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Frontiers review

Was the appearance of surfactants in air breathing vertebrates ultimately the cause of decompression sickness and autoimmune disease?

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ABSTRACT

All air breathing vertebrates are endowed with pulmonary surfactants, surface-active lipoprotein complexes formed by type II alveolar cells. Surfactants are deposited in clearly defined areas on the luminal aspect of blood vessels, producing hydrophobic spots. Gas nanobubbles measuring 5–100 nm form spontaneously on the smooth hydrophobic spot from dissolved gas. Bubbles nucleate and grow at these spots after decompression from high pressure. Proteins with hydrophobic regions circulating in the blood will adhere to the gas phase-plasma interface. Deformation of their secondary and tertiary configuration will present them as foreign molecules or autoantigens. Components of the intact protein which are also present in a deformed protein may be recognized as foreign too. This process is proposed as the trigger for autoimmune diseases. The presence of autoimmune disease in air breathing vertebrates, increased autoimmunity and the elevated risk of decompression sickness with age, as well as variable sensitivity to both diseases, can be matched with the appearance of surfactant spots. Eliminating these spots may provide protection against both diseases.

1. Introduction

We recently discovered hydrophobic spots on the luminal aspect of ovine large blood vessels at which nanobubbles are formed, later to become the gas micronuclei that develop into bubbles after decompression from high pressure (Arieli and Marmur, 2014). These spots should be covered by a permanent gas phase in the form of nanobubbles, with interaction between the free gas phase and proteins in the blood. A hypothesis is suggested to explain the possible development of autoimmune diseases, which connects the development of lung surfactants, hydrophobic spots on blood vessels, and the interaction between proteins and the gas phase.

2. Supporting data from various fields

2.1. Surfactants

2.1.1. Pulmonary surfactants

Pulmonary surfactants are surface-active lipoprotein complexes (phospholipoprotein) formed by type II alveolar cells. The proteins and lipids that make up the surfactants have both hydrophilic and hydrophobic regions. The water-insoluble hydrophobic group may extend out of the water phase, into the air, whereas the water-soluble head group remains in the water phase. In the lung this reduces surface tension, allows the hysteresis which maintains lung function, prevents infiltration of water into the alveoli, and prevents gas from small cavities being forced into large cavities. Surfactants are composed of ~40% dipalmitoylphosphtidylcholine (DPPC), 40% other phospholipids (phosphatidylcholine, phosphatidylglycerol), ~5% surfactant-associated proteins (SP-A, B, C and D), and cholesterol. In the human lung the main surfactant is DPPC (http://en.wikipedia.org/wiki/Dipalmitoylphosphatidylcholine), which also has a higher compaction capacity than the other phospholipids because the apolar tail is less bent. The SP proteins reduce the temperature required for transition from the gelatinous phase to liquid crystal from 41

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to 37 °C, and maintain the spread of DPPC at the interface. Alveolar surfactant has a half-life of 35 h once secreted (Janssen et al., 2002). It is mainly reabsorbed into the lamellar structures of type II pneumocytes.

2.1.2. Evolution

All air breathing vertebrates are endowed with surfactants. When vertebrates began to breathe air, the surface tension of their body fluids did not allow them to maintain small open gas cavities. This was enabled by the evolution of surfactants, which are to be found in lung fishes that are representative of the first air-breathing vertebrates. Disaturated phospholipid, purported to be the primary surface tension-controlling agent, is found in the lungs of the three living species from Australia (*Neoceratodus forsteri*), South America (*Lepidosiren paradoxa*), and Africa (*Protopterus annectens*) (Orgeig and Daniels, 1995). Phosphatidylcholine is the dominant phospholipid, phosphatidylglycerol is virtually absent, and there is a significant proportion of the combination of phosphatidylserine and phosphatidylinositol. Surfactant from the primitive Australian lungfish *N. forsteri* is almost identical to that of the primitive air-breathing actinopterygiian fish (Daniels and Orgeig, 2003).

