



Lung Proliferative Activity Following Pneumonectomy

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Background

- Following pneumonectomy (PNX), a rapid compensatory growth occurs in the remaining lung.
- It is not known, however, if hyperplastic growth occurs equally in each lobe of the lung.
- It is possible that relative changes in blood flow and lung expansion might be different among individual lobes of the remaining lung after PNX, influencing the proliferative response in each individual lobe.

Hypothesis

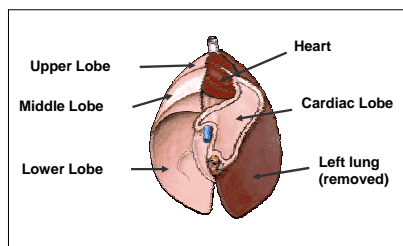
We hypothesized that individual lobe growth correlates with an increased proliferative activity during compensatory lung growth.

Study

The four lobes of the right lung were studied up to 21 days after surgery in the following two groups of rats (n=6/group/time):

PNX Group: Left pneumonectomy.

Sham Group: Sham left thoracotomy.



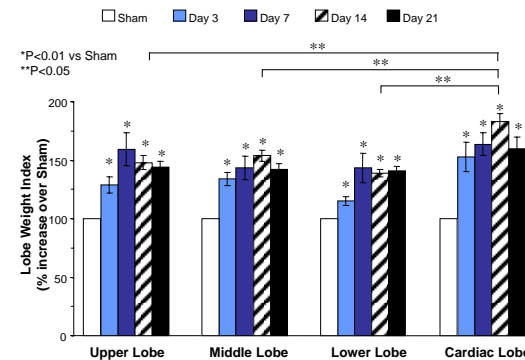
Methods

- Surgical Procedure:** Left PNX was performed through a small posterolateral thoracotomy (Kaza, AK et al. *Ann Thorac Surg* 71: 1645-1650, 2001). Animals in the sham group underwent a left thoracotomy only.
- Lung and lobe weight index:** Animals were recovered for 3, 7, 14 and 21 days after surgery and the lung weight (g) of each lobe was expressed as a ratio to the final body weight of the animal.
- Lung and lobe volume index:** A second group of rats was euthanized 7 and 21 days post-PNX. The lungs were fixed by intratracheal instillation of 4% paraformaldehyde at a constant pressure of 20 cm H₂O. Following fixation, Total lung volume and individual lobe volumes (ml) were measured by the volume displacement technique, and expressed as ratio to the body weight (g).
- PCNA immunostaining:** Total cell protein (100µg) was fractionated on a SDS polyacrylamide gel and transferred to nitrocellulose membrane. The membrane was incubated with a monoclonal anti-PCNA antibody (BD Biosciences, San Diego, CA) for 2 hrs, washed and incubated with goat anti-mouse IgG antibody coupled to horseradish peroxidase (Pierce, Rockford, IL). Protein bands were visualized by chemiluminescence and the autoradiograph quantitated by computerized densitometry.

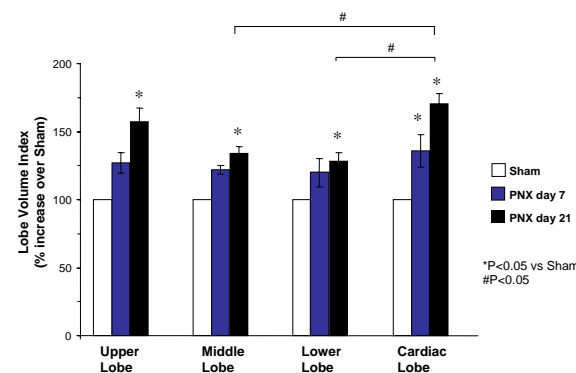
- Relative blood flow distribution:** Up to four radiolabeled microspheres (Ce-141, Sr-85, Nb-95 or Sc-46) were injected via tail vein in 2 groups: Acute (10 min after surgery, and 3, 24 and 48 hrs later) or chronic (3 hrs after surgery, and 3, 10 and 21 days later). The ratio between counts per minute (CPM) in each lobe and CPM in the whole right lung was used as an index to determine the relative distribution of blood flow between the lobes.

