Rocky Mountain Discussion Group

2018 Annual Meeting

Expanding the Frontiers of Pharmaceutical Sciences

Alder Auditorium
University of Washington
Seattle, WA
Tuesday, June 12 – Wednesday, June 13, 2018

Dear Colleagues,

Welcome to the University of Washington! We invite you to explore our beautiful campus, vibrant city and the Pacific Northwest.

Thank you to our generous sponsors, speakers and attendees for their support of this regional AAPS meeting. The AAPS RMDG annual meeting provides pharmaceutical scientists in the "expanded" Rocky Mountain geographical area with a forum for dissemination of cutting-edge research, discussion of important issues in pharmaceutical sciences, and networking for scientists in industry and academia from various Schools/Colleges of Pharmacy in Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, and Washington.

This year's theme, "Expanding the Frontiers of Pharmaceutical Sciences", reflects our diverse interests, research goals and aspirations. Here's to a successful 2018 AAPS RMDG meeting!

Local Organizing Committee
Yvonne Lin, Marc Vrana, and Mackenzie Bergagnini-Kolev,
Dept. of Pharmaceutics, University of Washington
Joe Zolnerciks, SOLVO Biotechnology

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To join the Rocky Mountain Discussion Group, please visit:
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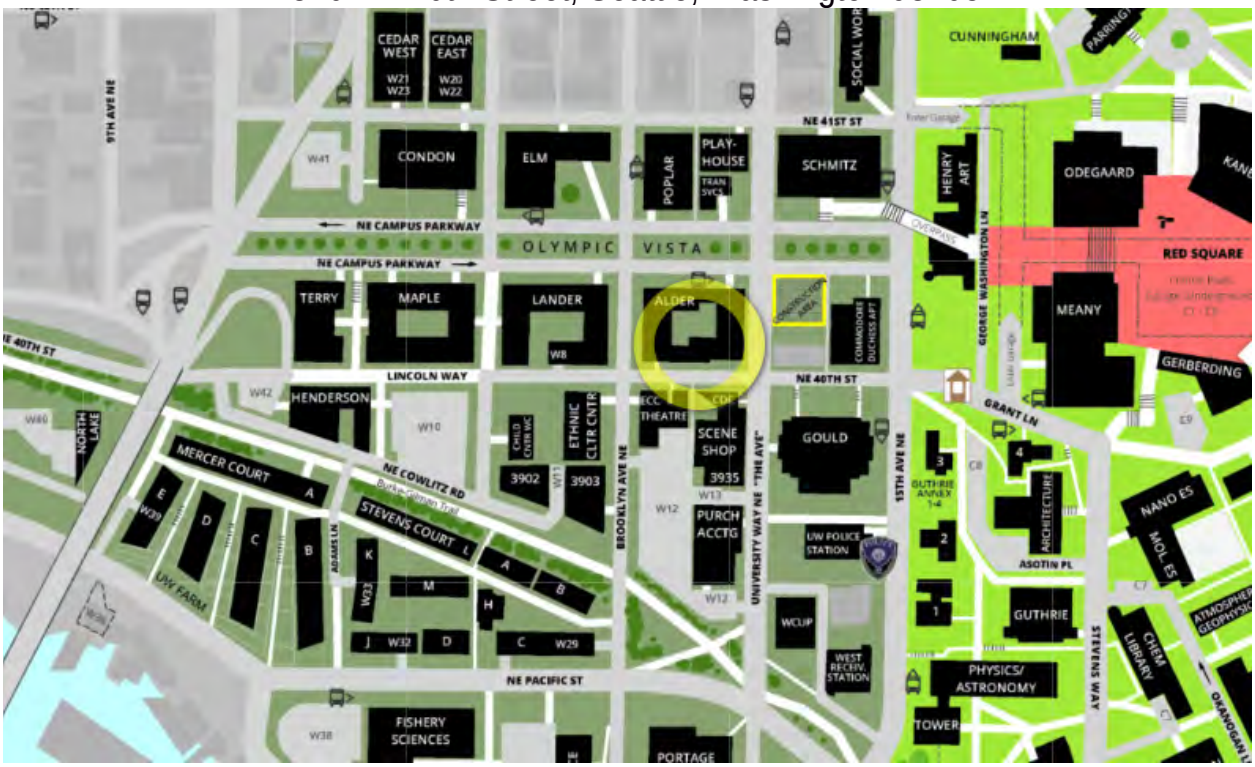
Meeting Location

University of Washington
Alder Auditorium – entrance on NE 40th St
1310 NE 40th Street, Seattle, Washington 98105



Parking

Parking is challenging on the UW campus. The UW parking fee is $15 per day.
http://www.washington.edu/maps

Central Plaza Garage.15th Ave NE and NE 41st St. The parking garage entrance is on the east side of the street.

W12 - Self-pay lot; limited number of spots. Between Brooklyn Ave NE and University Way NE south of NE 40th St.

