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RESEARCH ARTICLE

Dipalmitoylphosphatidylcholine in the hearts of mice does not correlate with expression of the autoimmune disease lupus

Nura Aburomi[1] Soliman Khatib[2,3] Eilam Palzur[1], Ran Arieli[1,4]

[1]Eliachar Research Laboratory, Western Galilee Medical Center, Nahariya,
[2]Department of Natural Compounds and Analytical Chemistry, MIGAL – Galilee Research Institute, Kiryat Shmona, Israel
[3]Laboratory of Analytical Chemistry, Tel Hai College, Kiryat Shmona, Israel
[4]The Israel Naval Medical Institute (INMI), Haifa, Israel

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ABSTRACT

Introduction The lung surfactant dipalmitoylphosphatidylcholine 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 contact between a large molecule in the blood at the gaseous/liquid interface would disrupt the molecule's tertiary structure. An exposed epitope may then prompt an autoimmune response. The double cause hypothesis suggests that high level of DPPC is a prerequisite for the development of autoimmune disease. The double cause hypothesis was supported in a study using diabetic mice.

Methods dipalmitoylphosphatidylcholine content was determined in the hearts of mice which suffered lupus: those which contracted lupus (lpr) and their control which would contract the disease much later on (mjp) and healthy control mice strain (C57/BL6).

Results dipalmitoylphosphatidylcholine content (mg/g, mean ± SD) in the three groups was: for lpr 32.1 ± 4.7, for mjp 36.1 ± 7.7 and for the control C57/BL6 40.3 ± 10.4. There was no significant difference between the three groups.

Discussion We suggest that the difference between diabetic mice and lupus is due to the nature of autoantigens: a protein in diabetic and nuclear, ribonucleoproteins and ribosomes in lupus. Possibly the distortion at the gaseous/liquid interface is more pronounced in proteins.

Keywords: lung surfactant, nanobubbles, heart, Active Hydrophobic Spot

Introduction

Microbubbles are formed at hydrophobic site at the luminal aspect of blood vessels. Large molecules are distorted at the gas/liquid interface and become autoantigens. This mechanism was suggested as the cause of autoimmune diseases. Study of diabetic mice supported the suggested mechanism: the hydrophobic material was higher in their hearts compared to controls[1]. The present study designed to expand this topic to mice affected by lupus.

ACTIVE HYDROPHOBIC SPOT – (AHS)

We succeeded in establishing the following 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, named "active hydrophobic spot" (AHS). Nanobubbles are formed from dissolved gas at the AHS[2]. These AHS can be found on any type of blood vessel: arteries, veins, capillaries and heart chambers.

DOUBLE CAUSE HYPOTHESIS – (DCH)

Considering the possibility that the blood is thus faced with a constant gas phase contained in the nanobubbles, we proposed that this may also affect autoimmunity[3]. 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 an autoantigen. The DCH is appealing because, if proved correct, it would enable a number of prophylactic procedures. Sometime in the future, the elimination of plasma DPPC or the removal of the AHS may prevent the development of an autoimmune disease.

THE LEAKAGE OF LARGE MOLECULES

Recently, different studies have identified target cells as the origin of autoimmune diseases[4]. Certain overactive genes code for disease related proteins associated with multiple sclerosis and rheumatoid arthritis. Many target cells are located in glands which pump hormones and possibly other proteins directly into the blood and are rich in blood vessels. The increased vulnerability of women to autoimmune diseases is related to activation of genes on both X chromosomes and increased protein production[5]. Women with lupus present activated genes on both X chromosomes and their activity correlates with the severity of the disease. All of the above considerations lead to increased chances of releasing large molecules into the blood stream.

AHS VARIABILITY

There is a large variability in the number and size of AHS in blood vessels of sheep, which corresponds to the variability of bubbling / non-bubbling divers[2]. We explained that the variability in AHS cause the variability in divers. According to the DCH it would be expected that individuals with high levels of AHS would be susceptible to autoimmune diseases. Because the heart contains many blood vessels and we have shown that AHS do exist in the chambers of the heart[2], we chose the heart to represent the total AHS of an animal. A limited study of mice (control n = 8 vs. lupus n = 5) pointed to an increased level of DPPC in the heart of mice affected by lupus [6]. Our study on Non-Obese Diabetic mice (NOD) proved that the hearts from NOD mice contain more DPPC than the hearts from control mice[1], which agree with the DCH suggestion.

