Nitroxides Modulate Neutrophil NADPH Oxidase Activity *Via* Pos-Translational Protein Phosphorylation

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In response to specific stimulation, neutrophils release reactive oxygen species (ROS) through NADPH oxidase complex activation. Although necessary to survival, this process sometimes cycle out of control, leading to the undesired consequences of autoimmune and inflammatory disease. It has been hypothesized that nitroxides could decrease the inflammatory response and attenuate the damage caused by ROS released by immune effector cells. The cell-permeable compounds 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo) and 4-9((-acridinecarbonyl)-amino)-2,2,6,6-tetramethylpiperidine-1-oxyl (Ac-Tempo) were used to investigate the hypothesys of nitroxides modulate signal trasduction pathway associated with NADPH oxidase activity. Inflammatory neutrophils were elicited from mice peritoneal cavity, incubated (10 min, 37 °C) with Tempo or Ac-Tempo and then stimulated with phorbol (PMA, 100 ng/10^6 cells). Superoxide anion ($O_2^{\bullet-}$) release was determined spectrophometrically through cytochrome *c* reduction (550nm). To discrimate superoxide disproportionation activity from NADPH oxidase inhibition by both nitroxides, we performed paralleled experiments with the xanthine-xanthine oxidase system. Tempo abolished oxidase activity (100 μM/10^6 cells), while Ac-Tempo only decreased superoxide release (70%) in a greather dose (400 μM) than Tempo. Cell viability was unaffect by nitroxides treatment or by PMA stimulation. Luminescent kinase assay (Kinase-Glo® Luminescent) point to decrease on protein kinase C activity achieved by nitroxides as regulatory cellular signal on the NADPH oxidase down regulation. These results were confirmed by blotting with monoclonal phospho-serine/threonine antibody, when PMA-induced protein phosphorilation was significantly decreased by neutrophils treatments with nitroxides.

Keywords: NADPH Oxidase, neutrophil, nitroxides

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