blood vessels stretched on microscope slides and photographed after decompression from hyperbaric exposure. We measured the diameter of 46 bubbles in 4 samples taken from 3 blood vessels (pulmonary artery, pulmonary vein, and aorta) in which both a “multi-bubble active spot” (MBAS)—which produces several bubbles at a time, and at least one “single-bubble active spot” (SBAS)—which produces a single bubble at a time, were seen together. The linear expansion rate for diameter in SBAS ranged from 0.077 to 0.498 mm/min and in MBAS from 0.001 to 0.332 mm/min. There was a trend toward a reduced expansion rate for bubbles in MBAS compared with SBAS. The expansion rate for bubbles in an MBAS when it was surrounded by others was very low. Bubble growth is related to gas tension, and under a flow regime, bubbles expand from a diameter of 0.1 to 1 mm in 2–24 min at a gas supersaturation of 620 kPa and lower. There are two phases of bubble development. The slow and disperse initiation of active spots (from nanobubbles to gas micronuclei) continues for more than 1 h, whereas the fast increase in size (2–24 min) is governed by diffusion. Bubble-based decompression models should not artificially reduce diffusion constants, but rather take both phases of bubble development into consideration.

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1. Introduction

We have shown that after decompression of ovine large blood vessels from high pressure, bubbles nucleate and expand at active hydrophobic spots on their luminal aspect (Arieli and Marmur, 2013, 2014; Arieli et al., 2015). We suggested that these active spots are the principal source of bubbles which cause the most severe decompression sickness. Various features of diving can be explained by this mode of bubble formation: bubblers–nonbubblers, adaptation to diving, a greater risk of decompression sickness on the second dive, and CNS decompression sickness (Arieli et. al., 2015). The rate of bubble expansion after decompression has been studied in the past, both theoretically and experimentally, in calm conditions (Arieli and Marmur, 2014; Kim et al., 2004; Kwak and Kim, 1998; Nikolaev, 2000; Papadopoulou et al., 2015). In some of these investigations, blood perfused the tissue encompassing the bubble. However, none of them studied the flow of the medium surrounding the bubble. Bubbles on the luminal aspect of blood vessels will be in the path of blood flow, which might replenish the supply of gas-supersaturated plasma in their vicinity. In such a case, gas transfer into the bubble will no longer be limited by diffusion through the surrounding medium, and the growth rate of a bubble adhering to the blood vessel wall should in reality be faster than previously estimated. Some studies refer to the competition for gas between adjacent bubbles (Chappell and Payne, 2006; Papadopoulou et al., 2015; Van Liew and Burkard, 1993). However, the flow of the medium may obliterate the diffusion distance prevailing in calm conditions, and therefore reduce or eliminate the competition for dissolved gas between adjacent bubbles. In our last report (Arieli et al., 2015), we photographed decompressed blood vessels in calm conditions for the first 30 min, and then in pulsatile flow of supersaturated saline with a mean velocity of 234 cm/min. Bubble expansion after decompression was

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extracted from the records of this study for the measurement of growth rate. Because there are active spots which produce a single bubble at a time, the single-bubble active spot (SBAS), and others which produce a number of bubbles at the same time, the multi-bubble active spot (MBAS), the growth of a single bubble can be compared with the growth of a number of bubbles adjacent to one another on the same active spot. In the present report, we describe the growth rate of bubbles under pulsatile flow of saline. We also suggest two steps of bubble expansion, a slow phase followed by a fast phase.

2. Methods

The methods were described in detail in our previous report (Arieli et al., 2015), and will be presented briefly here.

2.1. Tissue preparation

The complete heart and lungs from 6 slaughtered sheep (taken on separate days) were obtained at the abattoir. In the laboratory, under saline and without any exposure to air, samples from the blood vessels were gently stretched on microscope slides using metal clips with the luminal aspect exposed. Two slides were placed on the bottom of two Pyrex bowls (diameter 26 cm, height 5 cm) under 2.5 cm saline. The other two slides were kept under saline in the refrigerator for the next day.

2.2. 2 Protocol

The bowls containing the samples were transferred to two different 150 L hyperbaric chambers and were placed on double-walled metal plates with circulating water at 12° C for tissue preservation. Beforehand, the air conditioning in the small room containing the two hyperbaric chambers was used to take the room temperature down to 15° C. Staining for lipids following the 20 h exposure proved that the phospholipids layers at the active spot on the luminal aspect of the blood vessels remained intact (Arieli el al., 2015). Pressure was elevated at a rate of 200 kPa/min to 1013 kPa, 90 m sea water, and remained at that pressure overnight (20.5 ± 2.3 h, mean $\pm$ SD). In the morning, one of the chambers was decompressed at a rate of 200 kPa/min. The bowl was placed carefully on a nearby table for photography. We started automated photographing at 1-s intervals. The first 30 min of photography was conducted in calm conditions. Photography was resumed for the second 30 min with a flow of saline delivered horizontally over the blood vessel at a rate of 234 cm/min using a peristaltic pump. The same protocol was followed for the other samples.

