



Endothelial Nitric Oxide Synthase is Essential for Compensatory Lung Growth



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Background

- Endothelial nitric oxide synthase (eNOS) is expressed in endothelial cells and generates nitric oxide (NO) upon activation by Ca²⁺.
- NO has been shown to stimulate the proliferation and migration of endothelial cells, and vascular endothelial growth factor (VEGF) angiogenic effects are selectively linked to the NO pathway.
- Pneumectomy induces rapid hyperplastic growth of the remaining lung. This adaptive growth, referred to as compensatory lung growth, results in restoration of total lung volume, mass, DNA, protein, alveolar number, and normal lung cell populations.

Hypothesis

- Compensatory lung growth should necessarily occur in parallel with angiogenesis in order to maintain the interface between the growing alveoli and the neovasculature which is crucial in providing nutrients and gas exchange.
- Thus, we hypothesized that eNOS-generated NO is an important mediator of compensatory lung growth.

Methods

Animals:

Mature (14-16 wks) C57BL6 wild-type (WT-Pneum) and eNOS gene knockout (eNOS^{-/-}) mice underwent a left pneumectomy (n = 6/group).

Growth of the right lung was studied 14 days after surgery and compared to sham operated WT mice (WT-Sham).

Surgery:

Mice were anesthetized with ketamine and xylazine followed by tracheotomy and intubation, and ventilated with room air.

After an anterior thoracotomy the left lung was removed after ligation of the left hilum, the chest was closed, and the skin closed using surgical staples.

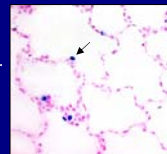
Mice were allowed to recover and extubated after initiation of spontaneous respirations.

Methods

Lung Harvests:

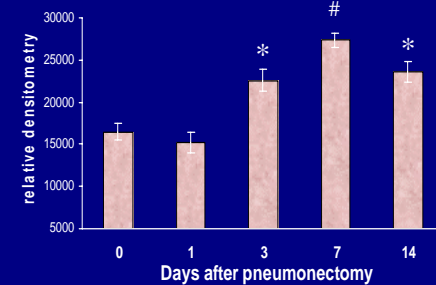
- 14 days after surgery mice were injected i.p. with 5-bromo-deoxyuridine (BrdU) at 50 mg/kg.
- Following anesthesia the right lung was removed, weighed, and received intra-tracheal instillation of 10% buffered formalin to a pressure of 25 cm H₂O.
- The lung weight and volume were expressed as a ratio to body weight to determine lung weight and lung volume index (LWI and LVI).
- The lung was paraffin-embedded and sectioned.
- Morphometric analysis was performed on H&E stained sections to measure alveolar surface density.

Cell proliferation index (CPI) of alveolar cells was measured on sections via BrdU immunostaining. An example is depicted here (arrow indicates a dividing type II alveolar cell).



Results

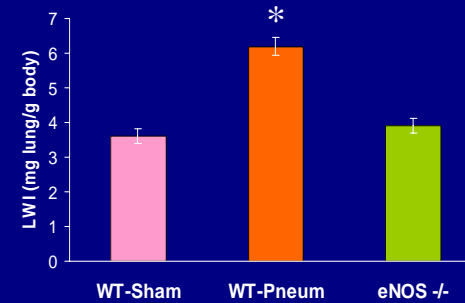
eNOS protein expression is upregulated after pneumectomy in wild-type mice:



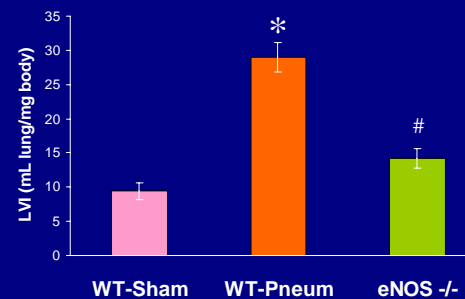
* p<0.001 vs day 0, # p<0.001 vs day 3 and day 14

Results

The increase in lung weight index is absent in eNOS^{-/-} mice.

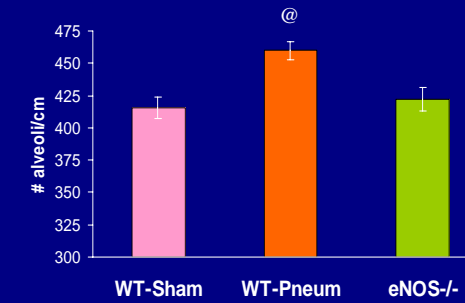


The increase in lung volume index is severely impaired in eNOS^{-/-} mice.

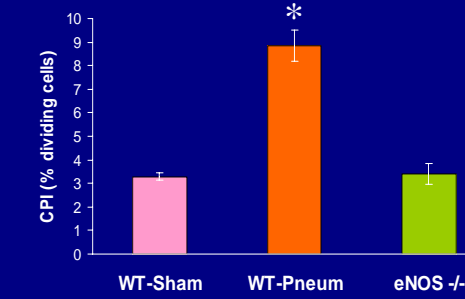


Results

The increase in alveolar surface density is absent in eNOS^{-/-} mice.



The increase in cell proliferation index is absent in eNOS^{-/-} mice.



*p < 0.01 vs. all, #p < 0.01 vs. WT-Sham, @p < 0.05 vs. all

Summary

- eNOS protein is significantly upregulated in WT-Pneum at 3, 7 and 14 days post pneumectomy.
- The eNOS^{-/-} mice had significantly impaired compensatory lung growth as indicated by reduced LWI and LVI.
- The eNOS^{-/-} mice had a significant reduction in alveolar surface density and alveolar CPI.

Conclusions

- Reduction in alveolar surface density suggests that eNOS is a positive effector of alveolar replication.
- Reduction in CPI suggests that eNOS is important in proliferation of alveolar cells.
- Taken together, these data strongly implicate a vital role for eNOS-generated NO in compensatory lung growth.

Future Directions

Future studies will be aimed at determining if impaired lung growth in eNOS-knockout mice is due to impaired angiogenesis and/or reduced capacity of type II cell proliferation and differentiation.