Rocky Mountain Discussion Group

2018 Annual Meeting

Expanding the Frontiers of Pharmaceutical Sciences

Tuesday, June 12

11:00 am Visiting Student Tour and Lunch (by invitation)
1:30 pm Registration
2:00 pm Welcome

2:15 pm **Navigating the Path from Graduate School into the BioPharmaceutical Industry**
Larry Wienkers
Former Vice President and Global Head, Department of Pharmacokinetics and Drug Metabolism Amgen

3:15 pm **Panel Discussion: Career Opportunities in Pharmaceutical Sciences**
John Hoekman, Co-founder and CSO, Impel NeuroPharma
Ian Templeton, Senior Consultant and Scientific Advisor, Certara
Melanie Joy, Associate Professor, University of Colorado

4:00 pm **Poster Session and Reception**
Odd numbered posters: 4:00 - 4:45 pm
Even numbered posters: 4:45 - 5:30 pm

6:00 pm End of day

Wednesday, June 13

8:30 am Registration

Frontiers of Designing New Therapies

9:00 am **Application of technological advancement in the design of safer polymyxins**
Anshul Gupta, Principal Scientist (PKDM)
Amgen

9:40 am **Cinnamaldehyde Analogs as Metabolism-dependent Inhibitors of CYP2A6**
John Harrelson, Associate Professor of Pharmaceutical Sciences
Pacific University

10:20 am **Structural assessment of the χ conotoxin PnID, a potential lead for treating neuropathic pain**
Michael Espiritu, Postdoctoral Fellow
Pacific University

10:35 am Break

Frontiers of Drug Delivery

10:50 am — Systems Approach to Targeted Drug Combination Therapy: Integrating Medical and Pharmaceutical Sciences
Rodney Ho, Professor of Pharmaceutics
University of Washington

11:30 am — Designing and Assessing Nanoscale Drug Delivery Systems for Chemotherapeutic Applications
Adam Alani, Associate Professor of Pharmaceutics
Oregon State University

12:10 pm — Mitigating doxorubicin induced cardiotoxicity in vitro and in vivo utilizing micellar polyphenols
Arthur Nguyen, PharmD Student
Pacific University

12:25 pm — Lunch

Frontiers of New Technology in Pharmaceutical Sciences

1:30 pm — Timing and Application of Transporter Assays in Drug Development
Krisztina Heredi-Szabo, Principal Scientist and Study Manager
SOLVO Biotechnology

2:10 pm — Developing Microorganisms for Drug Discovery
Jaclyn Winter, Assistant Professor of Medicinal Chemistry
University of Utah

2:50 pm — Formation and Distribution of cis- and trans-Epoxyeicosatrienoic Acids in Heart and Blood Tissue of Patients and Mice with Cardiomyopathy
Theresa Aliwarga, Graduate Student, Medicinal Chemistry
University of Washington

3:05 pm — Break

Frontiers of Translational Science

3:20 pm — DMPK Challenges and Considerations for the Development of Antibody-Drug Conjugates (ADC)
Baiteng Zhao, Principal
OptimumQuant, LLC

4:00 pm — Genetic Biomarkers of Drug Induced Adverse Events - Utility in Drug Development
Leslie Dickmann, Senior Scientist in Clinical Pharmacology
Genentech

4:40 pm — **Sequential Metabolism Kinetics of** Δ**9**-Tetrahydrocannabinol (THC) and its Psychoactive 11-OH-THC Metabolite
Gabriela Patilea-Vrana, Graduate Student, Pharmaceutics
University of Washington

4:55 pm — Closing remarks

5:00 pm — End of day

Abstracts

1. Theresa Aliwarga
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Formation and Distribution of cis- and trans-Epoxyeicosatrienoic Acids in Heart and Blood Tissue of Patients and Mice with Cardiomyopathy

[1]Theresa Aliwarga, [2]Xiaoyun Guo, [3]Scott Heyward, [2]Qinghang Liu, [1]Libin Xu, [1]Rheem A. Totah
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Cytochrome P450 (CYP) 2J2 is the major of CYP isozyme responsible in epoxyeicosatrienoic acids (EETs) biosynthesis in cardiac tissues. EETs are metabolites of arachidonic acid (AA) oxidation that have important cardioprotective and signaling properties. AA is an ω-6 polyunsaturated fatty acid that is prone to autoxidation. Although hydroperoxides and isoprostanes are major autoxidation products of AA, EETs are also formed from the largely overlooked peroxyl radical addition mechanism. While autoxidation yields both *cis*- and *trans*-EETs, CYP epoxygenases have been shown to exclusively catalyze the formation of all regioisomers of *cis*-EETs, on each of the double bond. In plasma and red blood cell (RBC) membranes, *cis*- and *trans*-EETs have been observed, and both have physiological functions.
We developed a sensitive ultra-performance liquid chromatography tandem mass spectrometry assay that separates *cis*- and *trans*- isomers of EETs and applied it to determine the relative distribution of *cis*- vs. *trans*-EETs in reaction mixtures of AA subjected to free radical oxidation in benzene and liposomes *in vitro*. We also determined the *in vivo* distribution of *cis*- and *trans*-EETs in RBC membranes and heart tissues from healthy and diseased human and from sham and myocardial infarction-subjected mice. Formation of EETs in free radical reactions of AA in benzene and in liposomes exhibited time- and AA concentration-dependent increase and *trans*-EET levels were higher than *cis*-EETs under both conditions. In healthy human tissues, *cis*-EET levels are higher than *trans*-EET levels. In contrast, disease state elevated cardiac EET levels and decreased RBC membrane EET levels in mice overexpressing CYP2J2. The ability to accurately measure circulating EETs resulting from autoxidation or enzymatic reactions in plasma and RBC membranes will allow for future studies investigating how these important signaling lipids correlate with heart disease outcomes.