EXPECTATIONS

We expect that, in similarity to the diabetic mice, DPPC in the heart of mice suffering lupus would be high compared to controls.

STUDY FRAMEWORK

In this study we sampled hearts from lupus affected mice (lpr) and their control strain which would suffer lupus much later on (mjp) and control mice (c57/BL6) which served as the control in our previous study[1].

Methods

ANIMALS AND TREATMENT

Mice were bred in a specific-pathogen-free vivarium and were fed a standard laboratory diet and water ad libitum. The study involved 11 c57/BL6 mice, 13 mjp and 12 lpr mice, and it was approved by the ethics committee of Bar-Ilan University.

PROTOCOL

Mice were sacrificed by dislocation. Excised hearts were carefully squeezed in saline to clear excess blood and stored at -20°C until analyses. Phospholipid extraction was done when all 36 samples had been completed. Phospholipids were extracted using an accepted procedure: Two µl of internal standard solution, 1 ml of chloroform and 2 ml methanol were added to the heart. After homogenisation for 2 min, another 1 ml of chloroform was added. Following another 30 s homogenisation, 1 ml double distilled water was added and 30 s homogenisation was performed. The homogenate was centrifuged (4 °C, 2,000 rpm, 10 min) and the lower chloroform phase was collected. After the addition of ~250 mg sodium sulfate and vortexing for 30 s, the test tube was kept still for 10 min and then filtered through cotton. The solvent was dried using a flow of nitrogen, and the test tube containing phosphatidylcholine was stored in a freezer at –20 °C for further analysis. The N_2-dried phospholipids were delivered to the MIGAL laboratory in Kiryat Shmona for the determination of DPPC using liquid chromatography–mass spectrometry. Samples were re-dissolved in 0.5 ml of methanol, vortexed and centrifuged at 2,000 rpm for 10 min at 4 °C. The supernatant was then collected and injected into a QTOF LC/MS (Agilent Technologies, Santa Clara, CA, USA) for the analysis of DPPC and the Internal standard (IS) 1,2-Diheptadecanoyl-PC. 10 µl of the supernatant was injected into a 1290 infinity LC system (Agilent Technologies, Santa Clara, CA, USA) connected to a C-18 reverse-phase column, XTerra C18 3.5 µm, 4.6×20 mm (Waters Corporation, Milford, MA, USA). The solvents used for separation of DPPC and the IS were solvent A (DDW with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid). Solvent B remained at 93% for 17 min, then increased from 93% at 17 min to 98% at 20 min, remaining at 98% for another 5 min, with a flow rate of 0.5 ml/min. The LC eluent was introduced directly into the electrospray ionisation (ESI^+) source connected to a UHD accurate-mass Q-TOF LC/MS 6540 (Agilent Technologies, Santa Clara, CA, USA). The ESI capillary voltage was set at 3,500 V, fragmentor 150 V, gas temperature 350 °C, gas flow 8 ml/min, and nebuliser 35 psi. The mass spectra (m/z 100–1700) were acquired in a Positive-ion mode. The calibration curve of DPPC and IS was prepared in methanol at a concentration range of 0.05 to 5 ppm and injected under identical conditions. A linear curve was obtained with R^2 = 0.9971 and 0.9955 for DPPC and the IS, respectively.

STATISTICAL ANALYSIS

A normality test (Shapiro-Wilk) and an equal variance test (Brown-Forsythe) were used for the concentration of DPPC in the 3 groups. Depending on these tests' results analysis of variance or Kruskal-Wallis tests were then used.

Results

The experimental mice and DPPC results (mean and SD) are presented in Table 1. The individual DPPC concentration is given in Table 2.

Table 1 - Experimental mice data

Group	n	Heart weight g	DPPC mg/g
lpr	12	0.170 (0.031)	32.1 (4.7)
mjp	13	0.139 (0.026)	36.1 (7.7)
Control C57/BL6	11	0.122 (0.023)	40.3 (10.4)

Data represent mean (SD)

Table 2. Concentration of DPPC in the hearts of mice.

lpr	mjp	Control C57/BL6
34.2	22.7	34.9
24.8	39.3	38.7
37.4	42.2	40.1
31.7	30.6	41.5
27.5	32.7	21.6
32.7	44.2	28.1
23.1	32.1	44.6
38.6	49.6	54.4
34.1	30.7	51.6
35.2	27.8	34.8
32.7	34.0	53.3
33.0	44.4	
	38.8	

In the statistical analysis for DPPC in the 3 groups, Normality test passed ($P = 0.901$), and the equal variance test failed ($P < 0.050$). The Kruskal-Wallis one way analysis of ranks presents no significant difference between the medians (c57/BL6 – 40.1, mjp – 34.0, lpr – 32.9, $P = 0.064$) of the 3 groups.