Results

Lobe weight index increased in all lobes at 3, 7, 14 and 21 days after PNX versus Sham surgery. At day 14, the cardiac lobe was significantly increased compared to the rest, but was not different at other time-points.



Lobe volume index was significantly increased in all lobes at 21 days after PNX and was significantly increased at day 7 only in the cardiac lobe. In addition, the cardiac lobe reached a significant increase over the middle and lower lobes.

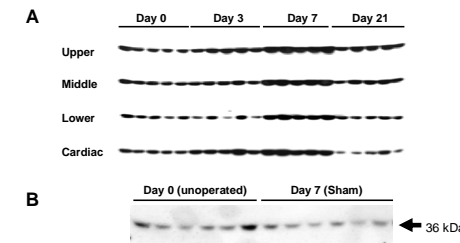


PCNA Expression.

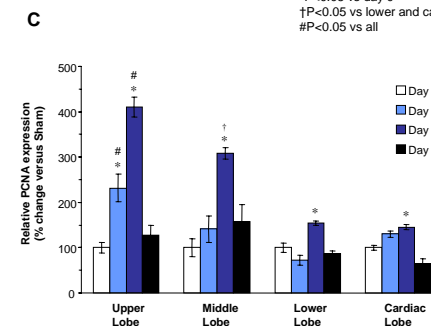
A. Representative Western blots for PCNA expression in each lobe at days 0, 3, 7 and 21 after PNX.

B. Representative Western blot of PCNA expression in the middle lobe at 7 days after Sham surgery versus the middle lobe from unoperated animals documenting that Sham surgery did not induce PCNA expression.

C. Histogram showing quantitation of relative PCNA expression by Western blot in each lobe at 3, 7 and 21 days after PNX as compared to corresponding lobes from unoperated animals (day 0). PCNA was elevated in each lobe after PNX, with peak levels at day 7 for each lobe. In addition, the upper lobe had greater relative increase in PCNA than the other three at days 3 and 7, and the middle lobe had a greater level of PCNA expression at day 7 versus the lower and cardiac lobes.



*P<0.05 vs day 0
†P<0.05 vs lower and cardiac
#P<0.05 vs all

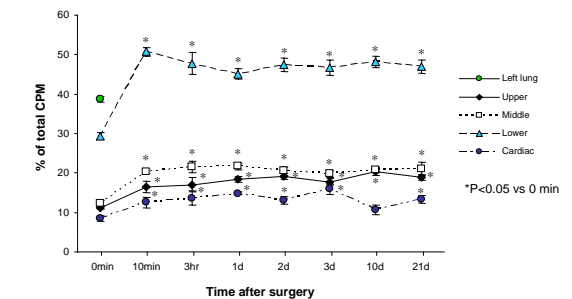


Pulmonary blood flow.

In unoperated animals, the right lower lobe received the highest portion of flow (30%); followed by middle (12%), upper (11%) and finally, the cardiac lobe (8%). The left lung accounted for 39% of the total pulmonary blood flow.

After left PNX, a large increase in blood flow to the right lung was observed. The lower lobe showed the highest increase in blood flow (73%) which was followed by the middle (66%), cardiac (49%) and upper (46%) lobes, and no evidence of further change was observed for each lobe at later time-points.

These increases in blood flow experienced by each lobe resulted in a final blood flow distribution in the lobes after 21 days: lower lobe (47%), middle lobe (21%), the upper lobe (19%) and cardiac lobe (13%). These post-PNX blood flow distributions were in very close ratio to unoperated rats, with each lobe experiencing an increase in blood flow ranging from 1.63- to 1.75-fold.



Summary

- Left-PNX induced compensatory growth in each of the four lobes of the right lung as illustrated by elevated weight and volume indices, as well as increased expression of PCNA.
- These elevations in growth and proliferative activity in the lobes were not uniform, especially by PCNA measurements.
- Measurements of relative lobar blood flow could not explain the disproportionate changes in lobar growth.
- Further studies of the relative changes in lung ventilation and distension will help understand the disproportionate lobar growth after PNX.