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RESEARCH ARTICLE

Testing the Double Cause Hypothesis for Autoimmune Diseases, Dipalmitoylphosphatidylcholine Should be Measured in Plasma or Blood Vessels of Diabetes Type 1?

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ABSTRACT

Introduction: The lung surfactant dipalmitoylphosphatidylcholine (DPPC) leaks into the blood, settling on the luminal aspect of blood vessels to create active hydrophobic spots. 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 active hydrophobic spots, its tertiary structure is disrupted. An exposed epitope may then prompt an autoimmune response. In a previous study, plasma DPPC in diabetes type 1 was higher in 2 samples from patients within 1.5 years in the disease compared to 8 others from longer time in the disease. Could these 1.5 years represent the previous time when active hydrophobic spots were formed?

Methods: DPPC was measured in plasma of 10 diabetes type 1 patients within 1.5 years in the disease compared to 10 controls.

Results: DPPC in the diabetic group was 1.17 ± 0.27 μg/ml , non-significantly higher than in the control group (1.51 ± 0.42 μg/ml).

Discussion: Leakage of DPPC from the lung to the plasma is not the limiting factor for buildup of the active hydrophobic spots. The heart of lupus mice contained more DPPC from the control mice. Further investigation, should explore the content of DPPC in the blood vessels from diabetic animals. If our hypothesis is proved true, it may open up considerable therapeutic potential.

Keywords: lung surfactant, nanobubbles, hydrophobicity, coagulation

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) leaks into the blood stream. Leaving the plasma, the DPPC settles on the luminal aspect of blood vessels to create an oligolamellar lining of phospholipids. We named this site an "active hydrophobic spot" (AHS). Nanobubbles are formed from dissolved gas at the AHS. During the dive, these nanobubbles become the gas micronuclei from which bubbles evolve on decompression [1].

Considering the possibility that the blood is thus faced with a constant gas phase at the nanobubbles, we proposed that this may also have an effect on autoimmunity [2]. The Double Cause Hypothesis – DCH suggests that the development of autoimmune disease may be due to two independent processes: 1. The existence of many and large AHS; and 2. The leakage of large molecules (potential autoantigen and specific for each disease) into the blood. This molecule will change its tertiary structure at the gas/liquid interface and be transformed into autoantigen.

The DCH is appealing because, if proved correct, it would enable a number of prophylactic procedures. At some time in the future, the elimination of plasma DPPC or the removal of the AHS may prevent the development of an autoimmune disease.

Until now, we did not know whether the rate limiting factor for production of AHS is the spillage of DPPC from the lung (which should be expressed by its concentration in the plasma) or the settling at the blood vessels. Our first report on concentration of DPPC in the plasma from diabetes type1 patients, did not revealed significant difference from the control [3]. However, a probable trend for reduction in the concentration of DPPC as function of years in the disease was observed. Because the two highest concentrations were found within 1.5 years in the disease, we suspected that this period represents the time before the appearance of the disease when AHS were formed. According to our hypothesis, the creation of AHS evidently takes place before the appearance of the disease.

DPPC in the plasma, which is correlated with the leakage of DPPC from the lung, may easily be measured, while sampling blood vessels from diabetic patients is out of question.

The aim of the present study is testing whether plasma DPPC is higher in diabetes patient within 1.5 y from diagnosis, as compared to controls. In the present study, we determined the concentration of DPPC in the plasma of patients suffering from diabetes type 1 within 1.5 y from the appearance of the disease, comparing them with a control group, in the expectation that we would see higher DPPC levels in the diabetic group. If such a difference would not be confirmed, the research focus should be diverted to the settled DPPC at blood vessels as the main factor.

Methods

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. Sample size was determined from our previous experience (3), expected difference of significant physiology of at least 30% (5) and the difficulty (time and effort) in getting enough subjects. Ten subjects suffering from diabetes type 1 within 1.5 y from the appearance of the disease 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.

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, 2,500 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 [4]. The N_2-dried phospholipids were kept at -20°C until delivery to the MIGAL laboratory in Kiryat Shmona for the determination of DPPC using liquid chromatography–mass spectrometry. Samples were analyzed as described in detail previously [4], 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.

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.

Results

Table 1–Subjects' demographic data

Group	Age	Male	Female
Diabetes type 1	13.6 (2.3)	4	6
Control	11.12(5.3)	3	7

Data represent mean (SD)

One sample from the diabetes group was too small for the analysis. The concentration of DPPC in the diabetic plasma was 1.17 ± 0.27 SD µg/ml, and in the control plasma 1.51 ± 0.42 SD µg/ml. Normality test (Shapiro-Wilk): passed ($P = 0.498$); equal variance test (Brown-Forsythe): passed ($P = 0.380$). Although a trend towards higher DPPC was noted in the control subjects, there was no statistically significant difference between the two groups (two-tailed *P*-value = 0.057). Results are presented in Fig. 1.

