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Is the probable spillage of the lung surfactant dipalmitoylphosphatidylcholine the ultimate source of diabetes type 1?

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ABSTRACT

The lung surfactant dipalmitoylphosphatidylcholine (DPPC) most probably leaks into the blood, settling on the luminal aspect of blood vessels to create active hydrophobic spots (AHS). Nanobubbles are formed at these spots from dissolved gas. We hypothesized that when a large molecule in the blood comes into contact with a nanobubble at the AHS, its tertiary structure is disrupted. An epitope not previously having undergone thymus education may then prompt an autoimmune response. There are thus two independent processes which may share the blame for autoimmune disease: spillage of large molecules into the blood, and the creation of AHS. DPPC was measured in 10 diabetes type 1 patients and 10 control subjects. DPPC in the diabetic group was 4.63 ± 0.68 μg/mL, non-significantly higher than in the control group (4.23 ± 0.94 μg/mL). However, in the diabetic group, DPPC was high when the samples were taken within 1.5 years of disease onset. This is closer to the time of AHS production, which takes place ahead of the disease. Further investigation, with sampling for DPPC as soon as possible after onset of the disease, may provide additional support for our hypothesis. If proved true, this may open up considerable therapeutic potential.

1. Introduction

In the search for the hypothesized gas micronuclei from which bubbles evolve during decompression after diving, one of us (R.A.) succeeded in establishing the chain of events. The lung surfactant dipalmitoylphosphatidylcholine (DPPC) most probably leaks into the blood stream. However, both DPPC and surfactant proteins SP-B and SP-C were detected in the plasma (Papaioannou et al., 2016; Arieli et al., 2019). Therefore, we believe that the main source of DPPC is the lung. Leaving the plasma, the DPPC settles on the luminal aspect of blood vessels to create an oligolamellar lining of phospholipids. Layering of DPPC is impossible without SP-B and SP-C, where all the three of them could have lung origin. We could not find in the literature another reasonable source for these three components. We named this site an "active hydrophobic spot" (AHS). During the dive, the nanobubbles formed at the AHS from dissolved gas become the gas micronuclei from which bubbles evolve on decompression (Arieli, 2017).

Considering the possibility that the blood is thus faced with a constant gas phase, we proposed that this may also have an effect on autoimmunity (Arieli, 2015). The hypothesis claims that the development of autoimmune disease may be due to two independent processes: 1. The existence of many and large AHS, which we suggested are common to a large number of autoimmune diseases; 2. The leakage of large molecules into the blood, which will be transformed into autoantigens specific for each disease.

When the large molecule (protein, ganglioside, ribonudeoprotein, nudeosomal DNA and other) in the blood stream comes into contact with the nanobubble at the AHS, its tertiary structure is disrupted. H-bonds will be broken, and whereas hydrophobic parts will bulge into the gas phase, the hydrophilic domain will remain within the aqueous plasma. An epitope not previously having undergone thymus education may then prompt an autoimmune response. Some autoimmune diseases have

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Table 1
Subjects' demographic data.

Group	Age	Male	Female	Age at diagnosis	Years in diabetes
Diabetes type 1	17.2 (4.7)	5	5	10.7 (4.3)	6.6 (3.9)
Control	8.1 (3.4)	5	5		

Data represent mean ± (SD).

been related to coagulation of proteins and exposure to a hidden antigen (Raghupathy, 2006), which is similar to the proposed mechanism. The novelty of our hypothesis is that it provides a precise indication of the possible cause of denaturation. The hypothesis is appealing because, if proved correct, it would enable a number of prophylactic procedures. Early detection of DPPC in the plasma could warn of a buildup of AHS. At some time in the future, the elimination of plasma DPPC may prevent autoimmune disease as may removal of the AHS.

The wide variability in the number and area of the AHS correlates with the variability in bubbling during decompression (Arieli and Marmur, 2017). Thus, differences in the distribution of AHS can explain the diversity in the characteristics of bubbling in divers. This variability may be related to either or both of the two steps in the production of AHS: leakage of DPPC from the lung into the circulation, and its settling on the luminal aspect of blood vessels. DPPC in the plasma, which is correlated with the leakage of DPPC from the lung, may easily be measured. According to our hypothesis, the creation of AHS evidently takes place before the appearance of the disease, and we are unaware whether DPPC leakage is temporary or a lifelong trait.

In the present study, we determined the concentration of DPPC in the plasma of patients suffering from diabetes type 1, comparing them with a control group, in the expectation that we would see higher DPPC levels in the diabetic group. As far as we know, this is the first time DPPC has been measured in human plasma.

2. Methods

2.1. Subjects

The study was approved by the ethics committee of the Ziv Medical Center in Safed. Each subject gave his/her signed informed consent after receiving a detailed explanation of the study and its purpose. Ten subjects suffering from diabetes type 1 and 10 control subjects participated in the investigation (Table 1). None of the volunteers for control sampling or their close family members suffered from an autoimmune disease.

