The Effect of Acetyl-DCA on LPS-induced activation of ERK and inflammatory cytokines in mouse RAW 264.7 macrophages

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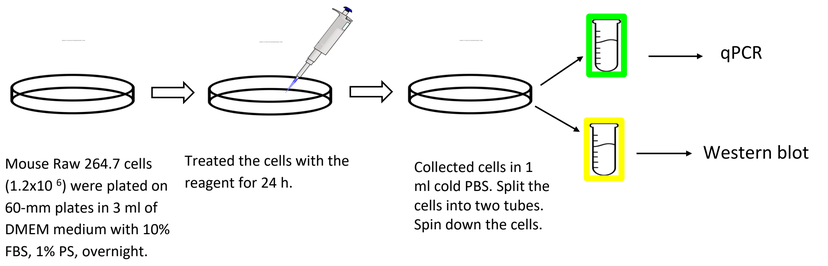
Abstract: Bile acids play a crucial role in the inflammatory response in mouse macrophages. The molecules, produced in hepatocytes in the liver, travel to the small intestine to assist in emulsifying and absorption of dietary triglycerides (Tungland, 2018). By binding to nuclear receptors in the liver like farnesoid X receptor (FXR) and Takeda G protein-coupled receptor (TGR5), bile acids are potent signaling molecules that regulate the expression of downstream factors influencing lipid, glucose, energy metabolism and inflammation (Tungland, 2018). The inflammatory effects of many bile acids, however, are unclear. This project aims to investigate the role of an acetylated bile acid, acetyl-deoxycholic acid (Acetyl-DCA), in immune response and cytokine expression in mouse macrophages in hopes to be a potentially viable therapeutic agent for inflammatory liver diseases and cholestasis. RAW 264.7 cells (macrophages) were initially plated with DMEM (3ml) containing growth factors (FBS) overnight. We then administered the bile acids DCA, Acetyl DCA, LPS + DCA, and LPS + Acetyl DCA onto the macrophage plates while isolating a plate to be the control. Post-treatment , cells were harvested and underwent centrifugation. RNA isolation and qPCR was then performed in one of the tubes to measure cytokines expression levels and other genes of interest. In the other tube, proteins were separated through a western blot, following which specific antibodies were added to detect for specific genes. Results indicated a substantial increase in mRNA expression pro-inflammatory cytokines in cells treated with LPS + Acetyl-DCA, compared to cells treated only with LPS (positive control). Conversely, isolated Acetyl-DCA on the inflammatory signaling pathways and cytokines was not significant compared to the control. Our findings suggest that Acetyl-DCA enhances LPS-induced inflammatory responses in mouse macrophages, leading to elevated expression and activation of pro-inflammatory cytokines and inflammatory signaling pathways. Due to limitations of our data, additional reliable experiments on this bile acid are necessary to validate our conclusions.

Introduction

Bile acids are extremely potent signaling molecules that can regulate various processes in the body, including immune response. Their impact on immune response is due to their ability to act as ligands for receptors like farnesoid X receptor (FXR) and Takeda G protein-coupled receptor (TGR5) which are highly expressed in macrophages and monocytes. LPS or lipopolysaccharide is a model that triggers robust inflammation in macrophages, making it a perfect control. The distinct modulation effect of bile acids on inflammasome activation may depend on diverse factors such as bile acid concentration, receptors involved, and the presence or absence of other signaling pathways **(Zhao, 2016)**. Hydrophobic bile acids like chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) are inflammatory agents that can injure the liver, intestine, and other tissue, while more hydrophilic acids, like ursodeoxycholic acid (UDCA) and tauroursodeoxycholic acid (TUDCA), are anti inflammatory **(Tungland, 2018)**. Different bile acids can also activate different receptors, leading to different immune responses. Previous studies have shown that activation of FXR and TGR5 suppresses inflammation in macrophages by reducing the expression of interleukin-1b (IL-1b), Tumor necrosis factor (TNF-a), and NLR family pyrin domain containing 3 (NLRP3); all of which are proinflammatory cytokines. Other receptors like pregnane x receptor (PXR) can upregulate immune response through increasing expression of toll like receptors (TLR) and the *NLRP3* gene. Previous data has shown that acetylated bile acids are potent ligands of PXR. Therefore, we hypothesize that acetyl acids, like acetyl-DCA, will produce a pro-inflammatory effect in mouse macrophages.

