Nitric Oxide Consumption by Circulating Neutrophils in Sickle Cell Disease

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ABSTRACT: The interaction of nitric oxide (NO) with enzymatic sources of reactive inflammatory mediators exerts modulatory actions on inflammatory signaling mechanisms. Therefore, we aimed to measure NADPH oxidase, total peroxidase and cylooxygenase (COX) activities and determine NO consumption in neutrophils isolated from sickle cell disease (SCD) patients. The expression of inducible nitric oxide synthase (NOS-2) and total nitrate/nitrite levels were also analyzed to assess NO production in SCD neutrophils. Functional assay of NADPH oxidase was performed by measuring neutrophil superoxide release which was quantified spectrophotometrically by CuZn SOD-inhibitable reduction of cytochrome c. Superoxide release by SCD neutrophils was similar to controls both at basal conditions and in response to 10 µM formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation. Peroxidase activity, as assessed spectrophotometrically, was not significantly different in SCD neutrophils when compared to controls. Total COX activity, measured via a COX activity assay kit, was significantly increased in neutrophils isolated from SCD patients. The increase in total COX activity observed in SCD neutrophils was due to enhanced activity of COX-2, differentiated by using the isoform-specific inhibitors DuP-697 and SC-560. Western blot analysis of COX-2 protein level in SCD and control neutrophils confirmed significantly increased enzyme activity in the diseased group. Western blot analysis of neutrophil lysates from SCD patients showed significantly increased NOS-2 protein content, compared to controls. Spectrophotometric measurement of total nitrate/nitrite levels affirmed increased levels of nitric oxide generation in SCD neutrophils. Electrochemical measurement of NO consumption performed in the presence of an NO donor (10 µM, PAPA NONOate) both under basal conditions and after 10 µM fMLP stimulation, revealed a significant decrease in SCD neutrophils compared to controls. The presented data suggests that decreased NO uptake by SCD neutrophils can alter redox signaling and impact on vascular inflammation associated with the disease. ***Acknowledgement*****: This study was supported by a grant from TUBİTAK (The Scientific & Technological Research Council of Turkey) No: SBAG-2797.**

KEYWORDS: **Sickle cell disease, neutrophil, cylooxygenase, nitric oxide.**

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