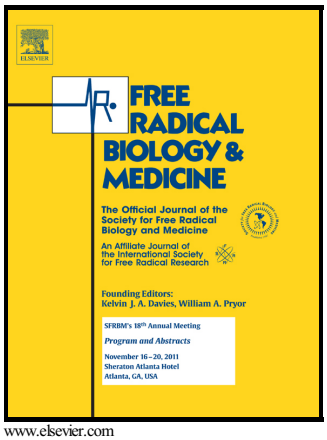

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NOX4-dependent hydrogen peroxide promotes shear stress-induced SHP2 sulfenylation and eNOS activation

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[1]Abbreviations used: 2-OH-E^+, 2-hydroxyethidium; BAEC, bovine aortic endothelial cell; BSA, bovine serum albumin; cGMP, cyclic guanosine monophosphate; cSrc, proto-oncogene tyrosine protein kinase Src; DHE, dihydroethidine; EDHF, endothelial-derived hyperpolarizing factor; eNOS, endothelial nitric oxide synthase; E^+, ethidium; FBS, fetal bovine serum; FAK, focal adhesion kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H_2DCFDA, H_2-dichlorofluorescein diacetate; LSS, laminar shear stress; IAM, iodoacetamide; MLEC, mouse lung endothelial cells; NEM, N-ethylmaleimide; NOX, NADPH oxidase; PECAM1, platelet endothelial cell adhesion molecule-1; PEG, polyethylene glycol; PFA, paraformaldehyde; PRX, peroxiredoxin; ROI, region of interest; ROS, reactive oxygen species; SHP2, SH2 domain-containing protein tyrosine phosphatase-2.

Abstract

Laminar shear stress (LSS) triggers signals that ultimately result in atheroprotection and vasodilatation. Early responses are related to the activation of specific signaling cascades. We investigated the participation of redox-mediated modifications and in particular the role of hydrogen peroxide (H_2O_2) in the sulfenylation of redox-sensitive phosphatases. Exposure of vascular endothelial cells to short periods of LSS (12 dyn/cm^2) resulted in the generation of superoxide radical anion as detected by the formation of 2-hydroxyethidium by HPLC and its subsequent conversion to H_2O_2, which was corroborated by the increase in the fluorescence of the specific peroxide sensor HyPer. By using biotinylated dimedone we detected increased total protein sulfenylation in the bovine proteome, which was dependent on NADPH oxidase 4 (NOX4)-mediated generation of peroxide. Mass spectrometry analysis allowed us to identify the phosphatase SHP2 as a protein susceptible to sulfenylation under LSS. Given the