Extracellular Vesicle Mediated Inflammation Under Obese Conditions

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Introduction: Over 1.9 billion people worldwide are clinically obese, subjecting them to a state of chronic low-grade inflammation that promotes development of insulin resistance, glucose intolerance, Non-Alcoholic Fatty Liver Disease (NAFLD), and more. Under these conditions, the metabolism of certain molecules can be dysregulated, leading to elevated levels. Two of these include palmitic acid (PA) and branched chain amino acids (BCAAs), both of which having been shown to play a role in promoting inflammation in the liver. However, the mechanism by which this is done is not fully understood. We hypothesize that PA and BCAA promote the formation of pro-inflammatory extracellular vesicles (EVs), which have been gaining interest for their abilities as inter-cellular messengers, leading to obesity-related inflammation.

Methods: Huh7 hepatocyte cultures were treated with elevated levels of PA, BCAA, or water for 24 hours. EVs were then isolated from culture media and quantified/measured using NanoSight analysis. Primary peritoneal macrophages were then isolated from mice and seeded into 24-well plates. Macrophages were treated with control EVs, BCAA EVs, PA EVs, or water. 24 hours later, RNA was isolated from treated macrophages and reverse transcription was performed to generate cDNA. qPCR of cDNA, using primers for inflammatory cytokines, was used as a measure of the inflammatory state of macrophages. Additionally, total EV RNA from control and PA treated groups was sent for RNA modification analysis using mass spectrometry.

Results: EV isolation from treated Huh7 cells resulted in an average EV concentration of $4.78x10^{10}$ EVs/ml (95% CI = $4.00x10^{10}$ – $5.56x10^{10}$) for control, $4.59x10^{10}$ EVs/ml ($3.24x10^{10}$ – $5.94x10^{10}$) for BCAA treated cells, and $3.78x10^{10}$ EVs/ml ($2.85x10^{10}$ – $4.71x10^{10}$) for PA treated cells. Repeating EV isolation on the same samples resulted in more EVs, suggesting that EV yield is limited by the kit. The mean diameters of EVs for control, BCAA, and PA treated cells were 111.8 nm (95% CI = 98.7 – 124.9), 95.34 nm (76.18 – 114.4), and 139.6 nm (130.3 – 149.0), respectfully, and the mode diameters were 106.0 nm (95% CI = 100.2 – 111.7), 111.1 nm (95.27 – 126.9), and 118.9 nm (104.4 – 133.3). Preliminary results show induction of expression of inflammatory cytokines, Il-1β, Il-6, and TNF-α in BCAA and PA treated groups compared to control, with no induction of MCP-1. Repeat trials are being performed to confirm these results. Total RNA samples have been sent for RNA modification analysis, but we are still awaiting the results.

Conclusions: Conditions of elevated BCAA or PA leads to the production of hepatocyte derived EVs that can induce an inflammatory response in macrophages. Therefore, the chronic inflammation associated with obesity may be due to pathogenic production of these pro- inflammatory EVs. Possible treatments for obesity-related inflammatory diseases, such as NAFLD and Type II Diabetes, could involve targeting of these EVs.

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