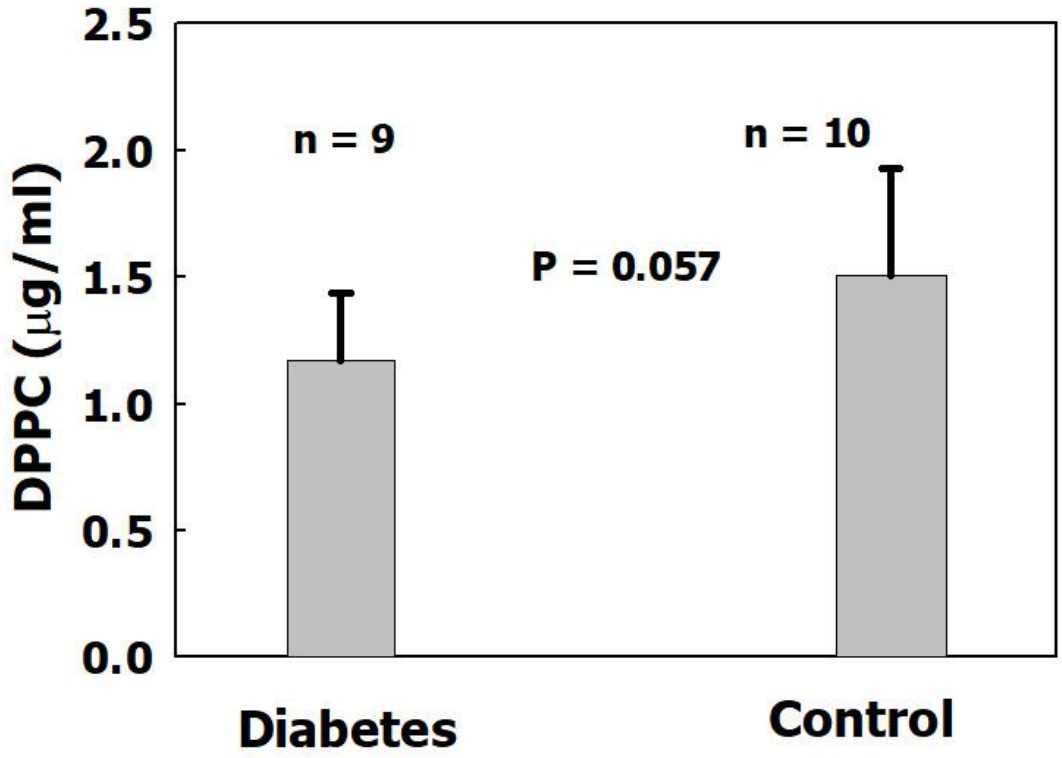

Figure 1–Concentration of DPPC in the plasma of diabetes type 1 and control subjects, mean - bar + SD

Discussion

The concentration of DPPC in the plasma from diabetes patients was not greater than that from the control. This is similar to the finding from our previous report [3]. We previously suggested (3) that during 1.5 y from diagnosis, plasma level of DPPC is high and may represents high plasma level before diagnosis when AHS was settled. This possibility was not supported in the present report. No correlation was found of the concentration of DPPC in the plasma of the sheep with the amount of DPPC at the blood vessels and the amount of bubbling [4]. However, the amount of DPPC was greater in the heart from lupus mice compared to controls [5]. Therefore, we conclude that the concentration of DDPC in the plasma does not reflect the amount of DPPC which was settled at the blood vessels. Because the DCH suggests that the

settled amount of DPPC at the blood vessel is one component of the double cause for autoimmune disease, further studies of the amount of settled DPPC at the blood vessels is needed. For example: the amount of DPPC in the heart including intact large blood vessels, in animal which naturally get diabetes type 1, before and after the induction of the disease. If the level of DPPC would be high before the appearance of the disease, it would assure that DPPC is most probably the cause and not the outcome of the disease. Another possible line of research is the extraction of blood vessels from amputated limbs from diabetes type 1 patients. Because of the huge clinical potential of the DCH: removal of plasma DPPC or removal of the AHS to prevent autoimmune diseases, further studies are encouraged.

Conclusion

In the double cause hypothesis (DCH) for autoimmune diseases, it was not determined which of the two: leaking DPPC from the lung to the blood or the settling of DPPC at blood vessels has the main effect. The finding that DPPC in the plasma of diabetes patients within 1.5 y from diagnosis is not higher than the control, with other considerations led to the conclusion that the main cause could be in DPPC at the blood vessels. The clinical potential of DCH should be a drive for further studies.

Competing Interests. The authors declare no competing interests

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