2. withdrawn

3. Mackenzie Bergagnini-Kolev
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Longitudinal Changes in 25-Hydroxyvitamin D3 and its Metabolites During Pregnancy

Mackenzie C. Bergagnini-Kolev[1], Laura Shireman, Brian Phillips[1], Kenneth E. Thummel[1], Thomas F. Easterling[2,3], Mary F. Hebert[2,3], Yvonne S Lin[1]
[1]Department of Pharmaceutics, [2]Department of Pharmacy, [3]Department of Obstetrics and Gynecology,
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Purpose: To understand how physiological changes that occur in pregnancy, including changes enzyme expression and plasma proteins may affect the circulating concentrations of 25-hydroxyvitamin D3 and its metabolites.
Methods: We conducted a longitudinal study in fifteen healthy women who were planning on becoming pregnant or in early pregnancy. Plasma samples were collected prior to pregnancy, during the first, second and third trimesters of pregnancy, at delivery, and postpartum. We analyzed the samples for: 25-hydroxyvitamin D3, 24,25-dihydroxyvitamin D3, 1α,25-dihydroxyvitamin D3, 4β,25-dihydroxyvitamin D3, 25-hydroxyvitamin D3 3-sulfate, and 25-hydroxyvitamin D3 3-glucuronide using liquid chromatography-mass spectrometry. A Mann-Whitney test was used to compare pregnancy, delivery, and post-partum measurements to pre-pregnancy measurements.
Results: Although no changes in the plasma concentration of 25-hydroxyvitamin D3 were observed, we found that plasma concentrations of 1α,25-dihydroxyvitamin D_3 were 2.0-fold higher in the first trimester and 2.5-fold higher in the second trimester through delivery compared to pre-pregnancy samples ($p < 0.01$). We also found that plasma concentrations of 24,25-dihydroxyvitamin D3 were 28% lower in the first trimester ($p < 0.05$) and returned to pre-pregnancy levels in the second trimester. No changes were observed in 4β,25-dihydroxyvitamin D3, 25-hydroxyvitamin D3 3-sulfate, or 25-hydroxyvitamin D3 3-glucuronide. Metabolite concentrations were normalized to 25-hydroxyvitamin D_3 to determine metabolite-to-parent ratios. When compared to pre-pregnancy ratios, 1α,25-hydroxyvitamin D3:25-hydroxyvitamin D3 ratios were 2-fold higher throughout pregnancy ($p < 0.01$), whereas 24,25-dihydroxyvitamin D3:25-hydroxyvitamin D3 ratios were 29% lower in the first and second trimesters ($p < 0.05$) and 25-hydroxyvitamin D3 3-sulfate:25-hydroxyvitamin D3 ratios were 33% lower in the third trimester and delivery ($p < 0.05$).

Conclusions: These data confirm that 1α,25-dihydroxyvitamin D3 is elevated as early as the first trimester of pregnancy. More mechanistic studies are needed to fully understand the dynamic processes that contribute to altered 25-hydroxyvitamin D3 throughout pregnancy.

This work was previously presented at the 21st Vitamin D Workshop on May 18th, 2018.

4. Stephen Black
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Alpha-chloro-*trans*-cinnamaldehyde, alpha-hexyl-*trans*-cinnamaldehyde and 3-phenylpropionaldehyde effects on CYP2A6 inhibition: evaluation of potential to alter nicotine metabolism

Stephen Black[1], Stephanie Longshaw[1], Lea Lambert[1], Ghina Moyeen[1], Madison Davis[1], Jeannine Chan[2], John Harrelson[1]
[1]Pacific University School of Pharmacy [2]Pacific University

Purpose: Nicotine dependence affects people worldwide contributing to serious health problems. CYP2A6 metabolizes ≈80% of a nicotine dose, making it a target for smoking cessation therapy. Previously we observed that *trans*-cinnamaldehyde is a mechanism-based inhibitor of CYP2A6. With the goal to develop more potent inhibitors and to understand the structure-activity relationships of *trans*-cinnamaldehyde analogs we investigated 3-phenylpropionaldehyde, alpha-chloro, alpha-methyl, and alpha-hexyl-*trans*-cinnamaldehyde as CYP2A6 inhibitors.
Methods: *In silico* modeling of ligand-CYP2A6 interactions via Autodock, IC_{50} values, spectral binding constants (K_S), and k_{inact}/K_I inhibition values were compared between analogs for inhibition potential.
Results: 3-Phenylpropionaldehyde (K_I = 85 μM; k_{inact} = 0.116 min^{-1}) and alpha-chloro-*trans*-cinnamaldehyde both demonstrated mechanism-based inhibition. The inhibition rates for the chloro analog fit best to a sigmoidal model (Hill coefficient = 3.2 +/- 1.1; R^2 = 0.9779), suggesting multiple ligand binding (K_I = 81.5 +/- 11.7 μM; k_{inact} = 0.115 +/- 0.014 min^{-1}). Comparatively, *trans*-cinnamaldehyde has a k_{inact} of 0.039-0.056 min^{-1} and K_I of 18.0-27.2μM. The alpha-methyl and alpha-hexyl analogs of *trans*-cinnamaldehyde did not demonstrate mechanism-based inhibition, and are suspected of having competitive inhibition, suggesting that an alkyl substitution at the alpha position prevents metabolism to an inhibitory reactive metabolite.
Conclusion: Structural changes at the alpha position of *trans*-cinnamaldehyde have profound effects on the mechanism/potency of CYP2A6 inhibition.

