MEETING REPORT

Regulation of drug metabolism and toxicity by multiple factors of genetics, epigenetics, IncRNAs, gut microbiota, and diseases: a meeting report of the 21st International Symposium on Microsomes and Drug Oxidations (MDO)

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1. Introduction

Efficacy and safety are two elements that determine the utility of a therapeutic drug for the treatment of a particular disease. Drug efficacy and safety profiles are governed by how the drug interacts with on-target and off-target molecules. Furthermore, how the drug is processed in the body will inevitably affect the accessibility of drug to its targets, by which the former consists of a number of critical processes namely absorption, distribution, metabolism and excretion (ADME). Mechanistically, drug-metabolizing enzymes and transporters (DMET) are the molecular determinants of the ADME processes. Therefore, pharmacokinetic properties of a drug, such as bioavailability, half-life and exposure, are dictated by the interactions of the drug with DMETs, which will ultimately determine drug efficacy and safety profiles following their actions on targets.

Variations in drug ADME or pharmacokinetics are common among a defined population as well as various groups, which may alter drug efficacy or cause toxicity. Indeed, the inhibition or activation of DMET protein functions often leads to remarkable variations in pharmacokinetics and consequently drug efficacy or toxicity. Secondly, changes in transcriptional gene expression of DMETs, which are governed by xenobiotic receptors or transcription factors in cells, have been revealed as another major cause of variations in drug metabolism and toxicity. Thirdly, genetic variations of ADME genes could lead to significant changes in DMET expression or activity in the metabolism and transport of drugs. In addition, recent studies have demonstrated that many other factors such as epigenetics, noncoding RNAs (ncRNAs), and gut microbiota may modulate ADME gene expression and cause variations in drug metabolism and toxicity.

The International Symposia on Microsomes and Drug Oxidations (MDO) are a well known series of conferences in the field of drug metabolism and related areas. The 21st MDO conference was held in Davis, California, USA, in October 2–6, 2016, to provide a unique opportunity for investigators and scientists to interact with each other. The programs consisted of 1 keynote lecture, 4 plenary lectures, 24 parallel sessions, and 120 posters. With a focus on the most recent advances in the fields of drug metabolism, the programs covered a wide range of important topics, including DMET structure and function, ADME gene regulation, drug development, and clinical pharmacology and toxicology. Some new merging fields, such as gene editing, gut microbiota, metabolomics, and IncRNAs, were also included. This report is to summarize some talks presented at the 21st MDO conference that are related to the regulation of drug metabolism and toxicity, particularly by multiple factors of genetics, epigenetics, IncRNAs, gut microbiota, and diseases.

2. Plenary lecture on “genetic and epigenetic regulation of ADME gene expression and drug response” by Dr. Magnus Ingelman-Sundberg

Dr. Ingelman-Sundberg started off by reviewing the current important pharmacogenomic biomarkers used in clinical medicine and in guidance by European Medicines Agency (EMA) and The US Food and Drug Administration (FDA). About 15% of medical products approved by EMA and 138 medicines approved by FDA contain pharmacogenomic labels. The most important pharmacogenomic biomarkers are related to genes encoding HLA molecules, enzymes, transporters, drug targets, specific markers and mutations in the somatic genome where mutations in the genes of ABL, ALK, BRAF, EGRF, HER2, Kras, Kit, and MET are of importance for selection of anticancer therapy.

Dr. Ingelman-Sundberg then explained the developmental origin of human polymorphisms. He pointed out genetic drift and genetic selection as the most important bases for the occurrence of today’s polymorphism. He gave examples of genetic selection from the animal world where tolerance to new environments has been developed through selection for CYP gene inducibility, expression and substrate specificities. They are exemplified, e.g., by the microsatellite 100-fold amplification of CYP6C73 in Mycetes persicace as adaptation for the tobacco plant as host, since the corresponding enzyme is active in nicotine metabolism. The genetic selection was also exemplified comparing the difference between mice and humans in alkaloid detoxification as well as the evolution of the CYP2D6 duplication in North East Africa.