Three blood vessels were examined, the aorta, pulmonary artery, and pulmonary vein, in which both an MBAS and at least one SBAS were seen together. The diameter of bubbles was measured under pulsatile flow at selected time intervals (0.25–1 min, according to their growth rate) over a period of 8–16 min. Because it usually takes time in the flow protocol until an MBAS is seen clearly, in three of the four samples the sampling period started some time after the pump had been switched on. Four samples were taken from 3 blood vessels, comprising 46 bubbles. Arieli and Marmur (2014) used a simple model to calculate bubble expansion after decompression. This model predicted linear growth of the diameter of a spherical bubble with time. The model assumed a constant diffusion distance and gas tension. The effect of surface tension was not considered. Although this is not the case in calm conditions, the actual diameters changed in a linear mode with time even in the calm state (Arieli and Marmur, 2014). When the medium flows over the bubbles, as in the present case, and it is well mixed, the diffusion path should be small. One would therefore expect linearity of the diameter with time, and the slope for diameter as a function of time was calculated for each bubble. Gas tension may decrease with time, mainly due to bubbles formed by the pump and depletion of the dissolved gas. Thus, the driving pressure for gas loading in the bubble should decrease with time.

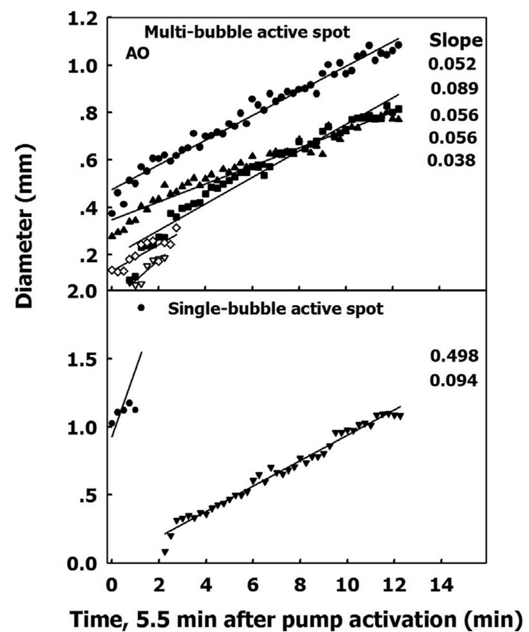

Fig. 1. Diameter of bubbles in the aorta (AO) for one single-bubble active spot (SBAS, lower panel) and one multi-bubble active spot (MBAS, upper panel) as a function of time. Each symbol represents a bubble. After the first bubble detached from the SBAS, another bubble developed. Slopes of the linear regression for each bubble are shown.

2.3. Statistical analysis

Univariate ANOVA and Levene's test for equality of variances were used to compare expansion rates on the SBAS and MBAS for the four samples.

3. Results

The size of bubbles in the sampled aorta is shown in Fig. 1, with the diameter of bubbles on the SBAS appearing in the lower panel. After detachment of the first bubble, another started developing on the same spot. Initially (over the first 3 measurements) the expansion rate of the second bubble (full triangles) was high, becoming lower as time progressed. The slope of the first bubble was steeper than that of the second, ranging from 0.094 to 0.498 mm/min (mean 0.296). A similar rate of expansion was observed over the same period (starting from the time the pump was activated) for 5 bubbles on the MBAS (upper panel), ranging from 0.038 to 0.089 mm/min (mean 0.058). Two bubbles which persisted for only a short time (empty symbols) did not detach, but merged with the larger bubble close by, resulting in a sudden steep increase in its diameter (full square symbols). In two bubbles (full square and full circle) a steep increase in diameter was seen at the start, with a more gradual slope as time progressed. The distance between the SBAS and MBAS was 5.0 mm.

Results from two SBAS and one MBAS in the pulmonary artery are shown in Fig. 2. Linear growth rates (slopes) were variable for the bubbles on both of the SBAS and for five bubbles on the MBAS, ranging from 0.057 to 0.123 mm/min (mean 0.090) on the SBAS and from 0.10 to 0.096 mm/min (mean 0.058) on the MBAS. The delay to sampling (time from pump activation) in this case was twice as

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