Red blood cells from patients with type 2 diabetes mellitus induce endothelial dysfunction through NADPH oxidase-derived reactive oxygen species

Authors: Z Zhou¹, A Mahdi¹, Y Tratsiakovich¹, J Yang¹, J Pernow¹, ¹Karolinska Institutet - stockholm - Sweden

Topic(s): Vascular Tone, Permeability, Microcirculation

Citation: Cardiovascular Research (2018) 114 (Supplement 1), S42

Funding Acknowledgements: Swedish Research Council; Swedish Heart and Lung Foundation

Background: NADPH oxidase (NOX) is a crucial source for reactive oxygen species (ROS) formation. NOX1 and NOX2 are the main isoforms and are activated in type 2 diabetes mellitus (T2DM) accounting for development of endothelial dysfunction. Previous studies showed that red blood cells (RBCs) from patients with T2DM (T2DM-RBCs) regulate vascular tone in response to hypoxia, suggesting a direct impact of RBCs on vascular function. Whether an imbalance in the NOX system in T2DM-RBCs is a cause of endothelial dysfunction remains to be determined.

Purpose: We aimed to investigate whether T2DM-RBCs cause endothelial dysfunction and whether this process involves NOX-derived ROS.

Methods: Aortae from healthy male Sprague-Dawley rats were isolated and incubated with T2DM-RBCs, RBCs from age-matched healthy subjects (HRBCs) or buffer for 18 h. Following the incubation and washing of the aortae, endothelium-dependent (EDR) and -independent (EIR) relaxations were stimulated with acetylcholine (ACh) and sodium nitroprusside (10⁻⁹-10⁻⁵ M), respectively. In separate experiments, the non-selective NOX inhibitor apocynin (100 µM), the selective NOX1 inhibitor ML171 (10 µM), the selective NOX2 inhibitor gp91 ds-tat (10 µM) and the ROS scavenger N-acetyl-L-cysteine (NAC, 10 µM) were used to study their roles in RBCs and the vasculature. NOX1 and NOX2 protein expression were determined by Western blot in RBCs and RBC-incubated aortae. ROS production was measured in RBCs using electron spin resonance.

Results: EDR was impaired in vessels following incubation with T2DM-RBCs (Emax: 41±3.0) but not HRBCs (Emax: 77±5.0) in comparison with buffer (Emax: 79±2.8; P<0.01 vs. T2DM-RBCs), while EIR was unaffected, indicative of endothelial dysfunction. The impairment in EDR was reversed by pre-incubation of RBCs with apocynin, ML171, gp91 ds-tat and NAC (Figure A). ROS production was increased in T2DM-RBCs in comparison with HRBCs (25.9±2.6 vs. 8.7±0.9 µmol/L/min; P<0.001). NOX2 protein expression was 1.6 fold higher in T2DM-RBCs than in HRBCs (P<0.05). At the vascular level, the impaired EDR was reversed by apocynin, ML171 and NAC but not by gp91 ds-tat (Figure B). Moreover, incubation with T2DM-RBCs induced increased NOX1 expression in aortae (1.8 fold vs. aortae incubated with HRBCs or buffer; P<0.05), while vascular NOX2 expression was unaffected by T2DM-RBCs.

Conclusions: RBCs from patients with T2DM induce endothelial dysfunction through increased ROS production that is likely derived from NOX1 and NOX2 in RBCs and NOX1 in the vasculature. Targeting RBCs and its regulation of vascular NOX may serve as a novel therapeutic tool for treatment of endothelial dysfunction in T2DM.
Abstract:
Red blood cells from patients with type 2 diabetes mellitus induce endothelial dysfunction through NADPH oxidase-derived reactive oxygen species.

Authors:
Z Zhou 1, A Mahdi 1, Y Tratsiakovich 1, J Yang 1, J Pernow 1
1 Karolinska Institutet - Stockholm - Sweden.

Topic(s):
Vascular Tone, Permeability, Microcirculation

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S42

Funding Acknowledgements:
Swedish Research Council; Swedish Heart and Lung Foundation

Background: NADPH oxidase (NOX) is a crucial source for reactive oxygen species (ROS) formation. NOX1 and NOX2 are the main isoforms and are activated in type 2 diabetes mellitus (T2DM) accounting for development of endothelial dysfunction. Previous studies showed that red blood cells (RBCs) from patients with T2DM (T2DM-RBCs) regulate vascular tone in response to hypoxia, suggesting a direct impact of RBCs on vascular function. Whether an imbalance in the NOX system in T2DM-RBCs is a cause of endothelial dysfunction remains to be determined.

Purpose: We aimed to investigate whether T2DM-RBCs cause endothelial dysfunction and whether this process involves NOX-derived ROS.

Methods: Aortae from healthy male Sprague-Dawley rats were isolated and incubated with T2DM-RBCs, RBCs from age-matched healthy subjects (HRBCs) or buffer for 18 h. Following the incubation and washing of the aortae, endothelium-dependent (EDR) and -independent (EIR) relaxations were stimulated with acetylcholine (ACh) and sodium nitroprusside (10⁻⁹⁻¹⁰⁻⁵ M), respectively. In separate experiments, the non-selective NOX inhibitor apocynin (100 µM), the selective NOX1 inhibitor ML171 (10 µM), the selective NOX2 inhibitor gp91 ds-tat (10 µM) and the ROS scavenger N-acetyl-L-cysteine (NAC, 10 µM) were used to study their roles in RBCs and the vasculature. NOX1 and NOX2 protein expression were determined by Western blot in RBCs and RBC-incubated aortae. ROS production was measured in RBCs using electron spin resonance.

Results: EDR was impaired in vessels following incubation with T2DM-RBCs (Emax: 41±3.0) but not HRBCs (Emax: 77±5.0) in comparison with buffer (Emax: 79±2.8; P<0.01 vs. T2DM-RBCs), while EIR was unaffected, indicative of endothelial dysfunction. The impairment in EDR was reversed by pre-incubation of RBCs with apocynin, ML171, gp91 ds-tat and NAC (Figure A). ROS production was increased in T2DM-RBCs in comparison with HRBCs (25.9±2.6 vs. 8.7±0.9 µmol/L/min; P<0.001). NOX2 protein expression was 1.6 fold higher in T2DM-RBCs than in HRBCs (P<0.05). At the vascular level, the impaired EDR was reversed by apocynin, ML171 and NAC but not by gp91 ds-tat (Figure B). Moreover, incubation with T2DM-RBCs induced increased NOX1 expression in aortae (1.8 fold vs. aortae incubated with HRBCs or buffer; P<0.05), while vascular NOX2 expression was unaffected by T2DM-RBCs.

Conclusions: RBCs from patients with T2DM induce endothelial dysfunction through increased ROS production that is likely derived from NOX1 and NOX2 in RBCs and NOX1 in the vasculature. Targeting RBCs and its regulation of vascular NOX may serve as a novel therapeutic tool for treatment of endothelial dysfunction in T2DM.

Figure: Acetylcholine (ACh)-induced endothelium-dependent relaxation (EDR) of isolated aortae following incubation with buffer and RBCs from patients with type 2 diabetes (T2DM-RBCs) for 18 h. The detrimental effect of T2DM-RBCs on EDR was abolished with inhibition of (A) RBC or (B) vascular NADPH oxidase or ROS. Values are mean±SEM, n=3-12; *P<0.05 vs. buffer; #P<0.05 effect of inhibitor vs. T2DM-RBCs.