THE GRADUATE COLLEGE OF THE

UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

TALEAH CHRISTINE FARASYN

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE

GRADUATE COLLEGE

GRADUATE PHARMACEUTICAL SCIENCES

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10:00 am | College of Pharmacy Building, Room 349, OUHSC

Assessing Impaired OATP1B1- and OATP1B3-Mediated Transport by mTOR Inhibitors, Multi-Kinase Inhibitor Vemurafenib and by Protein Kinase C Activation

COMMITTEE IN CHARGE: Wei Yue, PhD, Chair, Nathan Shankar, PhD, Doris M. Benbrook, PhD,Lucila Garcia-Contreras, PhD, Guangpu Li, PhD

ABSTRACT: Organic anion transporting polypeptides (OATP)1B1 and OATP1B3 are hepatic transport proteins that transport a wide variety of drug substrates including lipid-lowering statins. Inhibition of OATP1B1/1B3 is considered an important determinant of statin-induced myopathy. Unanticipated drug-drug interactions (DDIs) have been reported where patients developed severe adverse effects following concurrent administration of OATP substrates and inhibitors. My primary objective was to characterize OATP1B1/1B3 functional inhibition by sirolimus, everolimus, and vemurafenib and assess the DDI potential using static R-value and physiologically based pharmacokinetic (PBPK) models. Although the human sandwich-cultured hepatocytes (SCH) model is the most relevant *in vitro* model to study regulation, research is limited by the lack of commercially-available antibodies and available hepatocytes; therefore, I also aimed to develop a novel model to study OATP regulation. Following the US FDA guidance, the inhibition potency (IC_{50} value) against OATP1B1/1B3 was first determined for all potential inhibitors prior to calculating the R-Values [$R=1+(f_{u,plasma}\times I_{in,max}/IC_{50})$]. PBPK models were then used to assess the OATP-mediated DDI potential with statins. Pre-incubation with all inhibitors at clinical concentrations reduced the IC_{50} against OATP1B1/1B3. Everolimus and vemurafenib even increased the R-values to a value greater than the US FDA DDI cutoff value of 1.1. Static R-value and PBPK modeling predicted that sirolimus, everolimus, and vemurafenib have low potential to instigate OATP-mediated DDIs; however, taking an inhibitor preincubation step and protein binding for highly protein bound compounds into consideration was found to help establish a better *in vitro-in vivo* correlation for OATP inhibition and give a more accurate representation of the *in vivo* situation. Expression and function of FLAG-tagged-OATP1B3 (Ad-FLAG-1B3) in SCH was determined over days in culture. The effect of the PKC activator phorbol-12-myristate-13-acetate (PMA) on [^{3}H]CCK-8 uptake in Ad-FLAG-1B3-SCH was also investigated. On day 4, [^{3}H]CCK-8 accumulation was negligible in nontransduced-SCH and expression and function were substantial in Ad-FLAG-1B3-SCH. PKC activation significantly decreased function in both rat and human SCH. SCH exogenously expressing human OATP1B3 is a novel physiologically relevant *in vitro* model to study OATP1B3 regulation. Our studies emphasize the high efficiency and utility of adenoviral transduced rat and human SCH to assess OATP1B3 regulation under different conditions.