COLLEGE OF PHARMACY
PHARMACEUTICAL SCIENCES
UNIVERSITY OF MICHIGAN

The Pharmaceutical Sciences Department
is pleased to announce the
Ph.D. Dissertation Defense Seminar of

Dan Li
Pharmaceutical Sciences, Ph.D. Candidate
(Mentor: Dr. Anna Schwendeman)

Thursday, July 19 at 2:00 pm
NCRC building 10, Room ACR2

"Optimization and Application of Synthetic High-Density Lipoprotein (sHDL) System in Atherosclerosis and Glioma Therapy"

Abstract: Overwhelming evidence indicates that higher levels of high-density lipoprotein cholesterol (HDL-C) correlate with reduced risk of coronary heart disease (CHD). HDL can efflux excess of cholesterol by reverse cholesterol transport (RCT). Hence, reducing acute plaque accumulation by direct infusion of cholesterol-free synthetic HDL (sHDL) has generated considerable interest. sHDL consists of a phospholipid bilayer held together by apolipoprotein A-I (ApoA-I). Due to the high manufacturing cost of recombinant ApoA-I, ApoA-I mimetic peptides complexed with a variety of lipids have been studied as treatments for various pathologies. However, the best methods of administration and formulation remain controversial. For sHDL products consisting of ApoA-I mimetic peptides like ETC-642, rapid elimination can limit their clinical application and subsequent development. Thus, prolonging the circulation time of sHDL can potentially improve its anti-atherosclerosis effect. In addition, designing novel nanoparticles mimicking sHDL can eliminate the need for the ApoA-I protein/peptide component in sHDL while preserving the core pharmacological activity of sHDL.

We first studied the influence of administration route and lipidation of ApoA-I mimetic peptide 22A on plasma peptide levels, cholesterol mobilization, and lipoprotein remodeling *in vivo*. The mean circulation half-life for 22A-sHDL ($T_{1/2}$ = 6.27 h) was longer than for free 22A ($T_{1/2}$ = 3.81 h). The amount of 22A absorbed by the vascular compartment after intraperitoneal (IP) dosing was ~50% for both 22A and 22A-sHDL. The strongest pharmacologic response was observed after intravenous (IV) injection of 22A-sHDL. Both the route of administration and the formulation of 22A significantly affected the peptide's pharmacokinetic and pharmacodynamic properties. Following this study, sHDL surface modification with polyethylene glycol (PEG) was investigated for its potential to extend sHDL circulation *in vivo*. Various amounts and different chain lengths of PEG-modified lipids were incorporated into sHDL's lipid membrane. The circulation half-life of sHDL was extended both by adding more PEG or using PEG of longer chain lengths. Addition of PEG also increased the AUC for the phospholipid component of sHDL, leading to higher mobilization of free cholesterol in plasma due to prolonged circulation and increased stability.

To extend this research into biomimetic nanomaterial development, we formulated nanomicelles (NanoMCLs), structural nano-mimetics of sHDL with small particle size (12-14 nm) and a hydrophobic core and hydrophilic exterior. NanoMCLs were shown to be functionally similar to sHDL and exhibited up to 14-fold more efficient inhibition of inflammatory cytokine release *in vitro* compared to sHDL. When administered as a 6-week treatment to ApoE$^{-/-}$ mice fed a high-fat diet, sHDL and NanoMCL reduced atheroma by 21% and 40%, respectively. In addition, NanoMCL treatment significantly depleted atheroma macrophages.

Lastly, the application of sHDL as an anti-cancer drug delivery system was explored for treatment of glioma. Chemotherapeutic agent docetaxel (DTX) and immune-stimulatory toll-like receptor-9 (TLR-9) agonist cholesterol-CpG (cholCpG) were co-incorporated in sHDL nanoparticles. The sHDL composition was optimized to maximize DTX retention in plasma. In a murine glioma model, intracranial injection of DTX-sHDL-cholCpG system exhibited significant anti-tumor efficacy with 20% of animals surviving past 90 days. In addition, the mean survival time for animals on DTX-sHDL-cholGpG treatment was 55 days, compared to 38 and 47 days for DTX-sHDL and free DTX groups.
In summary, this thesis systemically studied the effect of sHDL lipid composition on cholesterol mobilization and established sHDL-PEG and NanoMCL systems to improve the anti-atherosclerosis effect of sHDL. In addition, DTX-sHDL-CpG nanoparticles were developed and applied in glioma therapy. We have shown that sHDL is a versatile nanoparticle with utility in atherosclerosis treatment and drug delivery.

Defenses are open to the public.