2.1.3. Surfactants on blood vessels

Using electron microscopy, Hills (1992) demonstrated an oligolamellar lipid lining on the luminal aspect of various ovine blood vessels. He also provided evidence of hydrophobicity, using the measured angle to a small (5 μl) drop of water. The hydrophobicity was reduced by rinsing these vessels with chloroform, which led Hills to ascribe it to phospholipids. He suggested that the deposition of lung surfactants created this hydrophobic lining. His claim was supported by increased hydrophobicity within blood vessels downstream, but not upstream, of the lung. However we demonstrated that hydrophobicity can be found in both the arterial and the venous circulation: in the pulmonary vein and artery, the left and right atria, superior vena cava, and aorta (Arieli and Marmur, 2013b). Hills and Butler (1981) also showed that when the pulmonary vasculature of the dog was flushed with microbubbles containing serum, the outflow contained surfactant. They could not determine whether the microbubbles caused the release of surfactant from within lung cells, or whether the surfactant was already present in the lumen of the vasculature. Arieli and Marmur (2013b) confirmed Hills' findings by establishing hydrophobic properties in various ovine blood vessels: the aorta, the pulmonary vein and artery, superior vena cava, and left and right atria. Using drops of saline (~100 μl), we found that hydrophobicity was highly variable between different areas of the same blood vessel, between blood vessels, and between animals. No difference in hydrophobicity was found between the six blood vessels. In a follow-up study, Arieli and Marmur (2014) found that there are clearly defined areas on the surface of blood vessels that fit the suggestion of hydrophobic spots at which bubbles nucleate and grow after decompression from high pressure. It is yet to be determined which of the various components of the pulmonary surfactants compose the hydrophobic spot.

2.2. The hydrophobic spot and creation of a gas phase

It has been shown that tiny, flat gas nanobubbles measuring 5–100 nm form spontaneously when a smooth hydrophobic surface is submerged in water containing dissolved gas (Tyrrell and Attard, 2001; Yang et al., 2007). The mechanism underlying this phenomenon was largely unexplained by the simple laws of physics describing the control of bubble stability, and the suspicion was that it was due to an artifact of the atomic force microscopy. However, a number of studies have confirmed the presence of these nanobubbles (Meyer et al., 2005; Singh et al., 2006; Stevens et al., 2005; Switkes and Ruberti, 2004). The use of both atomic force microscopy and optical techniques has proven that these nanobubbles are not an artifact (Karpitschka et al., 2012). This layer of gaseous nanobubbles is stable, their volume does not change with time, and did not change even when high pressure waves were applied (Brotchie and Zhang, 2011). A number of theories have been proposed in explanation of the formation and stability of these nanobubbles (Seddon et al., 2011; Weijs et al., 2012), and theoretical physics is still engaged in the search for an answer to these questions. In ultrasound irradiation, rectified diffusion increased the volume of nanobubbles (Brotchie and Zhang, 2011), suggesting that they might expand in a state of gas supersaturation. This agrees with our finding of bubble development on a smooth hydrophobic surface after decompression (Arieli and Marmur, 2011, 2013a). Lüderitz and von Klitzing (2012) showed that nanobubbles 30–60 nm in diameter are formed on patches of the surfactant solution which settle on the surface. A similar process of events may produce the hydrophobic spots on ovine blood vessels at which nanobubbles are formed, remaining there permanently. These nanobubbles grow into bubbles after decompression (Arieli and Marmur, 2014). We therefore suggest that a permanent layer of nanobubbles covers the hydrophobic spots on the luminal aspect of blood vessels. The hydrophobic–hydrophilic force at the gas–water interface is greater than that at the phospholipid–water interface.

2.3. Interaction of proteins with a gas phase

Proteins have evolved to perform their various tasks in an aqueous solution, and frequently also across the lipid bilayer in between aqueous media. Only certain specific proteins, such as keratin, are able to withstand dry gaseous exposure. Other proteins which are liable to come up against a gas phase within the body should have a liquid film for protection. The chain of amino acids in a protein may include hydrophobic acids such as alanine, valine, leucine, isoleucine, phenylalanine, tryptophan and methionine. The α-helices are also the most common structural element of the protein to cross biological membranes, because the helical structure can satisfy all backbone hydrogen bonds internally, leaving no polar groups exposed to the membrane if the sidechains are hydrophobic. Because the hydrophobic–hydrophilic force is high for a gaseous phase–water interface, the hydrophobic regions in proteins will react with the gaseous phase. Much has been studied regarding the interfacial denaturation of plasma proteins in oxygenator devices (Lee and Hairston, 1971). Usually, in the coiled folded protein, the polar groups are external and the non-polar groups are internal. In contact with a gas phase, because polar groups would face the aqueous side and the non-polar groups would protrude on the gas side, the weaker bonds (mainly hydrogen) would break and allow alteration of their secondary and tertiary configuration. The denatured protein would change its immunochemical properties. Exposure of hydrophobic domains would attract other molecules to produce aggregates of proteins and fatty acids, both in oxygenators in which blood was in direct contact with the gas phase, and in bubbles within the blood (Philp et al., 1972). Alteration of immunoglobulins was established after exposure to an oxygenator, when there was a high incidence of infection in patients after "open heart surgery" (Lee and Hairston, 1971).

3. Autoimmune diseases and hydrophobicity

3.1. Autoimmune diseases in animals

Autoimmune diseases are known in air breathing vertebrates, but not in gill breathers such as fish or sharks. Diabetes mellitus