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cGMP-mediated antioxidant signaling: a role for the c-Abl tyrosine kinase

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Background

Oxidant injury to the pulmonary endothelium contributes to acute lung injury. We have shown that activation of PKG_I by cGMP increases protein levels of the antioxidant enzymes catalase and glutathione peroxidase-1 (GPx-1) and ameliorates oxidant injury in mouse lung endothelium [1]. Catalase and GPx-1 mRNA was not increased. The pathway downstream of PKG_I that leads to increases in catalase and GPx-1 is unknown. The c-Abl tyrosine kinase has been reported to regulate catalase and GPx-1; fibroblasts deficient in c-Abl have increased levels of catalase and resist oxidant injury [2-4]. We hypothesized that 1) activation of PKG_I would decrease c-Abl protein levels; and 2) inhibition of c-Abl with imatinib would increase antioxidant proteins and hydrogen peroxide (H_2O_2) degradation, attenuate H_2O_2-induced endothelial permeability, and decrease H_2O_2-induced cell death in mouse lung microvascular endothelial cells (MLVMEC).

Methods

MLMVEC were isolated from wild-type (wt) and PKG_I knock-out (PKG_I -/-) C57Bl/6 mice. MLMVEC were treated for 4 hours with 8pCPT-cGMP (50 μM) or imatinib. H_2O_2 scavenging was measured with a H_2O_2 electrode after addition of known concentrations of H_2O_2 to cells in suspension. Nuclear condensation was assessed using fluorescence microscopy. Transendothelial resistance (TER) was measured using electric cell-substrate impedance sensing (ECIS). Proteins were quantified by Western blotting.

Results

Treatment of wt MLMVEC with 8pCPT-cGMP significantly decreased protein levels of c-Abl by 29% ($p=0.02$). 8pCPT-cGMP had no effect on cAbl in PKG_I -/- MLMVEC. 7 days of sildenafil treatment (100mg/kg/day) [5] significantly decreased whole lung c-Abl protein by 37 % and increased whole lung catalase protein by 40% ($p<0.05$ for both). Treatment of wt MLMVEC with 10 and 20 μM imatinib increased catalase protein by 16% and 37% respectively, and GPx-1 protein levels by 24% and 36%, respectively ($p<0.05$ for all values). Imatinib (10 μM) decreased the measured peak H_2O_2 after addition of extracellular H_2O_2 (20, 50, and 100 μM) by 18%, 23%, and 9%, respectively ($p \leq 0.05$ at all concentrations). Imatinib (10 μM) significantly attenuated 100 and 250 μM H_2O_2-induced nuclear condensation by 15% ($p<0.05$). Finally, imatinib (20 μM) attenuated the H_2O_2-induced TER decrease in monolayers of MLMVEC ($p<0.05$ by ANOVA).

Conclusion

These data suggest that cGMP, through PKG_I, increases MLMVEC antioxidant activity by down regulating the cAbl tyrosine kinase. Inhibition of c-Abl with imatinib attenuates oxidant-induced MLMVEC death and dysfunction, mimicking the antioxidant effects of cGMP-PKG_I signalling. The mechanism linking activated PKG_I and cAbl expression is unknown.

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