The American Chemical Society Northwest Regional Meeting (NORM), June 25-28, 2017

5. Kuan-Fu (Freddy) Chen
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The Impact of Proximal Roux-en-Y Gastric Bypass Surgery on Acetaminophen Absorption and Metabolism

Kuan-Fu Chen,[1] Taurence Senn,[2] Brant K. Oelschlager,[3] David R. Flum,[3] Danny D. Shen,[1] John R. Horn,[4] Yvonne S. Lin[1] and Lingtak-Neander Chan[4]
[1]Department of Pharmaceutics; [2]Department of Medicinal Chemistry; [3]Department of Surgery; [4]Department of Pharmacy, University of Washington, Seattle, Washington

Bariatric surgery is one of the most effective medical interventions for the treatment of obesity and 45% of patients undergo Roux-en-Y gastric bypass surgery (RYGBS) that surgically alters the stomach and length of small intestine. The aim of this study was to determine how Roux-en-Y gastric bypass surgery (RYGBS) affects the absorption and metabolism of acetaminophen. Twelve morbidly obese received 1.5 g of acetaminophen (APAP) orally on three separate pharmacokinetic study days (i.e., pre-RYGBS baseline, 3-month, and 12-month post-RYGBS). Plasma was collected at pre-specified time points over 24 hrs and the samples were analyzed using liquid chromatography-mass spectrometry for APAP, APAP-glucuronide (APAP-gluc), APAP-sulfate (APAP-sulf), APAP-cystein (APAP-cys), and APAP-N-acetylcystein (APAP-nac). Peak concentrations of APAP increased by over 2-fold following RYGBS. Peak concentrations of APAP-gluc and APAP-sulf were increased to a smaller extent (range: 1.2 to 1.5-fold) following RYGBS, whereas peak concentrations of APAP-cys APAP-nac were unchanged. In contrast to peak concentrations of APAP, there were no major differences in weight-normalized clearance, weight-normalized volume of distribution or terminal half-life of APAP pre- and post-RYGBS. Interestingly, the metabolite-to-parent ratios of all four metabolites were decreased at 3 and12-months post-RYGBS. In conclusion, RYGB caused a rapid increase in the rate of absorption of APAP and a possible decrease in the activities of CYP2E1 and Phase II enzymes.

6. Michael Espiritu
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A Mechanistic Study on the Inhibition of Nicotine Metabolism by *Trans*-Cinnamaldehyde

Michael Espiritu[1], Stephen Black[1], Casey Johnston[2], Jeannine Chan[2], and John Harrelson[1]
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Purpose: *Trans*-cinnamaldehyde has previously shown to selectively inhibit cytochrome P450 2A6 (CYP2A6) via metabolism based inactivation using assays involving coumarin as a reporter substrate. In this study we aimed to investigate the mechanism by which CYP2A6 is inactivated by *trans*-cinnamaldehyde, with the ultimate goal of developing *trans*-cinnamaldehyde as a smoking cessation agent. We have also determined the level of inhibition of nicotine metabolism when nicotine is incubated with *trans*-cinnamaldehyde, human liver microsomes and cytosol.
Methods: CYP2A6 activity and heme loss were monitored in the presence of *trans*-cinnamaldehyde and NADPH *in vitro* using human liver microsomes and reconstituted CYP2A6 respectively. The effect of reactive oxygen species (ROS) generation on CYP2A6 activity and heme loss was studied using the reactive oxygen scavengers. To determine if heme loss was observed due to heme destruction or adduct formation, predictive multiple reaction monitoring (MRM) was performed using a liquid chromatography tandem mass spectrometry (LC-MS/MS) protocol.
Results: In activity studies using HLM and coumarin all reactive oxygen scavengers protected against activity loss, with N-acetyl cysteine and catalase offering the most protection (20% respectively). Heme monitoring using LC-MS/MS resulted in a significant amount of heme loss (97%) with a standard deviation (SD) of 3% after 18 minute incubations with *trans*-cinnamaldehyde and NADPH in comparison to enzyme only controls. Inclusion of glutathione resulted in approximately 46% (SD of 7%) protection of the heme. Incubations with an NADPH regenerating system yielded a 30% (SD of 21%) loss of heme in comparison to controls without *trans*-cinnamaldehyde. Predictive MRM yielded no detectable alkylated or acylated heme adducts or their respective hydroxylated forms.
Conclusions: This study demonstrated for the first time that nicotine metabolism is inhibited by *trans*-cinnamaldehyde and that ROS generation and heme destruction are major contributors to the inactivation of CYP2A6.

This work was presented at the Experimental Biology conference on April 24th 2018.

7. withdrawn

8. Kendan A. Jones-Isaac
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Modeling the health effects of aristolochic acid using linked liver-kidney microphysiological systems

Kendan A. Jones-Isaac[1], Shih-Yu (Shirley) Chang[2], Kirk P. Van Ness[1], Jonathan Himmelfarb[3], & Edward J. Kelly[1,3]
[1]Department of Pharmaceutics, [2]Department of Pharmacy, and [3]Department of Medicine, Division of Nephrology, Kidney Research Institute, University of Washington, Seattle, Washington, USA.