Methods

We first plated 23 microliters of RAW 264.7 cells (macrophages) onto 55 mm dishes with 3 ml of DMEM growth medium. After letting them sit overnight, we added the following reagents to one dish separately: Dimethyl Sulfoxide + Control, Deoxycholic acid (DCA), Acetyl Deoxycholic acid (Ac-DCA), LPS+DMSO, LPS+DCA, and LPS+Ac-DCA. After 24 hours, we removed the medium and stored the cells on each dish in two tubes with phosphate buffered saline solution. One tube was used for RNA isolation and qPCR to analyze the effects of the bile acids on expression of pro-inflammatory genes mTNFa, mIL6, and mIL1b. The other tube was used for western blot to detect phosphorylation of the ERK (Extracellular signal regulated kinase) pathway.



Results

1. a

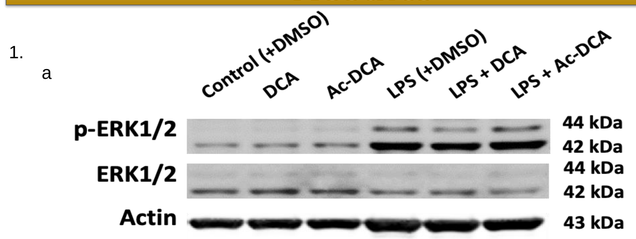
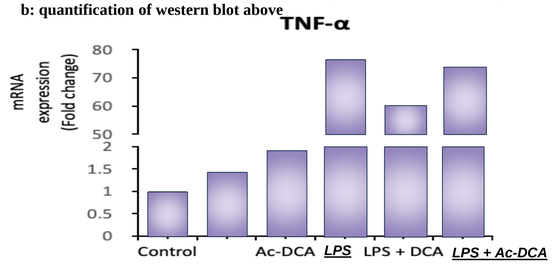
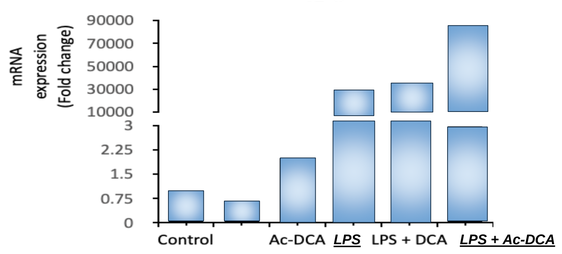


Figure 1. a: Western blot detecting phosphorylation of ERK pathway and total ERK pathway + Actin b: quantification of western blot above

2. a



b



c

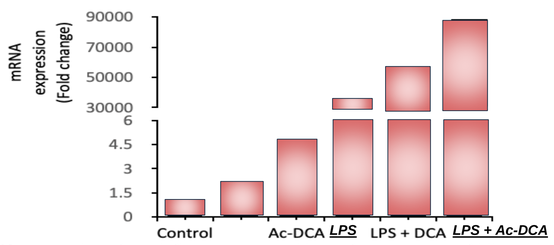
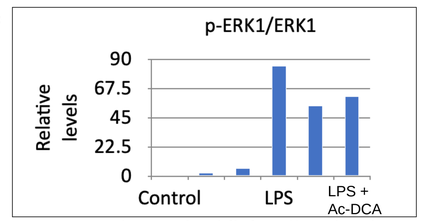
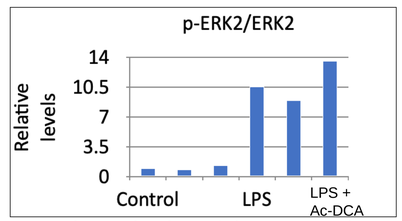


Figure 2. mRNA expression of pro-inflammatory cytokines after treatment of bile acids. A. treatment on mTNFa B. treatment on mIl6. C. treatment on mIL1b

Results/Discussion

b





- Ac-DCA and DCA had little effect by themselves on the activation of the ERK pathways, important signaling pathways that can influence the expression of inflammatory cytokines and other inflammatory mediators. LPS + Acetyl DCA increased relative levels of ERK2, resulting in more inflammatory responses, however significance is unknown.
- The qPCR revealed that DCA had a miniscule effect in mRNA expression while Acetyl DCA paired with LPS induced a substantial increase in mRNA expression in all 3 graphs.

Conclusion

- Our experiment suggests that Acetyl-DCA may act as a potential inflammatory agent in the context of LPS-induced inflammation in macrophages, supporting our hypothesis.
- Although it appears that the combination enhanced the inflammatory response a lot, our data was somewhat limited due to only one trial, so significance cannot be shown. Future studies should be conducted to expand on this topic and provide statistically reliable data along with repeated trials as well.

Acknowledgements

I would like to thank Dr. Zhou, Nan Wu, and the rest of the Zhou lab for their assistance and guidance. I would also like to thank VCU MSIP codirectors Sanjana, Aditya, Mabia, Elena, and Anaya for their wonderful organization of the program.

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