VITAMIN D_3 ALTERS NETOSIS INTENSITY THROUGH REGULATION OF PADI2 EXPRESSION IN HUMAN NEUTROPHILS

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Introduction. Neutrophils, the most numerous group of granulocytes, play a fundamental role in innate immunity. One of the most effective mechanisms for capturing and neutralizing pathogens is through the deployment of "neutrophil extracellular traps" or NETs. While the molecular mechanism triggering netosis is not completely understood, several key proteins involved in this process have been identified. The process is initiated by the generation of free radicals by NADPH oxidase, which increases the activity of enzymes like peptidyl arginine deiminases (PADI2 and PADI4). This results in citrullination of histones in the neutrophil nucleus, leading to chromatin decondensation and the formation of "trapping threads." Since excessive NET formation in body tissues often accompanies various pathological conditions, understanding the regulation of NETosis can have significant therapeutic implications. One of the most potent regulators of immune cell functions, including neutrophils, is the hormonally active form of vitamin D_3 – 1,25(OH)2D3.

The aim of our study was to investigate the influence of $1,25(OH)_2D_3$ on the expression level of the PADI2 enzyme and the intensity of NETosis in human neutrophils.

Material and methods. Primary human neutrophils for our research were isolated using the standard gradient method from the blood of healthy donors at the Bogomolets University Clinic. Isolated neutrophils were seeded on 0.01% poly-l-lysine coated coverslips at $0.5*10^6$ cells/mL for immunocytochemistry or were planted in 6-well plate for $3*10^6$ cells/well for immunoblotting approach. The cells were preincubated during 3h with a different concentrations of $1,25(OH)_2D_3$ and then stimulated or not with 100 nM of PMA for 3h at 37°C in 5% CO_2 for NETosis induction. Cells were then stained with 8 μM of Hoechst 33342and mounted on slides for immunocytochemistry. The percentage of NET-releasing cells was calculated by normalizing the total amount of cells use immunofluorescence microscope. Immunoblotting was used to study the PADI2 expression level. The present study complied with the Declaration of Helsinki, study protocol was approved by an Ethical Committee at Bogomolets National Medical University (№ 128, 23.12.2019 p.).

Results. Our study showed that preincubation of primary neutrophils with $1,25(OH)_2D_3$ at all experimental concentrations (10^{-7} M, 10^{-8} M and 10^{-9} M) tended to reduce the level of NETosis among the cell population, while the difference was statistically significant after incubation only with 10^{-8} M concentration of vitamin (-14.3±1.1%, $p<0.05$). Incubation PMA-nonstimulated neutrophils with $1,25(OH)_2D_3$ at a concentration of 10^{-8} decreased the expression level of the PADI2 enzyme by 1.48 times compared to control ($p<0.05$). At the same time, in the group with PMA stimulation, the PADI2 expression level was 1.29 times lower than in the control group ($p<0.05$).

Conclusions. The obtained results show the probable involvement of vitamin D_3 in the molecular mechanisms of NETosis regulation by influencing the expression level of the PADI2 enzyme.