Discussion

The amount of DPPC settled at the AHS could be determined by two stages: leaking DPPC from the lung to the blood and settling of DPPC at the AHS. In two previous studies we showed in diabetic type I patients, that leakage of DPPC from the lung to the plasma is not the limiting factor for buildup of the AHS[7,8]. Therefore, the settling of DPPC is the factor which determines the strength of the AHS. The concentration of DPPC in the hearts of mice which are prone to lupus and control was unlike our expectation and unsimilar to the results we had with diabetic mice. As compared to diabetic mice, the autoantigens of lupus are mainly nucleosomal DNA, small nuclear ribonucleoproteins and ribosomes[9].

We suggested that distortion of a protein (proto-antigen) at the gas/liquid interface reveal new epitope to the immune system. It could have been that, as compared to protein, nuclear molecules are less sensitive to the gas/liquid interface and thus the mechanism of autoimmunity of lupus is different. Our present research does not disprove the DCH and we suggest that further studies should address proteins as autoantigen. For example, in our previous study, DPPC in the hearts of NOD mice was high in in mice suffering from diabetes as well as in those not suffering from active disease compared to controls. We suggested for a further study to test whether a protein (a proto-antigen) leaked into the blood of mice suffering from diabetes.

Conclusions

The concentration of DPPC in the hearts of mice suffering lupus was not higher than that of the controls. It is suggested that autoantigenic nuclear molecules are less sensitive to the gas/liquid interface than proteins.

Competing Interests:

The authors declare no competing interests.

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Author Contributions:

R.A contributed to the design and management of the study, and the analysis and interpretation of the data. N.A, E.P. and R.A. collected the samples and extracted the phospholipids and S.K. analyzed the DPPC. R.A. wrote and edited the manuscript. All authors have read and reviewed the manuscript. R.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References:

1. Aburomi N, Khatib S, Piran R, Arieli R. The Double Cause Hypothesis for Autoimmune Diseases is Supported in Diabetic Mice (NOD). Medical Res Arch S.I. 11: 12, 2023. ISSN 2375-1924. doi.org/10.18103/mra.v 11i12.4844

2. Arieli R. Nanobubbles Form at Active Hydrophobic Spots on the Luminal Aspect of Blood Vessels: Consequences for Decompression Illness in Diving and Possible Implications for Autoimmune Disease-An Overview. *Front Physiol.* 2017;8:591. Published 2017 Aug 15. doi:10.3389/fphys.2017.00591

3. Arieli R. Was the appearance of surfactants in air breathing vertebrates ultimately the cause of decompression sickness and autoimmune disease?. *Respir Physiol Neurobiol.* 2015;206:15-18. doi:10.1016/j.resp.2014.11.008

4. Sutherland S. How Auto-Immunity Starts. Sci Am. 2021;325(3):34. doi:10.1038/scientificamerican0921-34

5. Moyer M. Women at Risk. Sci Am. 2021; 325(3):40. doi:10.1038/scientificamerican0921-40.

6. Arieli R, Khatib S. Dipalmitoylphosphatidylcholine in the heart of mice with lupus might support the hypothesis of dual causes of autoimmune diseases. *Respir Physiol Neurobiol.* 2022;300:103871. doi:10.1016/j.resp.2022.103871

7. Arieli R., Khatib S., Khatib A., Bukovetzky E., Gottfried O.D. (2020). Is the probable spillage of the lung surfactant dipalmitoylphosphatidylcholine the ultimate source of diabetes type1? *Respir. Physiol. Neurobiol.* 286: 103615. doi: 10.1016/j.resp.2020.103615.

8. Arieli R, Khatib S, Tarabeh A, Bakry D, Bukovetzky E, Gottfried OD (2023). Testing the Double Cause Hypothesis for autoimmune diseases, dipalmitoylphosphatidylcholine should be measured in plasma or blood vessels of diabetes Type 1? Medical Res. Arch. 11(4). doi.org/10.18103/mra.v11i4.3562.

9. Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J Exp Med. 1994;179(4):1317-1330. doi:10.1084/jem.179.4.1317.