Exposures to environmental toxins pose a significant threat to human health. However, evaluating the impact of chronic exposure to such toxins can be difficult in humans due to ethical concerns. Plant-derived aristolochic acids, potent nephrotoxins and carcinogens, are etiologic agents in the clinical syndromes Balkan Endemic Nephropathy and Chinese Herb Nephropathy. Using primary human cells, microfluidically linked liver and kidney 3D microphysiological systems (MPS) have been used previously to demonstrate the significant contribution of hepatocyte-specific metabolism to the bioactivation of aristolochic acid I (AA-I) and resulting nephrotoxicity. During acute 24-hour exposure in the linked system, AA-I showed moderate nephrotoxicity at 10 µM and high nephrotoxicity at 25 µM. In contrast, kidney MPS displayed low to moderate nephrotoxicity when directly exposed to 25 µM AA-I. The exposure to high concentrations of AA-I is similar to the incident in in a Belgian weight loss clinic, where individuals exhibited acute kidney injury after ingesting AA-I containing Chinese herbal products. In the case of Balkan Endemic Nephropathy, individuals are exposed to lower concentrations of aristolochic acid from tainted wheat. To discriminate the acute high dose from chronic low dose effects of AA-I, we exposed our MPS organs to 1 and 10 µM AA-I over the course of three weeks. In both kidney only and liver linked kidney systems, we observed substantially increased cytotoxicity toward human kidney proximal tubular epithelial cells with exposure to both 1 µM and 10 µM. This integrated microphysiological system provides an ex vivo approach for investigating both organ-organ interactions, whereby the metabolism of a drug or other xenobiotic by one tissue may influence its toxicity toward another and represents an experimental approach for studying chronic toxicity and carcinogenicity arising from such interactions.

9. Max Lee
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Investigation of anti-inflammatory effects of natural products in murine macrophages

Max Lee*, Deepa A. Rao, Joe Su†

Purpose: Chronic inflammation is fundamentally related to the top two killers in the United States: cancer and cardiovascular disease. As dysregulation of molecular pathways is associated with chronic inflammation, we hypothesize that natural products (NP) such as resveratrol (RES), quercetin (QUE), curcumin (CUR), 18-glyccheretic acid (18-GA), Epigallocatechin gallate (EGCG), cinnamaldehyde (CIN), piperine (PIP), and naringenin (NAR) individually or in combination will inhibit chronic inflammation.
Methods: Murine macrophage cell lines RAW 264.7 and J774A.1 will be seeded at 50,000 cells/well and treated individually or in combination with the NP for 24 and 48 hr at various attachment times. The cell viability will be assessed using CellTiter-Blue® assay. Cells will be seeded and treated with NP or their combinations and challenged with lipopolysaccharide (LPS) to induce inflammation. Caspase-1 activity will be assessed to determine the magnitude of inflammation mitigation by NP.
Results: Mean IC50 values for NP are presented in Figure 1. The ideal attachment and treatment times for RAW 264.7 and J774A.1 are 24 hrs and 24 hrs, respectively.
Combination studies and Caspase-1 activity studies are currently underway.
Conclusion: The lifetime of macrophages varies from days to months and therefore the treatment time with NP is critical to ensure inflammation is halted without inducing apoptosis in macrophages. Based on previous studies investigating the influence of NP on inflammatory molecular pathways such as interleukin-6 (nitric oxide synthase), interleukin-1β (inflammasome), and receptor tyrosine kinase (arachidonate 5- lipoxygenase), we anticipate that one or more combinations will be effective in attenuating LPS-induced inflammation.

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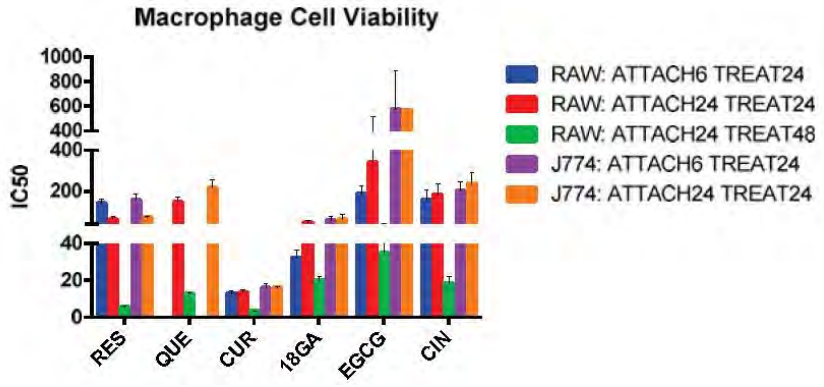

	RAW: ATTACH6 TREAT24			RAW: ATTACH24 TREAT24			RAW: ATTACH24 TREAT48			J774: ATTACH6 TREAT24			J774: ATTACH24 TREAT24		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
RES	141.675	20.894	4	60.460	18.938	4	5.595	0.495	3	156.875	31.660	4	70.438	4.845	4
QUE				145.625	26.631	4	12.615	0.446	4				217.450	39.462	4
CUR	12.925	1.751	4	13.535	1.616	4	3.359	0.115	4	15.950	2.574	4	15.800	0.700	4
18GA	32.240	4.253	3	45.310	13.025	4	20.145	1.848	4	58.360	19.001	4	62.878	22.078	4
EGCG	188.850	40.234	2	339.800	175.645	2	34.595	5.720	2	574.100	314.097	2	565.700	0.000	1
CIN	158.770	50.346	4	183.975	52.821	4	18.483	3.557	4	199.475	47.804	4	236.825	52.584	4

Figure 1. Cell Viability of Murine Macrophages with Natural Products. n = 4 studies are ongoing.

