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STEARYLAMINE INDUCES ROS-INDEPENDENT NEUTROPHIL EXTRACELLULAR TRAPS

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Neutrophils are capable to form extracellular traps consisting of chromatin and granular proteins, in which bacteria get stuck like in the nets. This process is called netosis and was studied in detail in a model induced by exposure of neutrophils to Phorbol 12-myristate 13-acetate (PMA). In this case it takes 2–3 hours and requires reactive oxygen species generated by NADPH oxidase (the so-called classical netosis). The aim of this work was to study the features of netosis induced by stearylamine (SA) in comparison to netosis caused by PMA. Stearylamine was dissolved in DMSO or introduced into the liposomes. Neutrophils were isolated from peripheral blood of healthy donors and incubated with SA dissolved in DMSO (SA 0.2 mg/ml, DMSO 2%) or with cationic phosphatidylcholine liposomes containing SA (PC-SA-liposomes; PC 1.8 mg/ml, SA 0.2 mg/ml). Neutrophils were fixed with methanol and observed by confocal fluorescence microscopy. It has been shown that SA both dissolved in DMSO and in liposomes

evoked the release of neutrophil extracellular traps. The kinetics of netosis was studied on living cells in real time using fluorescent labeled PC-SA-liposomes. It was found that PC-SA-liposomes get adsorbed on distinct sites of the cytoplasmic membrane and with increase of the incubation period liposomes cover all membrane surface. It has been shown that chromatin decondensation, fusing of nuclei and cytoplasm contents occur during the netosis induced by SA, as well as during the netosis induced by PMA. But netosis caused by SA proceeds at a much higher rate (30–90 min) compared to netosis caused by phorbol ester. It should be noted that SA did not induce production of reactive oxygen species, as was shown by the luminol-dependent chemiluminescence. The catalase and the NADPH oxidase inhibitors apocynin and DPI did not affect the release of neutrophil extracellular traps induced by PC-SA, which also shows that SA stimulates ROS-independed release of neutrophil extracellular traps.

Keywords: stearylamine, neutrophils, neutrophil extracellular traps, NETosis, fluorescence confocal microscopy, phosphatidylcholine liposomes, luminol-dependent chemiluminescence