THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

# Alexandra Crowe

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE
GRADUATE COLLEGE
Graduate Pharmaceutical Sciences

Wednesday, May 1, 2019 | 2:00 pm | College of Pharmacy | Room 101

## *Regulation of Organic Anion Transporting Polypeptide (OATP) 1B1 Transport Function and Phosphorylation by a Single Nucleotide Polymorphism c. 521 T>C and Protein Kinase/Phosphatase Modulators*

COMMITTEE IN CHARGE: Wei Yue, PhD; Jessie L.S- Au, Pharm.D, PhD, Lucila Garcia-Contreras, PhD; Sukyung Woo, PhD; Doris Benbrook, PhD and Jie Wu, PhD

ABSTRACT: Organic anion transporting polypeptide (OATP) 1B1 is a hepatic membrane transport protein that mediates the hepatic uptake of many drugs (e.g. lipid-lowering statins). Inhibition of OATP1B1-mediated transport is an important determinant of statin-induced myopathy and transporter-mediated drug-drug interactions (DDIs). The c.521 T>C (V174A) non-synonymous polymorphism of OATP1B1 is the most robust predictor of statin-induced myopathy. Currently, there is limited understanding of how transport function of OATP1B1 is regulated beyond competitive transporter inhibition, especially at the post-translational level. The goals of the current studies are twofold: 1) to characterize the phosphorylation status and plasma membrane localization of the V174A-OATP1B1 variant and 2) to determine the effects of protein kinase C (PKC) activation on OATP1B1 phosphorylation, surface expression and transport kinetics.

Transport function of OATP1B1 was determined in a FLAG-tagged HEK293 stable cell line using [$^{3}$H]-$E_2$17βG as the probe substrate. To determine the phosphorylation status of OATP1B1, cells were labelled with $^{32}$P-orthophosphate, followed by immunoprecipitation and immunoblot with FLAG and autoradiography. Expression of OATP1B1 on the plasma membrane was determined by surface biotinylation, immunofluorescent staining, and confocal microscopy. Genotyping of the c.521 T>C SNP of OATP1B1 in formalin-fixed paraffin embedded (FFPE) liver tissue was conducted by extraction of genomic DNA and PCR, followed by Sanger sequencing. Expression of OATP1B1 in human liver tissue was determined by immunohistochemistry (IHC).

V174A-OATP1B1 is predominantly expressed on the plasma membrane of hepatocytes in human liver tissue and *in vitro*. The reduced transport function of V174A-OATP1B1 is associated with an increase in phosphorylation. PKC activation rapidly increased phosphorylation of OATP1B1, and was associated with a decrease in surface expression after prolonged treatment. The current studies report novel findings that impaired transport function of OATP1B1 is associated with its increased phosphorylation status in the clinically significant V174A variant and after treatment with a PKC activator. These studies provide insight into understanding of the mechanism(s) underlying altered transport function of OATP1B1 by post-translational regulation.