

INTRODUCTION

- Fat embolism syndrome (FES) is a lung process following bone fracture, surgery, and liposuction and expressed by inflammation, vasculitis, and fibrosis.
- A rat model of FES is induced by IV injection of Triolein (T). A second mild injury (i.p. injection of lipopolysaccharides (LPS) exacerbates this condition.^{1,2}
- In previous studies, we observed that mast cell (MC) numbers increased in T-treated rats vs. saline controls and even more when LPS was added to T.³ This MC increase was also blocked by losartan treatment.
- In view of the mast cells' interaction with macrophages in the inflammatory process leading to fibrosis, we extended our study to the identification of macrophages in the lung and looked to see if losartan decreased the number of macrophages

METHODS

- Thirty-six Sprague Dawley rats were treated with T (0.2 ml i.v.) or saline.
- After 6 weeks, half of the two groups were given saline or LPS 0.1 mL (3 mg/kg) i.p. followed one hour later with saline or LOS (10 mg/kg i.p.), and then by LOS in the drinking water (50 mg/L).
- 4 weeks later (10 weeks after T), the rats were necropsied after isoflurane anesthesia. Lungs were fixed in 10% formalin and stained with H&E for histological evaluation, Masson Trichrome for fibrosis and CD68 for macrophage counts.
- Two pathologists unaware of the slides' identity took 10 photographs at random in each slide at 400x and counted the number of macrophages.

RESULTS

- Triolein injection induced severe fibrosis, with even more fibrosis with LPS addition. Such fibrotic damage as well the number of the mast cells were reduced by administration of Losartan (4).
- Two types of macrophages were observed: one population with compact cytoplasm and a size similar to mast cells, and a second population with a larger round vacuolated cytoplasm.
- Contrarily to what was observed for mast cells³ no increase was seen for the small macrophages nor did LOS reduce their number in any of the study groups.
- Large vacuolated macrophages however were markedly increased vs controls in T + saline and LPS + saline groups ($p < 0.05$ and $p < 0.01$).
- However the group receiving both T + LPS treatment did not show any increase in these types of macrophages.
- LOS reduced the number of large macrophages in all groups with exception of the T + LPS treated group.

SUMMARY

	Pulmonary fibrosis	Number of Small Macrophages vs Control	Number of Large Macrophages vs Control	Effect of Losartan on Number of Large Macrophages
T+Saline	↑	-	↑	↓
LPS+Saline	+/-	-	↑	↓
T+LPS	↑	-	-	-

Fig 1. Macrophage Count and Effect of Losartan

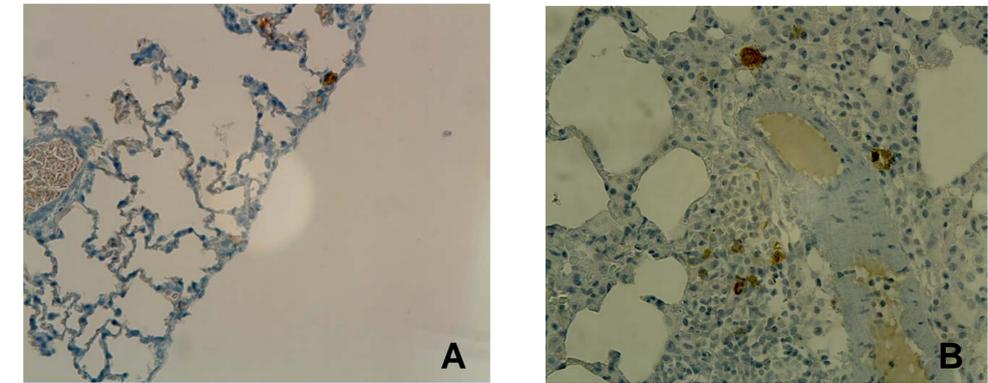


Fig-2A Control showing small size macrophages, Fig-2B T+Saline highlights the presence of round large macrophages. Stain CD68+ macrophages (400x)

CONCLUSION

- The findings suggest the existence of different cellular responses to the inflammatory and fibrotic stimuli given to the animals.
- It is worthwhile to notice that each of the insult given separately increase the number of macrophages and LOS treatment is effective in reducing their number.
- The combined treatment however of T and LPS doesn't induce a synergistic action on these cells.

CREDITS/DISCLOSURE/REFERENCES

1. McIlff T., Poisner A., Herndon B et al: J of Trauma 70 (5) 1136-1191,2011
 2. Poisner A., Adler F., Uhal B.: J of Trauma 72 (h)992-998,2012
 3. Kesh S., Fletcher A., Voelker D et al. Proc. XIX ICLAF Meeting, Dublin, Ireland, Sept 22-26, 2016. Q.J.M 109 Suppl. S48, 2016.
 4. Molteni A., Poisner AM., Adler F., McIlff T., Proc. XVI ICLAF Meeting, Busselton, Australia, Oct 30 – Nov 3, 2010.
- ACKNOWLEDGEMENTS: The histopathology laboratory, Truman Medical Center, Kansas City, MO.
Supported by the Catherine G. Geldmacher Foundation, St.Louis, MO.