Acetyl cholic acid effect on LPS-induced activation of ERK and inflammatory cytokines in mouse macrophages and cholangiocytes

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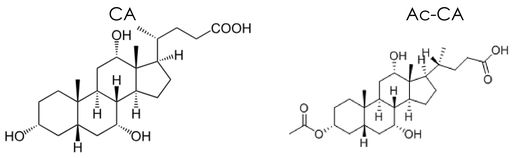
ABSTRACT: Numerous gut microbes produce four novel cholic acid (CA) derivatives. The most notable among these microbes is *Christensenella minuta*, a bacteria that alleviates metabolic diseases. 3-acetyl cholic acid (Ac-CA) is a CA derivative that inhibits the critical Farnesoid X Receptor (FXR), a nuclear receptor that modulates multiple pathways including immune response and inflammation. This study explores the impact of Ac-CA on lipopolysaccharide (LPS)-induced activation of extracellular signal-regulated kinases (ERKs) and inflammatory cytokines in mouse macrophages and cholangiocytes. First, we cultured the macrophages *in vitro* using 3 ml of DMEM medium. To detect the phosphorylation levels of ERK downstream of LPS activation, we performed a Western blot. To measure the inflammatory response of the macrophages, we used quantitative polymerase chain reaction (qPCR) to detect the upregulation of three different proinflammatory cytokines, including TNF-a, IL-1β, and IL-6. We found that Ac-CA noticeably increased LPS-induced activation of ERK in cholangiocytes, but not in macrophages. Additionally, Ac-CA had no effect on the transcriptional expression of inflammatory cytokines in macrophages, but we did not measure the inflammatory cytokines in cholangiocytes. Therefore, the use of *C. minuta* to alleviate metabolic disease without incurring inflammatory diseases is promising. However, we should first research *C. minuta's* 3 other cholic acid derivatives. Because the role of FXR is cell type specific, Ac-CA's effects in resident immune cells in the gastrointestinal tract is more relevant. Future studies should include ELISA assays to directly access cytokine secretion rather than mRNA expression alone. Lastly, we need to conduct at least two more trials of the experiment before we can begin to run statistical tests to determine significance.

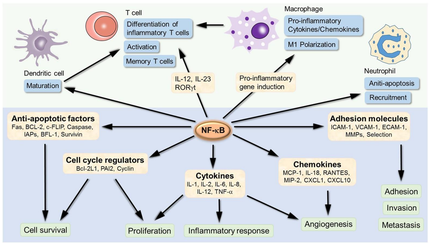
BACKGROUND

C. minuta - a gut commensal that primarily produces Ac-CA, which modulates whole body metabolism. However, other bacteria also produce Ac-CA.

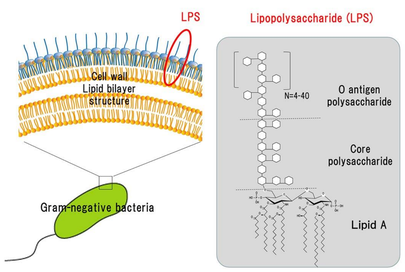
C. minuta exhibits preferential acetylation of primary bile acids over secondary bile acids.



- Ac-CA is a known FXR antagonist
- FXR is a nuclear receptor found in macrophages and other tissues that modulates inflammation.
- FXR suppresses the NF-κB signaling pathway to reduce the transcriptional expression of pro-inflammatory cytokines such as TNF-a, IL-1β, and IL-6.
- Simulation of FXR promotes M2-like macrophage polarization, releasing anti–inflammatory cytokines.

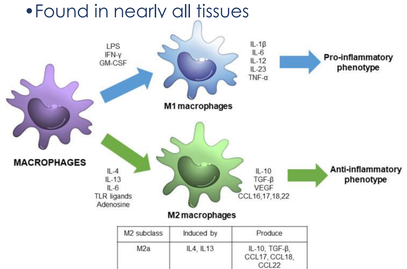


- ERK (Extracellular signal-Regulated Kinase) is a crucial protein kinase within the MAPK (Mitogen-Activated Protein Kinase) signaling pathway that responds to various external stimuli by transmitting signals from the cell surface to the nucleus, regulating cell division, differentiation, and survival.
- The external stimuli this study focuses on is LPS, which is the outer-membrane of gram-negative bacteria that binds to toll-like receptor 4 (TLR4). It triggers downstream signaling including MAPK and NF-κB. LPS also stimulates FXR. We used LPS derived from *Escherichia coli* for this study.



