INHIBITORY ACTION OF NITROXIDE TEMPOL ON PROTEIN DISULFIDE ISOMERASE ACTIVITY AND NEUTROPHIL NADPH OXIDASE ACTIVATION

Santos, G.B1.; González-Perilli, L2.; Mastrogiovanni, M2.; Trostchansky, A2.; Brigagão, M.R.P.L1

1Departamento de Bioquímica, Instituto de Ciências Biomédicas, Universidade Federal de Alfenas, Alfenas, Brazil
2Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo-Uruguay

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Introduction: PDI is a 57 kDa oxidoreductase that forms, breaks and rearranges disulfide bonds in nascent proteins reaching the endoplasmic reticulum. Previous studies support the idea that PDI may regulate the phagocytic NADPH oxidase complex (NOX2) of relevance for inflammatory processes.

Objectives: The aim of this study is to determine whether the nitroxide 4-hydroxy-2,2′,6,6′-tetramethyl-1-piperidinyloxy (Tempol) inhibits PDI activity with consequent modulation of NOX2.

Materials and methods: PDI activity was evaluated by using the spectrophotometric insulin method. PEG-SWITCH assay and mass spectroscopy analysis of the protein after reacting with Tempol were performed to study the covalent reaction of the nitroxide with the protein. Inflammatory neutrophils from mice peritoneal suspensions were incubated with Tempol, bacitracin or DTNB and stimulated with PMA. PMA triggered NOX2 activity in neutrophils as determined by oxygen consumption and cleavage of a fluorescent probe was correlated with the PDI reductase activity. To corroborate Tempol inhibitory effect on NOX2 activity, neutrophil kinases activities associated to NOX2 were performed through luminescent assays. After neutrophils disruption by sonication and protease and phosphatase inhibitors cocktails additions, sample were analysed by dot blot to evaluate Tempol action on protein phosphorylation levels. Also experiments with the well known PDI inhibitors bacitracin and dithionitrobenzoic were included.

Results and conclusion: The insulin reduction test showed that Tempol inhibited PDI reductase activity with an IC_{50} of 35 μM. PEG-SWITCH and MS studies showed a possible interaction of Tempol with PDI. NOX2 and PDI reductase activities detected in neutrophils were significantly inhibited in a concentration-dependent manner when neutrophils were pre-treated with Tempol having similar IC_{50} (45 μM Tempol). Assays with bacitracin and dithionitrobenzoic acid were performed to confirm their ability to decrease the neutrophil respiratory burst to correlate PDI and NOX2 activities. These studies also show that Tempol’s inhibition of NOX2 activity is correlated with lower levels of protein phosphorylation and PDI activity at inflammatory neutrophils, suggesting a novel anti-inflammatory action of Tempol.