10. Antonio Lopez-Quinones
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Interaction of the Plasma Membrane Monoamine Transporter and Organic Cation Transporters with Meta-iodobenzylguanidine (mIBG)

Antonio J López Quiñones, David Wagner, and Joanne Wang

Meta-iodobenzylguanidine (mIBG) is a radiopharmaceutical used as both a diagnostic imaging agent (^{123}I-mIBG) and a targeted frontline radiotherapy (^{131}I-mIBG) for neuroblastoma. Scintigraphic imaging of mIBG in human and biodistribution studies in xenografted animal models have shown that alongside its uptake into neuroblastoma tumor tissues, mIBG is also significantly transported into normal organs and tissues including the liver, salivary glands, and kidney. ^{123}I- or ^{131}I-mIBG accumulation in normal tissues can interfere with its tumor imaging quality and contribute to peripheral toxicities. While mIBG uptake into the neuroblastoma tumors has been shown to be facilitated predominantly by the human norepinephrine transporter (hNET), the molecular mechanisms involved in its uptake into the peripheral tissues have been poorly understood. We hypothesized that the organic cation transporters 1-3 (hOCT1-3) and the human plasma membrane monoamine transporter (hPMAT) are involved in mIBG transport and accumulation in normal tissues. Thus, the purpose of this project is to characterize the interaction and transport kinetics of mIBG with these transporters and compare them with hNET. This was done by executing uptake assays in human embryonic kidney cells stably expressing hPMAT, hNET, or hOCT1-3, followed with mIBG quantification by liquid chromatography-tandem mass spectrometry. Time-dependent studies showed that mIBG is an excellent substrate of hPMAT, hNET, and hOCT1-3. Concentration-dependent studies were fitted to the Michaelis-Menten equation, resulting in Km values for hPMAT, hNET, and hOCT2 of 42.0 ± 10.7, 35.7 ± 8.91, and 17.2 ± 2.77 μM, respectively, suggesting that mIBG has a similar apparent affinity towards these transporters. Our data demonstrated that mIBG is a novel substrate for hPMAT and hOCTs, providing a molecular basis for these transporters as potential facilitators for mIBG uptake into normal tissues.

This work is supported by NIH Grants GM066233 and T32-GM007750.
This work was previously presented at the Experimental Biology 2018 Meeting, San Diego, CA

11. Arthur Nguyen
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Mitigating doxorubicin induced cardiotoxicity *in vitro* and *in vivo* utilizing micellar polyphenols

Arthur Nguyen[1], Karen Seo[1], Adel Al Fatease[2], Brianna Cote[2], Lisa Janssen Carlson[3], Vidhi Shah[2], Adam WG Alani[2], Deepa A. Rao[1]*
[1]Pacific University, School of Pharmacy, Hillsboro, OR, [2]Oregon State University, Portland, OR, [3]Legacy Day Treatment Center, Vancouver, WA

Purpose: Doxorubicin (DOX) is used to treat cancers by intercalating into nucleotide bases to form a Top2-DOX-DNA complex and reactive oxygen species (ROS). This mechanism occurring in cancer cells as well as cardiomyocytes leads to cardiomyopathy. Natural products such as resveratrol (RES), quercetin (QUE), and curcumin (CUR) are know free radical scavengers and chemosensitizers, and when used in combination with DOX can lead to a decreased cardiotoxicity and increased effectiveness in tumor tissue. We hypothesize loading Pluronic® F127 micelles (mRQ & mRC) with RES, QUE, CUR in combinations DOX will mitigate DOX's cardiotoxic side effects while also acting as a chemosensitizer *in vivo* and *in vitro* ultimately extending its life-time dosing limit.
Methods: Micelles (mRQ and mRC) were prepared and characterized for loading & stability. Using ovarian (SKOV-3) and heart (H9C2) cells, *in vitro* cell viability and ROS studies were conducted along with western blot studies for Topo II**β**. Afterwards, *in vivo* studies using healthy & xenograft mice were conducted to determine efficacy and cardioprotectivity, treating them with DOX in combination with mRC or mRQ.
Results: Using micelles loaded with RC and RQ in combination with DOX showed to have a synergistic effect on SKOV-3 cells meaning the combinations therapy was more potent, while having a antagonistic cardioprotective effect in H9C2 cells. In vivo animal models (healthy mice) exhibited healthy left ventricular ejection fraction and a significantly lower cardiac troponin I levels when treated with mRQ and mRC with DOX.
Conclusion: Treatment using DOX with mRQ and mRC lead to a effective strategy to mitigate cardiotoxicity from DOX while also acting synergistically as a chemosensitizer.

