Honors Capstone Critique: Cytochrome P450 Function and Pharmacological Roles in Inflammation and Cancer

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Inflammation is a global issue that continues to rise. Sustained inflammation has been linked to pathologies such as obesity, cancer, and type II diabetes. My honors capstone project, under the mentorship of Dr. Christmas, investigated the role of proteins called cytochrome P450s (CYPs) in the regulation of inflammation.

I worked with human cell lines that provided experimental models to study these enzymes *in vitro*. I specifically investigated an enzyme called CYP4F3. The hypothesis of my project was that CYP4F3 uses a lipid called geranylgeranyl pyrophosphate (GGPP) as a substrate and regulates a process called protein prenylation in cells. Prenylation involves the transfer of a lipid chain from GGPP to proteins to enable the proteins to attach to membranes and become functionally active. The hypothesis further suggested that CYP4F3 metabolizes excess GGPP and maintains its levels within a range that allows for normal prenylation. In addition, it was hypothesized that sustained inflammation downregulates CYP4F3 expression in cells leading to increased levels of GGPP and excessive prenylation, which could contribute to many of the pathologies associated with chronic inflammation. The goals of the project were to demonstrate that CYP4F3 increases GGPP metabolism and reduces protein prenylation in cells, and to show that inflammatory cytokines such as interleukin-1 (IL-1) downregulate CYP4F3 expression.

My mentor and I established a simple assay for GGPP-dependent prenylation using human umbilical vein endothelial cells (HUVECs). Lipopolysaccharide (LPS) stimulates an increase in E-selectin expression in the cells, which was measured by real-time PCR. This is sensitive to a geranylgeranyl transferase inhibitor (GGTI). Transfection of HUVECs with an expression vector for CYP4F3 resulted in a change in LPS-dependent E-selectin expression that was comparable to the effects of GGTI, but the change is reversed by addition of excess GGPP, suggesting that CYP4F3 reduces levels of GGPP available for prenylation. I also demonstrated that CYP4F3 is expressed in differentiated HepaRG cells, a human liver cell line. A longer-term goal is to show that inflammatory downregulation of CYP4F3 results in increased GGPP-dependent prenylation and changes in cell function.

This capstone gave me the opportunity to gain research experience and present my findings at the national level. I presented my capstone at the American Society for Biochemistry and Molecular Biology national conference, and I will remember that experience forever. While intimidating at first, this project pushed me outside my comfort zone and I am beyond grateful. This opportunity sparked an interest in graduate research and I hope to continue exploring similar areas. As I pursue Physician Assistant school after graduation, the skills gained from this capstone will be advantageous and help set me apart from others. Radford honors, and specifically their capstone program has enhanced my Radford University experience and prepared me for what lies ahead!