Acat-1 Inhibition Limits iNOS in an *In Vitro* Model of Macrophage Activation

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Each year in the United States, there are 190,600 cases of acute lung injury (ALI), associated with a mortality rate of over 74,000 deaths. Data shows acyl-coenzyme A acetyltransferase-1 (ACAT-1) inhibition improves pulmonary inflammation in an in vivo murine model of ALI. We hypothesize that ACAT-1 inhibition has anti-inflammatory effects beyond its intended use to reduce cholesterol esterification. The purpose of this study is to establish an in vitro bone marrow-derived macrophage (BMDM) model to investigate the effect of ACAT-1 inhibition in macrophage activation by inducing an inflammatory response through lipopolysaccharide (LPS), and selectively inhibiting ACAT-1 with K-604. This model will provide insight on target cell metabolism, reduce interference from whole-body effects, and minimize animal use. Monocytes were harvested from the bone marrow of 6-8 week old wild-type mice C57BL/6J (Jackson Laboratory) and stimulated with M-CSF on d0, 3, and 7 to induce macrophage differentiation. To examine if ACAT-1 inhibition limits macrophage activation, K-604 was co-administered with M-CSF. Then, the cells were treated with LPS on d7 and harvested after 24h. Nitrite, NOS2 expression, and iNOS protein were determined through nitrite colorimetric measurement, RT-qPCR, and western blot, respectively. Nitrite was measured as a proxy for nitric oxide (NO) production and was observed to decrease in LPS-stimulated cells chronically treated with K-604. NOS2 was also reduced in LPS and K-604 conjunctive treatment. Compared to LPS, iNOS was reduced with chronic K-604 as measured by iNOS protein. LPS-induced macrophage activation was suppressed and NO production was hindered due to lack of the iNOS protein. This aligns with the in vivo model, where K-604 reduced pulmonary inflammation in a rodent model of ALI. As LPS is known to increase cellular reliance on glycolysis, we will examine the effect of chronic K-604 on GLUT-1 transporter activity and PFKB1 protein expression. Supported by R25ES020721