BMC Pharmacology

BioMed Central

Oral presentation

Open Access

# Janus-faced signaling of cGMP in acute lung injury

R Scott Stephens, O Rentsendorj, Eric P Schmidt, Paul Hassoun, Aigul Moldobaeva and David B Pearse*

Address: Division of Pulmonary and Critical Care Medicine, Department of Medicine, Johns Hopkins University, Baltimore, Maryland, USA

Email: David B Pearse* - dpearse@jhmi.edu

* Corresponding author

*from* 4th International Conference of cGMP Generators, Effectors and Therapeutic Implications
Regensburg, Germany. 19–21 June 2009

Published: 11 August 2009

*BMC Pharmacology* 2009, **9**(Suppl 1):S31 doi:10.1186/1471-2210-9-S1-S31

This abstract is available from: http://www.biomedcentral.com/1471-2210/9/S1/S31

© 2009 Stephens et al; licensee BioMed Central Ltd.

The effect of increasing pulmonary endothelial cGMP concentration on endothelial function in acute lung injury appears to depend on 1) the presence of specific cGMP targets, 2) intracellular cGMP compartmentalization and 3) the timing of the increase in cGMP relative to the injury onset [1-4]. For example, we recently showed that pretreatment of pulmonary artery endothelial monolayers with 8pCPT-cGMP attenuated oxidant-induced barrier dysfunction by a cGMP-dependent kinase-1 (cGKI)-dependent mechanism [1,2]. More recently, however, we found that the increase in endogenous lung cGMP resulting from increased NO production in a ventilator-induced lung injury (VILI) mouse model caused lung endothelial barrier dysfunction [4]. The injurious effect of sGC-derived cGMP in VILI was mediated by the simultaneous generation of phosphodiesterase 2A (PDE2A), which was stimulated by cGMP to hydrolyze cAMP. Interestingly, in the same model, pretreatment with BAY 41-2272 (1.5 μM) to stimulate sGC before injurious tidal volume ventilation attenuated VILI.

Recent evidence suggests that endothelial apoptosis may contribute to VILI [5] so we wondered if the protective effect of increasing lung endothelial cGMP before injury could be mediated by an anti-apoptotic effect of cGMP signalling. Mouse lung microvascular endothelial cells (MLMVEC) were isolated and purified by flow cytometry and shown to express cGK1 by Western blot and phosphorylation of VASP $Ser^{235}$. A 6 hr pretreatment with 8pCPT-cGMP (50 μM), significantly attenuated $H_2O_2$-induced cell death assessed by flow cytometry (Annexin-, 7AAD-) and nuclear condensation. A similar protection was not observed in human pulmonary artery endothelial cells (HPAEC) which lack cGK1 expression in vitro. Restoration of cGK1 expression in HPAEC resulted in cGMP-mediated protection against oxidant cell death suggesting a cGK1-mediated effect. To determine if this protective effect was upstream of apoptotic signaling, MLMVEC from C57BL6 mice were treated with 8p-CPT-cGMP (50 uM) before exposure to increasing concentrations of $H_2O_2$. The extracellular $H_2O_2$ concentration ($[H_2O_2]_{ext}$) was continuously measured with a $H_2O_2$ electrode. Compared with untreated cells, wildtype MLMVEC pre-treated with 8p-CPT-cGMP for 2 or 4 hrs (but not 30 min) significantly decreased the maximal $\Delta[H_2O_2]_{ext}$ by 33 ± 11, 32 ± 10 and 25 ± 10% in cells exposed to 20, 50, and 100 μM $H_2O_2$, respectively (N = 8, $P < 0.01$). Consistent with this effect, 8pCPT-cGMP pretreatment attenuated $H_2O_2$-induced H2DCF fluorescence as well as p38MAPK and Akt phosphorylation suggesting that intracellular $H_2O_2$ concentration was also decreased. MLMVEC isolated from cGK1-/- mice failed to enhance $H_2O_2$ uptake suggesting cGK1-mediated signaling was responsible. An assessment of the major $H_2O_2$ degrading enzyme systems revealed a significant cGMP-mediated increase in catalase expression without an increase in catalase mRNA suggesting a post-translational effect.

We conclude that the effects of cGMP signalling on lung endothelial function in acute lung injury are complex and

include both injurious and protective mechanisms depending on the specific downstream signalling pathways that are present. Activation of lung microvascular endothelial cell cGK1 by cGMP protects against oxidant-mediated cell death possibly through an increase in endothelial antioxidant function.

## Acknowledgements

Research supported by NIH/NHLBI, American Heart Association and the Flight Attendant Medical Research Institute. The cGK1 adenovirus and cGK1-/- mice were kindly provided by Drs. Ken Bloch and Alexander Pfeifer, respectively.

## References

1. Moldobaeva A, Servinsky LE, Shimoda LA, Tuder RM, Verin AD, Pearse DB: **Role of protein kinase G in barrier protective effects of cGMP in human pulmonary artery endothelial cells.** *Am J Physiol Lung Cell Mol Physiol* 2006, **290:**L919-L930.
2. Rentsendorj O, Mirzapoiazova T, Adyshev D, Servinsky LE, Renne T, Verin AD, Pearse DB: **Role of vasodilator-stimulated phosphoprotein in cGMP-mediated protection of human pulmonary artery endothelial barrier function.** *Am J Physiol Lung Cell Mol Physiol* 2008, **294:**L686-L697.
3. Dodd-o M, Hristopoulos ML, Kibler K, Gutkowska JA, Mukaddam-Daher S, Gonzalez A, Welsh-Servinsky LE, Pearse DB: **Role of natriuretic peptide receptor-A signaling in unilateral lung ischemia-reperfusion injury in the intact mouse.** *Am J Physiol Lung Cell Mol Physiol* 2008, **294:**L714-L723.
4. Schmidt EP, Damarla M, Rentsendorj O, Servinsky LE, Zhu B, Moldobaeva A, Gonzalez A, Hassoun PM, Pearse DB: **Soluble guanylyl cyclase contributes to ventilator-induced lung injury in mice.** *Am J Physiol Lung Cell Mol Physiol* 2008, **295:**L1056-L1065.
5. Le A, Damico R, Damarla M, Boueiz A, Pae HH, Skirball J, Hasan E, Peng X, Chesley A, Crow MT, Reddy SP, Tuder RM, Hassoun PM: **Alveolar cell apoptosis is dependent on p38 MAP kinase-mediated activation of xanthine oxidoreductase in ventilator-induced lung injury.** *J Appl Physiol* 2008, **105:**1282-90.

Publish with **BioMed Central** and every scientist can read your work free of charge

*"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."*

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp