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Ciclesonide protects against LPS-induced lung endothelial inflammation and acute lung injury

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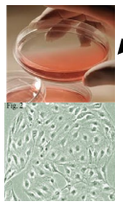
Background and Methods

Background:

- Postnatal sepsis is a major risk factor for BPD in premature infants. Coalson, Kallapur, Jobe
- Cell wall of gram –ve bacteria (LPS) can cause TLR4-induced inflammation and neonatal lung injury. Menden, et al.
- CIC is known for being a brain-sparing steroid in neonates. Jaumotte, et al. 2021

✓ CIC will reduce LPS-induced inflammation and acute lung injury in the developing lung.

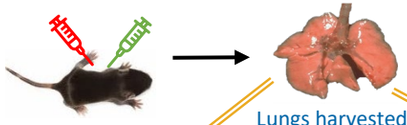
Primary Human Pulmonary Microvascular Endothelial Cell (HPMEC, ScienCell) experimentation



- Around 18wks gestation, passages 3 to 4 used.
- Control cells and 100ng/mL LPS (Invivogen)
- Incubation with 100nM CIC (Selleck Chem) 6hrs prior to LPS

Cells processed for:
Viability (Trypan blue); qPCR, Western Blot (WB); 2D Angiogenesis by Matrigel

Mouse (C57BL6) lung experimentation



- i.p injection with 2mg/kg LPS and 2.5mg/kg CIC Jaumotte, et al. 2021

Lungs harvested

Short term (48 hours)
- LPS at time 0
- CIC at +2 and 24 hours
- PCR (Harvest at day of life (DOL) 8)
- Bronchoalveolar Lavage (at DOL 11)

Long term (7 days)
- LPS at time 0
- CIC at +24 and 48 hours
- Inflations and Morphometry (Harvest at DOL 15)

Results

LPS-induced TLR activation, cell death, & inflammation is suppressed by CIC

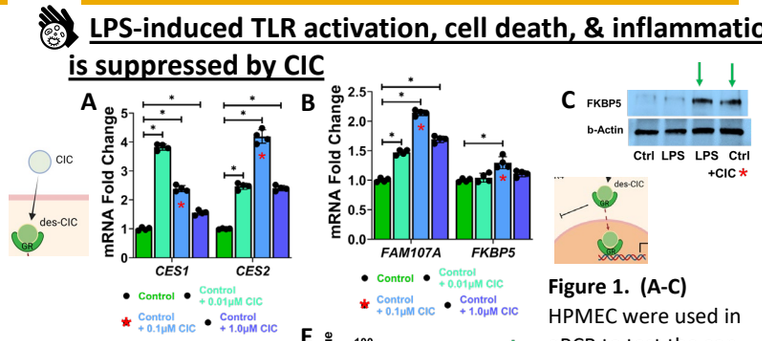


Figure 1. (A-C) HPMEC were used in qPCR to test the conversion of CIC to des-CIC **(A)** and the activation of the receptor **(B)**, by western blot (WB) **(C)**. **(D)** WB of TLR signaling after CIC and LPS. **(E)** Live cells after assessing viability by Trypan blue stain. **(F)** WB showing pro-inflammatory signaling after CIC and LPS. **(G-H)** 2D Angiogenesis by Matrigel, showing tube formations **(G)** and images **(H)**.

LPS-induced lung inflammation repressed by CIC

DOL 8

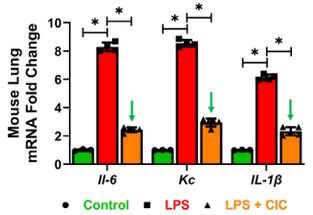


Figure 2. Cytokine gene expression in DOL 8 lung lysates by PCR 48hr after CIC and LPS injections. *p<0.01

Results and Conclusion

Systemic LPS induced lung vascular permeability and inflammatory influx is inhibited by CIC

DOL 11

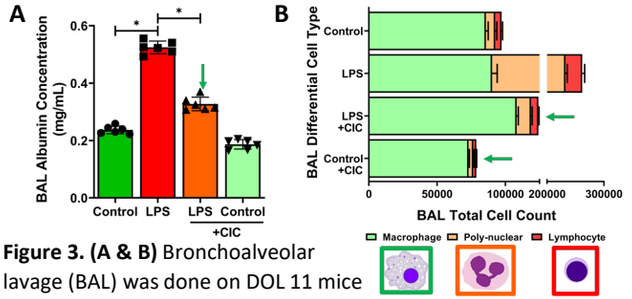


Figure 3. (A & B) Bronchoalveolar lavage (BAL) was done on DOL 11 mice following 48hr LPS and CIC treatments, with the levels of albumin quantified **(A)** and immune cell differential shown **(B)**. *p<0.01.

LPS-induced lung simplification rescued by CIC

DOL 15

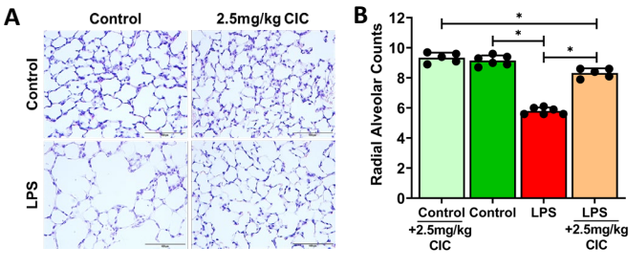


Figure 4. (A-B) H&E stained images from inflation-fixed lungs at DOL 15 **(A)**, with radial alveolar counts (RAC) quantified **(B)**. *p<0.01.

Conclusion:

- In HPMEC, the LPS-induced cell death, inflammation, and angiogenesis was rescued by Ciclesonide.
- In neonatal mice, Ciclesonide suppresses LPS-induced cytokine expression, inflammatory cell influx, and chronic alveolar remodeling.

✓ *This study provides mechanistic insight into the decrease of LPS-induced sepsis injury after the use of Ciclesonide, the safer steroid for neonates.*

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