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12. Hung Nguyen
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The binding of α-alkyl analogs of *trans*-cinnamic aldehyde to cytochrome P450 2A6

Hung Nguyen[1], Tia Uehara[1], Blessing Cao[2], Jeannine Chan[1] and John Harrelson[2]
[1] Pacific University Chemistry Department, [2] Pacific University School of Pharmacy

Purpose: Approximately one-third of the global population smokes with quit rates of only 19-36%, which highlights the need for alternative smoking cessation aids. Inhibition of nicotine metabolism would maintain nicotine concentrations in the blood for longer periods and decrease the frequency of the urge to smoke. *trans*-Cinnamic aldehyde (tCA) is a known inhibitor of cytochrome P450 2A6 (CYP2A6), the major nicotine metabolizing enzyme. Our objectives were to purify CYP2A6 and to understand the binding affinities of α-alkyl analogs of tCA to CYP2A6, specifically the α-ethyl, α-propyl, and α-isopropyl analogs.
Methods: We conducted ligand binding assays to see which analog, α-ethyl, α-isopropyl, or α-propyl, had the highest binding affinity to CYP2A6. Data was analyzed using GraphPad Prism, which calculated dissociation constant (K_d) values.
Results: We found that out of all the alkyl analogs tested, the α-ethyl analog had the highest affinity with the lowest K_d, and the α-propyl analog had the lowest affinity with the highest K_d.
Conclusion: The α-alkyl analogs tested had lower K_d values on average than tCA. Thus adding an alkyl group to the alpha position of tCA appears to increase the binding affinity to CYP2A6.

Portland ACS Undergraduate Poster Symposium and Career Fair: October 22, 2017
26th Annual Murdock College Science Research Conference (2017): November 5, 2017

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SEQUENTIAL METABOLISM KINETICS OF Δ^9-TETRAHYDROCANNABINOL (THC) AND ITS PSYCHOACTIVE 11-OH-THC METABOLITE

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Aims: Use of marijuana during pregnancy has been associated with negative fetal outcomes. These risks may be perpetrated by fetal exposure to the most abundant and psychoactive component in marijuana, Δ^9-tetrahydrocannabinol (THC) and its psychoactive metabolite 11- hydroxy-Δ^9-THC (11-OH-THC). Since fetal cannabinoid exposure, and therefore fetal risk, will be driven by maternal concentrations, it is necessary to predict maternal cannabinoid disposition. Here, we report hepatic metabolism kinetic parameters (V_{max} and K_m) necessary to predict maternal-fetal cannabinoid exposure through our published maternal-fetal physiologically-based pharmacokinetic (PBPK) model.
Methods: Substrate depletion studies of THC (0.01 – 14 μM) and 11-OH-THC (0.01 – 60 μM), were conducted using pooled (n=50) human liver microsomes (HLMs) in the presence of co-factors NADPH (CYP enzymes) and UDPGA (UGT enzymes). Substrate depletion and metabolite formation was monitored via LC-MS/MS. Final parameters (V_{max} and K_m) were estimated using a population PK approach, where datasets from 3-4 independent experiments were simultaneously analyzed using a comprehensive sequential metabolism model (Figure 1) built in Phoenix (Certara). The final model was selected based on the following criteria: -2LL, AIC, BIC, visual predictive check, and diagnostic goodness-of-fit plots. The tube adsorption method was used to quantify microsomal binding (fu_{mic}) of THC/11-OH-THC (n=4).
Results: Table 1 shows the final population estimates and fu_{mic} values. 11-OH-THC formation accounts for 71% of THC depletion. Formation of COOH-THC via CYPs accounts for 4.3% of 11-OH-THC depletion. UGT and CYP enzymes account for 72% and 28% depletion of 11-OH- THC, respectively.
Conclusions: Kinetic results (CL_{int}) are similar to the fractional contribution (fm) values we previously estimated for THC/11-OH-THC. In those depletion studies, at clinically relevant plasma concentrations of THC/11-OH-THC in the presence of selective inhibitors, we found that CYP2C9 primarily depletes THC (fm = 0.97) and forms 11-OH-THC (fm = 0.90), CYP3A4 primarily depletes 11-OH-THC (fm = 0.75) and UGT and CYP enzymes contribute to 60% and 40% of total 11-OH-THC depletion, respectively. The reported fraction unbound of THC in plasma is comparable to the fu_{mic} values reported here. Considering the different THC plasma concentrations achieved via inhalation (0.09 – 0.73 μM) versus oral administration (0.001 – 0.045 μM), 11-OH-THC formation will be saturated in the former but not the latter.

Interestingly, COOH-THC was not the main metabolite of 11-OH-THC, even though it is the most abundant circulating plasma metabolite. The mechanistic data presented here, along with cannabinoid fractional enzyme contribution, will be incorporated into our maternal-fetal PBPK model to predict cannabinoid maternal and fetal exposure throughout pregnancy.

Supported by NCATS TL1 TR000422 and NIDA P01DA032507.

