Angiotensin II and its Role in Fat Embolism-induced Lung Fibrosis

Background

- Fat embolism from long bone fracture or other trauma is known to cause acute lung injury, but long term effects are unknown.
- Previous studies in our lab found lung pathology extending 3 and 6 weeks when i.v. triolein was used as a fat embolism model.
- A progressive collagen increase in rat lungs was seen, with increased histological staining of tissue for angiotensin peptides and smooth muscle actin.
- Angiotensin II blockers reduce effect of triolein on lung.
- The lung histology is compiled by both lab pathology extending 3 and 6 weeks when i.v. triolein was used as a fat embolism model.

Methods

**MY RESEARCH:** DETERMINE ANGIOTENSIN II PEPTIDE LEVELS IN TRIOLEIN TREATED RAT LUNG

- **ANIMAL GROUPS:** ~60 rats were given 0.2 mL triolein (or saline). i.v. Two triolein groups were also given Angiotensin II blockers Losartan or Captopril.
- Rats were euthanized at 24, 48, 96 hr, or 11d, 21d, or 42d and lungs harvested/frozen or formalin-fixed.
- My study involved grinding 3-2mm pieces of lung treated with triolein (kept on dry ice) in a micro homogenizer with cold protein homogenizing buffer, purifying the peptides on C18 columns then analyzing the eluate by ELISA for angiotensin II.

**GRINDING:**
- Dounce homogenizer with loose and tight pestles, dry ice to keep lung frozen, cold. "Tpers" (protein homogenizing solution, Fisher)
- Break off chunks of frozen lung, add Tpers and homogenize with pestles; add to labeled microfuge tube on dry ice.
- Ground lung fluid kept on ice and then stored in freezer.

**PURIFICATION:**
- Lung homogenate thawed, clarified by centrifugation; supernatant added to small C-18 "spin columns" (Pierce).
- Activation and equilibration solutions were washed through each lung sample in its C18 column, then each peptide was eluted with a final wash.
- Lung peptides were evaporated to dryness in the cold then frozen until ELISA test (Mouse/rat angiotensin II enzyme immunoassay kit, AssayPro #EA3S01).

**RESULTS:**
- Whole lung angiotensin II peptide, purified by a C18 column from total lung homogenate and tested by ELISA, shows an increase in angiotensin II peptide by animals receiving both inhibitors after triolein.
- Histochemical staining of lungs by anti-angiotensin II peptide demonstrated lowered presence in lungs harvested after receiving captopril in vivo. Lungs after Losartan dosage stained at control level.
- Many steps are involved in angiotensin II peptide presence; histochemistry for the reactants as well as tissue analysis may clarify the differences in measuring this interesting peptide.

Results & Discussion

- Our data, showing both histological identification and ELISA after peptide purification to identify presence in lung of angiotensin II peptide, show some dissimilarities.
- Whole lungangiotensin II peptide, purified by a C18 column from total lung homogenate and tested by ELISA, shows an increase in angiotensin II peptide by animals receiving both inhibitors after triolein.
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Strengths & Weaknesses

**Strengths:**
- Sophisticated techniques to investigate new topics of medical importance.

**Weakness:**
- Study finds significant input of an angiotensin type 1 receptor antagonist (Losartan) and an ACE inhibitor (Captopril) to levels of lung ACE2 in a rat model of fat embolism. DATA SHOW CORRELATION ONLY, NOT MECHANISMS.

Summary

- These experiments have shown that fat embolism produced experimentally by i.v. triolein in rats leads to development of lung collagen in vascular and airway smooth muscle.
- By demonstrating change in lung angiotensin II peptide when fat embolism is modeled and blockers administered, results suggest a mechanism by (and treatment for) lung fibrosis of fat embolism.
- The change induced in angiotensin II following blocking or inhibiting the peptide deserves more evaluation to look at mechanisms of the fat embolism and angiotensin II relationship.

References