2.2. Protocol

On arrival at the diabetes clinic in Ziv Medical Center, a 4 ml venous blood sample was taken from each subject in a Li-heparin tube. Plasma was separated by centrifugation at 4 °C for 10 min, 2500 rpm, and stored at −20 °C. When all 20 samples had been completed, they were transferred to the Western Galilee Medical Center for phospholipid extraction. Phospholipids were extracted using an accepted procedure, as described in Arieli et al. (2019). The N_2-dried phospholipids were kept at −20 °C until delivery to the MIGAL laboratory in Kiryat Shmona for the determination of DPPC. Samples were analyzed as described in detail previously (Arieli et al., 2019), using Dionex Ultimate 3000 UHPLC system, equipped with a heated electrospray ionization (HESI-II) source connected to a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer (Thermo Scientific) instead of Q-TOF LC–MS. ESI capillary voltage was set to 3900 V, capillary temperature to 350°C, aux gas heater temperature to 350°C, sheath gas flow rate to 35 and aux gas flow rate to 10. The mass spectra (m/z 67–1000) were acquired using both positive ion mode.

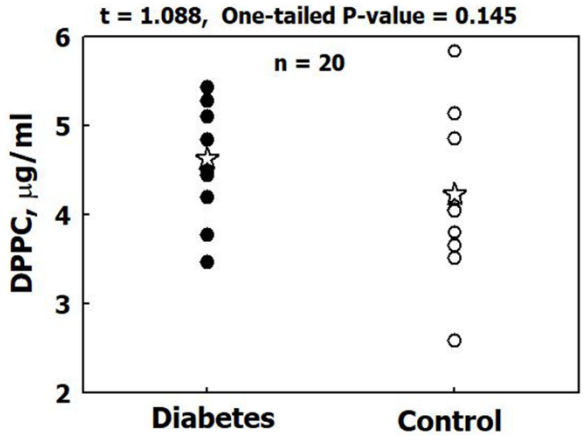

Fig. 1. Concentration of DPPC in the plasma of diabetes type 1 (full circles) and control subjects (empty circles) Stars indicate the mean.

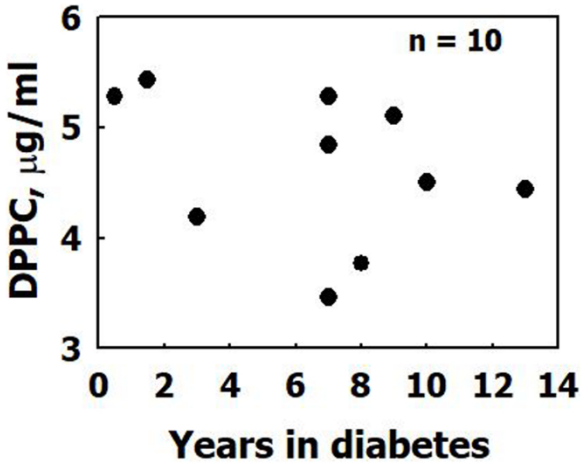

Fig. 2. Concentration of DPPC in the plasma of diabetes type 1 patients as a function of time in diabetes.

2.3. Statistical analysis

A normality test (Shapiro-Wilk) and an equal variance test (Brown-Forsythe) were used for the concentration of DPPC in the diabetic and control groups. The Student t-test was then used for the equality/inequality of the results.

3. Results

The concentration of DPPC in the diabetic plasma was 4.63 ± 0.68 μg/mL, and in the control plasma 4.23 ± 0.94 μg/mL. Normality test (Shapiro-Wilk): passed (P = 0.893); equal variance test (Brown-Forsythe): passed (P = 0.481). The t-test yielded t = 1.088 with 18 degrees of freedom. Although a trend towards higher DPPC was noted in the diabetic subjects, there was no statistically significant difference between the two groups (one-tailed P-value = 0.145). Results are presented in Fig. 1. The concentration of DPPC in the diabetic group is plotted against time in diabetes in Fig. 2. A trend may be seen towards a decrease in DPPC concentration with time in diabetes.

4. Discussions

The concentration of DPPC in both the diabetic and control groups in the present study was higher than the data obtained from sheep plasma (2.15 ± 0.87) in a previous investigation (Arieli et al., 2019). These

results provide continued support for the generality of the presence of DPPC in mammalian plasma. Our hypothesis regarding the development of autoimmune disease suggests that the layering of DPPC to create the AHS and nanobubbles precedes the induction of the disease. Thus, the high concentration of DPPC within a short time (less than 1.5 years) of the diabetes surge, may represent the high leakage of DPPC before the induction of diabetes type 1. It may be that later on in life, the leakage is reduced. Diabetic autoantigens and antibodies are also known to decline in long-term diabetes type 1. Thus, the results of the present study do not contradict our hypothesis regarding the origin of autoimmune diseases. A further study of DPPC concentrations closer to the induction of diabetes type 1 may reveal the actual spillage of DPPC from the lung into the blood.

Declaration of Competing Interest

The authors report no declarations of interest.

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