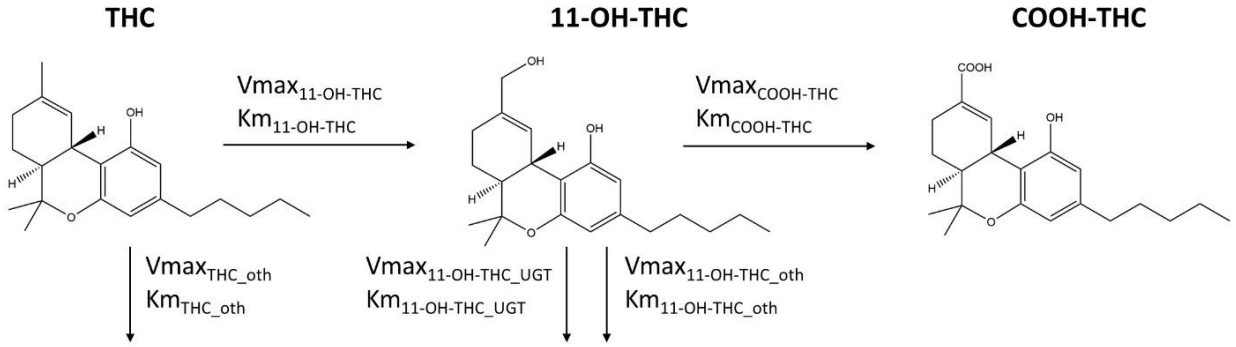

Figure 1. Sequential metabolism model for THC/11-OH-THC

Table 1. Final population mean (CV%) estimates for metabolism of THC/11-OH-THC in HLMs				
	Estimate (CV%)	**CL_{int} (ml/min/mg)**	**fu_{mic} (mean±SD)**	**$CL_{int,u}$ (ml/min/mg)**
$Vmax_{11\text{-}OH\text{-}THC}$ (nmol/min/mg)	0.699 (7.2%)	6.79	THC 0.04 ± 0.02	168
$Km_{11\text{-}OH\text{-}THC}$ (μM)	0.103 (12%)			
$Vmax_{THC\_oth}$ (nmol/min/mg)	3.86 (23%)	2.83		70.8
Km_{THC_oth} (μM)	1.36 (45%)			
$Vmax_{COOH\text{-}THC}$ (nmol/min/mg)	0.005 (7.2%)	0.009	11-OH-THC 0.06 ± 0.03	0.156
$Km_{COOH\text{-}THC}$ (μM)	0.577 (20%)			
$Vmax_{11\text{-}OH\text{-}THC\_oth}$ (nmol/min/mg)	2.07 (34%)	0.211		3.46
$Km_{11\text{-}OH\text{-}THC\_oth}$ (μM)	9.78 (41%)			
$Vmax_{11\text{-}OH\text{-}THC\_UGT}$ (nmol/min/mg)	0.412 (16%)	0.506		8.30
$Km_{11\text{-}OH\text{-}THC\_UGT}$ (μM)	0.814 (22%)			

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A Thorough Characterization of Subcellular Localization and Evaluation of the Impact of Processing Variables on Recovery and Enrichment of Drug Metabolizing Enzymes During Subcelular Fractionation

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In the early stages of drug development, drug metabolism data are generated using human in vitro models such as liver microsomes, cytosol, and S9. These subcellular fractions are isolated from liver tissue by a combination of low- and high-speed centrifugation steps which enrich drug metabolizing enzymes (DMEs). While the localizations of traditional hepatic DMEs (CYPs, UGTs) are well characterized, localization of other important DMEs including carboxylesterases (CESs), aldehyde oxidase (AOX), paraoxonases (PONs), etc., is not well known. This knowledge gap is a major limitation in using the data for in vitro to in vivo extrapolation (IVIVE).

Using samples procured from the Therefore, the aims of this study were to characterize localization of non-CYP and non-UGT DMEs in human liver subcellular fractions, and to evaluate the impact of processing variables on the apparent localization of these DMEsUniversity of Washington liver bank (n=3), we followed a standard procedure for the isolation of microsomes, changing key variables (centrifugation speeds) in the fractionation process in order to assess their impact on the a) enrichment and b) recovery of 28 important DMEs, including CYPs, UGTs, CESs, PONs, ALDH, cholinesterases (CHes), arylacetamide deacetylase (AADAC), sulfotransferases (SULTs), AOXs, and EPHXs. The protein quantification of these DMEs was performed in each fraction using validated LC-MS/MS proteomics methods [1]. The protein expression was normalized to the total protein concentration in each fraction and the enrichment and recovery were calculated.

CES1 and CES2 were found to be significantly enriched in both the cytosol and microsomes (166±15% and 77±10%). Other esterases, including PONs, ACHes, and AADAC were much more highly enriched in the microsomes, demonstrating localization to the endoplasmic reticulum. The soluble enzymes likewise showed a predictable pattern of recovery, with ADH1A, 1B, 1C, and ALDH1A1 having an average enrichment of 185±9% and 14.6±2% in the cytosol and microsomes, respectively. As expected CYPs and UGTs were found at low relative concentrations in the cytosol fraction as contamination, and were highly enriched in the microsomes when compared to the homogenate (0.2 vs. 4.2 fold respectively). Using a lower centrifugation speed in the initial separation of bigger organelles (6000 vs. 15000xg) generally produced better recovery on average, but also produced much greater variability. Further, a significant and variable loss of microsomal DMEs was observed in the mitochondrial pellet as well as the final microsomal wash step.

Conclusions: LC-MS/MS proteomics is an accurate and high-throughput technique to characterize localization of DMEs. CESs were confirmed at similar levels in both microsomes and cytosol, while other esterases (CHes, AADAC, PONs) were highly enriched in the microsomal fraction. Additionally, it was determined that the initial low speed centrifugation has a marked effect on the recovery of proteins. Further, a significant loss of microsomal DMEs occurred during the processing that must be mitigated or otherwise accounted for in IVIVE calculations to ensure physiological relevance.

1. Vrana, M., et al., CPT Pharmacometrics Syst Pharmacol, 2017. 6(4): p. 267-276.

Presented at ISSX 2017

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