MACROPHAGES

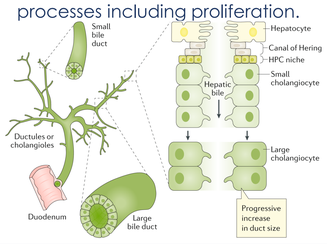
- Type of white blood cell
- Fall into two phenotypes: M1 (proinflammatory) and M2 (anti-inflammatory).
- Destroys pathogens and cancer cells.
- Activates other immune cells by secreting cytokines
- Involved in inflammation and tissue repair
- Found in nearly all tissues



M2 subclass	Induced by	Produce
M2a	IL4, IL13	IL-10, TGF-β, CCL17, CCL18, CCL22
M2b	LPS + IL-1β	TNF-α, IL-1β, IL-6, IL-10
M2c	Glucocorticoids, IL-10 and TGFβ	IL-10, TGF-β, CCL16, and CCL18
M2d	IL6, adenosine	IL10, VEGF

LARGE CHOLANGIOCYTES

- **Large Cholangiocytes**: specialized epithelial cells lining the common bile duct
- **Inflammatory response roles**: Involved in modulating liver inflammation, with ERK signaling part of broader regulatory networks.
- **ERK pathway activation:** Cholangiocytes activate ERK signaling in response to various stimuli; important for cellular processes including proliferation.



OBJECTIVES

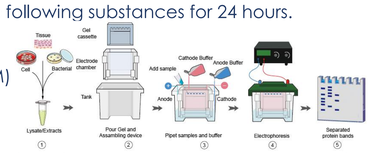
- To elucidate the inflammatory effect of Ac-CA in macrophage proinflammatory function
- Compare the levels of LPS-induced ERK between different bile acids in macrophages and cholangiocytes.
- Measure the transcription levels of inflammatory cytokines in macrophages after being exposed to various combinations of bile acids and LPS.

HYPOTHESIS

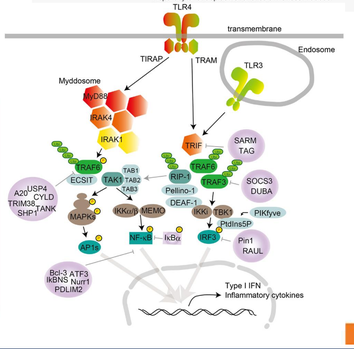
We hypothesized that Ac-CA suppression of FXR will reduce LPS-mediated ERK activation and proinflammatory cytokine gene expression in cholangiocytes and macrophages.

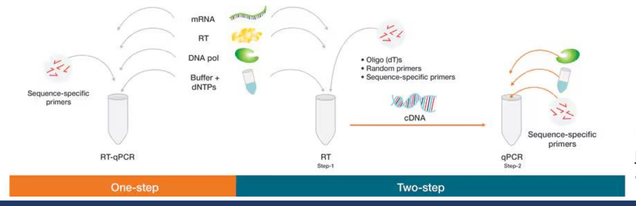
METHODS

1. Cultured the RAW cells in vitro using 3 ml of DMEM medium with 10% FBS, 1% PS overnight.
2. Treated cells with the following substances for 24 hours.
   1. Control
   2. CA (50 mM)
   3. Ac-CA (50 mM)
   4. LPS (50 ng/ml)
   5. LPS+CA
   6. LPS+Ac-CA



3. To measure LPS activation of ERK, we performed a Western blot to detect the phosphorylation levels of ERK proteins, thereby indirectly assessing LPS activation.
4. We used quantitative polymerase chain reaction (qPCR) to quantify the mRNA expression for two inflammatory cytokines, including tumor necrosis factor-alpha (TNF-a), interleukin-1 beta (IL-1β), and interleukin





