2993 Cannabinoid Receptor 1 Blockade Alters Dysbiosis in Gut Microbiota Contributing to Its Anti-Obesity Effects

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Obesity and insulin resistance (IR) are key precursors for type 2 diabetes and cardiovascular disorders. Excessive energy intake results in elevated adiposity, IR, chronic low-grade inflammation, endocannabinoid system hyper-activation, and gut microbial dysbiosis. Pharmacological intervention of diet-induced obesity (DIO) using cannabinoid receptor 1 (CB_1) antagonists ameliorates obesity, IR, and inflammation; however, its effects on microbial dysbiosis are yet-to-be fully explored. CB_1 is mainly expressed in the central nervous system where it can regulate appetite but is also expressed peripherally in adipose tissue and the gastrointestinal tract. A healthy gut microbiome comprises of commensal flora, which aid in proper food digestion and intestinal barrier function. Environmental factors, such as high-fat diet (HFD) and obesity, are associated with gut dysbiosis, which can cause inflammation, gut leakage, and increased energy harvest. In the current study, we sought to determine if CB_1 blockade alters DIO-associated dysbiosis in gut microbiota, then if these microbial changes contribute to improvement in obese phenotype. Six-week-old C57Bl/6 mice were fed HFD for 12 weeks then treated with the selective CB_1 antagonist AM251 daily for 4 weeks. Treated mice showed significant loss of fat mass, improved glucose homeostasis, decreased adipose tissue inflammation, and modestly increased colon length. Stool was collected at weeks 0, 2, and 4 of treatment for 16S rDNA metagenomics sequencing. Analysis revealed AM251 treatment increased alpha-diversity while decreasing richness of gram-positive Firmicutes belonging to the Peptostreptococcaceae and Lactobacillaceae families. Correlation of these data with short chain fatty acid profile from cecal contents and colon sections interrogated for mucin layer thickness and localization of bacteria will provide insights into the mechanistic role of AM251 on gut microbiota in treatment of obesity. Furthermore, corroborative fecal transplantation studies should shed light on interplay between gut microbial alteration and obesity phenotype. This study implies novel microbial therapies may be developed to treat obesity, IR, and related diseases, and may be used to circumvent adverse side effects of CB_1 antagonist treatment. *(Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755 and P20GM103641)*

2994 Characterization of IL-6 Levels and Endocannabinoid Metabolizing Enzyme Activity in Mouse and Human Lymphocytes under Conditions of Inflammation

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Resolution of inflammation is a critical part of the inflammatory process, the perturbation of which could be part of a mechanism by which compounds exhibit toxicity. Previously our laboratory demonstrated that in response to the toll-like receptor ligand, lipopolysaccharide (LPS), IL-6 production was induced and endocannabinoid metabolizing activity was inhibited. This pathway has the potential to increase endocannabinoid levels and serve as a possible as a negative feedback system to reduce inflammation. We investigated this pathway in mouse splenocytes and human peripheral blood mononuclear cells (PBMCs) by evaluating the response to LPS or another TLR ligand, CpG. We hypothesized that high levels of IL-6 would inhibit the activity of the endocannabinoid metabolism enzymes monoacylglycerol lipase (MAGL) and carboxylesterase (CES). Human PBMCs were isolated from whole blood of healthy donors and mouse splenocytes were harvested from naïve C57BL/6 mice. Cells were treated for 3.5 hours with LPS, CpG, CpG plus anti-IL-6 to neutralize levels of IL-6, or control. IL-6 levels were assessed using ELISA. PBMC and splenocyte endocannabinoid hydrolysis was measured using liquid chromatography mass-spectrometry. CpG and LPS both induced IL-6 production from PBMCs and splenocytes that was blocked by anti-IL-6 neutralizing antibody. Hydrolytic activity of the endocannabinoid 2-arachidonoylglycerol (2-AG) was not affected by treatment with CpG or CpG plus anti-IL-6 in the human PBMCs, but was blocked by LPS in mouse splenocytes. Current studies are focused on effects of LPS in human PBMCs since the IL-6 response was higher as compared to CpG. Together these data suggest a possible IL-6-dependent negative feedback system to control inflammation. *(Funded by MSU CVM Office of Research and Graduate Studies)*

