Captopril and Losartan Mitigate the Time Dependent Histopathology Associated with Triolein Induced Fat Embolism

INTRODUCTION:
Emboli of fat from fractured long bones, pelvis and soft tissues occurs in 90% of major trauma cases and can be a major cause of morbidity and mortality. Fat Embolism Syndrome (FES), a serious manifestation of fat embolization, occurs in up to 10% of these cases and includes hypoxia, petechiae and mental disturbances. The mortality rate of FES has been reported to range from 2.5% to 20%. This syndrome has its primary effect on the lungs and is a significant cause of Acute Respiratory Distress Syndrome (ARDS). Currently, treatment of FES is most often non-specific, supportive, and directed to tissue oxygenation.

Many models of experimental pulmonary injury have been shown to be prevented or ameliorated by interference with angiotensin II (Ang II) or other components of the renin-angiotensin system (RAS). This appears to be the case whether the initial insult is a chemical, physical or biological stimulus. Furthermore, beneficial effects have been found whether the pathological changes developed fairly rapidly or more slowly, with a delayed time course.

In the present study the effects of interference with the RAS by an ACE inhibitor, captopril, and an Ang II type 1 receptor blocker (ARB), losartan, were studied in unanaesthetized rats, employing i.v. injection of the neutral fat triolein in order to determine if the RAS was similarly involved in FE. Triolein is a neutral fat, the major component of bone marrow, and has been considered to be an appropriate insult for the modeling of fat embolization caused by traumatic injury. It was hypothesized that both the ACE inhibitor and the ARB could reduce the pathological changes to the lungs caused by triolein injection, just as they do in other models of pulmonary injury.

METHODS:
A total of 17 Sprague-Dawley rats (280-300 g) were obtained with IACUC approval. Pure triolein (glyceryl trioleate, Sigma Grade) at a dose of 0.2 ml was injected into the caudal vein of 15 unanaesthetized rats. An hour after the triolein injection, five rats received intraperitoneal injections of captopril (50 mg/kg), another group (n=5) were given losartan (10 mg/kg), and the rest were given 0.2ml normal saline solution (n=5). One additional control group of 2 rats received i.v. saline, no triolein. Rats in the captopril group were given drinking water with losartan at a concentration of 0.1 mg/ml. Rats in the losartan group were given drinking water with losartan at a concentration of 0.1 mg/ml. Rats were given ad libitum access to food and water.

Following euthanasia, at 48 hours post injection, the lower lobe of the right lung was collected and placed in 10% buffered formalin. After ten days of fixation, the specimens were paraffin embedded. Another portion of the lungs was frozen at -20o C and used to stain for fat. Sagittal lung sections were stained with H & E, Red Oil O which stains non-specifically for fat, Masson Trichrome for collagen, and immunohistochemistry for SMA.

Histomorphometric scores and ratios were derived from histological evaluation. The degree of injury was blind scored by two researchers. The pulmonary damage was scored using the changes observed in the thickening of the alveolar septa, the presence of inflammatory cells both in the septa and the alveolar space, the presence of hemorrhages in the lung parenchyma, and the severity of inflammation and scarring. A subjective rating ranging from 5 to 40 (minimal to severe lung damage) was assigned to each component of the organ. The value of 5 represented borderline damage, and then larger multiples of 5 given based on the severity of damage. Likewise, collagen and smooth muscle actin were evaluated in relation to their presence and intensity in the vasculature, septa, and the peri-bronchial musculature.

The patency of the lumen of small caliber pulmonary arteries and arterioles was evaluated by measuring the luminal diameter of the vessel divided by their external (vascular) diameter. The percentage of the occlusion of the lumen of the small arteries (diameter range 20 to 100 micrometers) as well as the thickness of the arteriole and arteriolar wall was measured in at least three photographs at 100x and 400x magnification per slide. Score and ratio data were analyzed statistically using ANOVA. Comparisons were made using Fisher LSD. Significance was set at p < 0.05.

RESULTS:
Animals sacrificed following triolein injection alone had extremely severe vascular occlusion with an increased presence of monocytes and eosinophils, as well as mild edema in the adventitia (Fig 1 & 2). There was a marked thickening of the bronchial musculature and the peribronchial arterioles. Collagen was very prominent in the peribronchial musculature, the septa, and the small caliber arteries and arterioles of the animals treated with triolein alone (Fig 3). The lungs had intensive expression of SMA in the wall of small caliber arteries and arterioles and in the peribronchial musculature and in the peribronchial arteries.

Both drugs reduced the inflammatory, vasoconstrictor and profibrotic effects present at 48 hours. There was a reduction in the number of infiltrating leukocytes, macrophages, myofibroblasts and eosinophils, along with a decrease in hemorrhage and collagen deposition (Fig 3). Pathologic changes in bronchial epithelium were also diminished. Figures below demonstrate the mitigating effects of both Captopril (Cap) and Losartan (Los). Error bars represent 95% confidence intervals. Asterisks denote significant difference from triolein group.

DISCUSSION:
Our study examined the effects of treatment with the rescue drugs administered one hour after the triolein injection and terminated at 48 hrs. In preliminary studies with this model, we found that the peak histopathological changes due to fat embolism induced by i.v. triolein occurred at 48 hours 1. These results suggest that the utilization of drugs which act on the renin-angiotensin system (RAS) might provide an effective and targeted therapy for FES.

REFERENCES:

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