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

Sung Jae Lee, Emily Stevenson, Elena Abramova, Changjiang Guo, Andrew Gow
Rutgers, The State University of New Jersey

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.