2995 Disentangling the Spatiotemporal Tissue Response to Inflammation Caused by a Physical Stressor

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Investigations of molecular and signaling responses involved in physical injuries have contributed greatly to the advancement of our knowledge regarding inflammation. Intra- and extracellular regulators, such as cytokines, signaling proteins, and growth factors, possess significant roles in facilitating recovery from physical injury. This study explored 30 spatiotemporal responses comprised of cytokines (IL-1α, IL-1ß, IL-2, IL-6, TNF-α, and MIP-1α), proteins (Akt, c-Jun, CREB, ERK1/2, JNK, MEK1, p38, p53, and p90RSK), phosphorylated proteins (p-Akt, p-c-Jun, p-CREB, p-ERK1/2, p-GSK-3α/ß, p-HSP27, p-IκBα, p-JNK, p-MEK1, p-p38, p-p70S6K, p-p90RSK, p-STAT2, and p-STAT3), and Caspase-3, measured in rat skeletal muscle tissue following a traumatic fracture injury. The dataset was examined using network centrality parameter analysis to assess the impact of each protein response in relation to all other co-measured molecular and signaling responses. The results from the network analysis allowed us to determine the progression of tissue response (from inflammation through new tissue formation), while also identifying the proteins that appear to be regulatory within this complicated framework. Notably, severely damaged tissue showed cellular indications of inflammation and new tissue formation by 168 h post-injury, while tissue 1-cm away from the site of injury (that experienced minor injury) exhibited signs of new tissue formation as early as 24 h post-injury. Extracellular hallmarks of inflammation, cytokines IL-1ß, IL-6, and IL-2 appear to have a pronounced impact at earlier time points (0-24 h post-injury), while intracellular proteins involved in cell proliferation, differentiation, or proteolysis (c-Jun, CREB, JNK, p38, p-c-Jun; p-MEK1, p-p38, p-STAT3) are more significant at later times (24-168 h). Overall, this study offers a prospective spatiotemporal depiction of the intra- and extracellular signaling involved in tissue response to inflammation, and additionally demonstrates the advantages of using a network analyis approach to extract significant information from a complex, multifaceted dataset.

2996 Mitigation of Clinical Injection Site Reaction by Preclinical Testing of Intramuscular Route of Administration

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Delivery of nucleic acids as a therapeutic intervention for disease encompasses various approaches from virus mediated DNA transduction to delivery of DNA and RNA in micellar particles such as liposomes. We have focused on the delivery of mRNA using lipid nanoparticles (LNPs) by parenteral routes of administration (ROAs). Of particular interest to our ventures are the intramuscular (IM) and subcutaneous (SC) ROAs. Unfortunately, these methods of mRNA/LNP delivery are often associated with an inflammatory injection site reaction (ISR) characterized by a dose-limiting innate immune response leading to injection site swelling, infiltration of neutrophils and macrophages and destruction of surrounding tissue. ISRs in preclinical species are highly translatable to humans and, depending upon severity, might be an unacceptable adverse event during clinical trials. Here we compared six novel LNPs to an ISR positive control in a single-dose *in vivo* rat model for ISR. These lipids were injected IM in Sprague Dawley rats at 10 or 100µg in 100µL and ISR as assessed by histopathology and cytokine panel. The cytokine panel showed no correlation with the severity of ISR. However, histopathology of the skin and muscle at the site of injection showed varying degrees of inflammatory cell infiltrate, neutrophil degeneration and muscle fiber necrosis. These reactions were graded by blind assessment which enabled rank ordering LNPs for ISR severity and aided the selection of an IM delivery vehicle.


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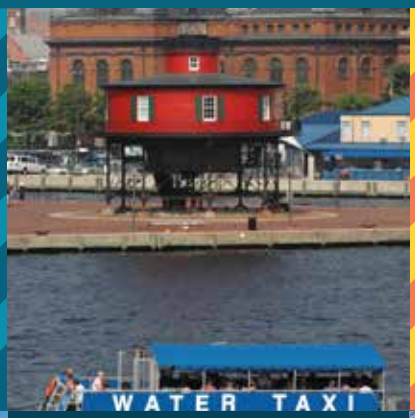

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