RESULTS

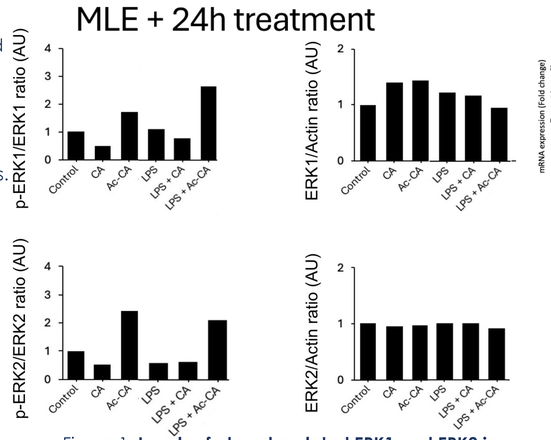
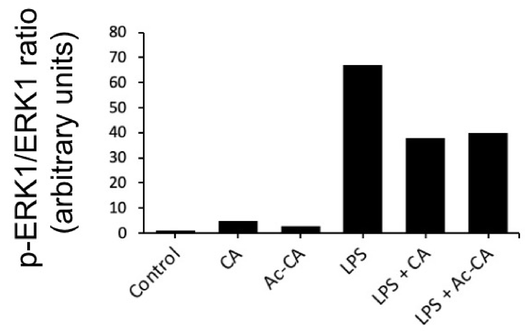


Figure 1: **Levels of phosphorylated ERK1 and ERK2 in cholangiocytes normalized to their respective total ERK levels (p-ERK1/ERK1, p-ERK2/ERK2) and the total ERK1 and ERK2 levels normalized to the housekeeping protein Actin (ERK1/Actin, ERK2/Actin)** across various treatments. AU stands for "arbitrary unit." Ac-CA has a notable effect on ERK1/2 activation with and without LPS.



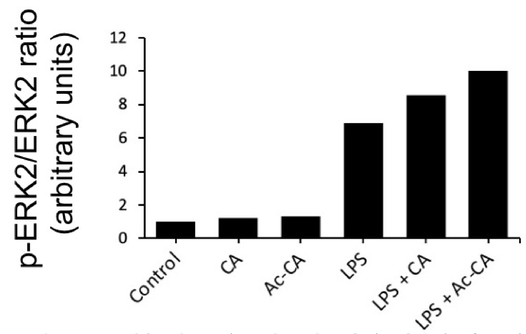


Figure 2: **Western blot detecting phosphorylation levels of ERK in macrophages** relative to the control group. Ac-CA has no effect on ERK1/2 activation, with or without LPS.

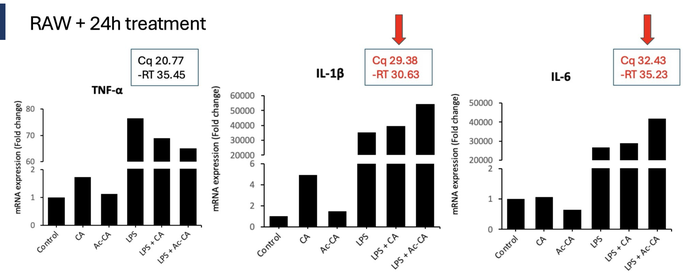


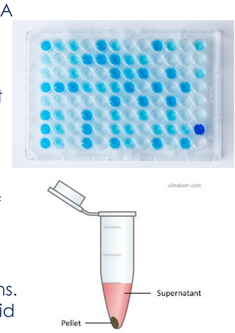
Figure 3: **qPCR of mRNA expression of TNF-a, IL-1β,and IL-6 respectively in macrophages** after 24 hours of the same treatments as Fig 1. We split the Y-axis to more accurately show the variations in mRNA in macrophages without LPS. Ac-CA has no impact on the gene expression of proinflammatory cytokines.

CONCLUSION

The hypothesis was partially correct: Ac-CA had a noticeable effect on LPS-induced activation of ERK in cholangiocytes, but no effect on LPS-induced activation of ERK or mRNA expression of proinflammatory cytokines in macrophages. For macrophages, the addition of LPS was a much more crucial factor. This may be because macrophages express more TLR4 than cholangiocytes, making them more responsive to LPS, while cholangiocytes express more FXR, making them more responsive to Ac-CA.

FUTURE DIRECTION

- Dimethyl sulfoxide (DMSO) cell concentration ≥ 0.1%; cell death by toxicity could be confounding variable
- An experiment testing the effects of Ac-CA in resident immune cells in the gastrointestinal tract would be more relevant.
- Perform direct ELISA assay on supernatant to directly measure secretion of inflammatory cytokines.
- Measure inflammatory cytokines in cholangiocytes
- Dissolve Ac-CA in DMSO concentration of 0.01%.
- Perform at least two more times to run meaningful statistical tests
- Analyze macrophage polarization patterns.
- Perform tests on the three other cholic acid derivatives.



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ACKNOWLEDGEMENTS

I'd like to thank Nan Wu, Kaitlyn Jackson, Dr. Zhou, the rest of the Zhou lab, MSIP Co-directors, and sponsors for making this project possible.