Dr. Ingelman-Sundberg further presented the €15 million Ubiquitous Pharmacogenomics project (http://upgx.eu/) that will...
3. Parallel session on “disease effect on drug metabolism and disposition”, chaired by Drs. Wen Xie and Lauren M. Aleksunes

3.1. Regulation of drug transporters and drug disposition by fatty liver disease, Nathan J. Cherrington from University of Arizona, USA

Numerous drug-induced and environmental exposure–related toxicities are the result of inter-individual variations in the ADME processes of absorption, distribution, metabolism, and elimination that control the fate of these compounds from the body. Alterations in these processes provide the mechanistic basis for individual variability in response to drugs and environmental exposures. A common perception is that variability in response is due to genetic polymorphisms within the drug metabolizing enzyme and transporter genes. While there are numerous examples of these differences that play a major role in the susceptibility of genetic subpopulations for specific toxicities, the potential for transient phenotypic conversion due to temporary environmental changes, such as inflammation and diseases, are often overlooked. Due to the ensuing liver damage caused by the progressive stages of non-alcoholic fatty liver disease (NAFLD), gene expression patterns can change dramatically resulting in a phenocconversion resembling genetic polymorphisms. Because the liver plays such a key role in the metabolism and disposition of xenobiotics, it is well recognized that liver diseases can alter drug disposition and require dose adjustment to maintain drug concentrations within the therapeutic window. This temporary phenocconversion could lead to the inability of patients to properly metabolize and excrete drugs and environmental toxicants, increasing the risk of some adverse drug reactions and environmental toxicities. Dr. Cherrington's laboratory has made significant strides in identifying liver-specific disturbances in the expression and function of xenobiotic bio-transformation enzymes and membrane drug transporters. Importantly, these molecular alterations in the expression and functions of drug transporters and biotransformation enzymes lead to in vivo perturbations in the disposition of numerous xenobiotics. Therefore, Dr. Cherrington suggests that patients with NAFLD present as a subpopulation of individuals that are at higher risk for developing adverse drug reactions, due to aberrant disposition of drugs and other xenobiotics. Specifically, Dr. Cherrington's laboratory has documented individual differences in ADME genes and proteins, such as metabolizing enzymes and transporters that cause a profound alteration in the pharmacokinetics, overall exposure, and toxicity of clinically relevant drugs and xenobiotics.

3.2. Endobiotic and xenobiotic disposition in pregnancy and maternal cholestasis, Lauren M. Aleksunes from Rutgers University, USA

Pregnancy is a critical period with high nutritional demands in order to support fetal growth and development. To accommodate these needs, the enterohepatic, renal, and cardiovascular systems undergo a number of adaptive molecular and physiological changes. Circulating hormones and growth factors modify global transcription factor signaling and drug disposition. Dr. Aleksunes...
described adaptive changes in the expression of drug and bile acid metabolizing enzymes as well as transporters in pregnant mice\textsuperscript{15,16}. To enhance the absorption of lipids during pregnancy, bile acid synthesis is increased and transport is reduced\textsuperscript{16}. An enhanced supply of bile acids coupled with reduced enterohepatic circulation predisposes pregnant women to develop intrahepatic cholestasis. Interestingly, in pregnancy, Aleksunes demonstrated a down-regulation of the fibroblast growth factor 15 (mice)/19 (human), an ileal endocrine factor that represses hepatic bile acid synthesis\textsuperscript{19}. Ex vivo studies using primary hepatocytes and serum from pregnant mice further supports the critical role of circulating hormones as modulators of the classic bile acid synthesis enzyme, CYP7A1. Treatment with recombinant FGF19 restored the expression of Cyp7a1 in primary hepatocytes cultured with serum from pregnant mice\textsuperscript{21}. Additional data pointed to a potential role for 17β-estradiol to down-regulate FGF19 in cultured human intestinal cells primed with the bile acid, chenodeoxycholic acid. Using pharmacological activators of the farnesoid X receptor GW4064 and the constitutive androstane receptor TCPOBOP, the Aleksunes laboratory has demonstrated restored expression of bile acid and drug metabolizing enzyme and transporter pathways in the livers and intestines of pregnant mice\textsuperscript{19}. These data point to a novel pharmacological approach to regulate liver–intestine bile acid crosstalk during pregnancies complicated by maternal cholestasis.

### 3.3. Role of nuclear receptors and microRNAs in the regulation of drug metabolism by inflammation, Ulrich M. Zanger from Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Germany

Variability in drug response is caused by various genetic and nongenetic factors\textsuperscript{22}. Data from the genome-wide expression studies in human liver (n = 150) from Dr. Zanger's laboratory\textsuperscript{21} show that the clinical inflammation marker C-reactive protein (CRP) is associated with a broad “negative acute phase response” that comprises most drug metabolizing enzymes and transporters (DMET), supporting a well-documented negative influence of inflammation on drug metabolism\textsuperscript{22}. To investigate mechanisms leading to coordinated DMET downregulation, Dr. Zanger's laboratory treated human primary hepatocytes (PHH) with IL-6 and selective signal transduction inhibitors. Reverse-phase phosphoproteomics analysis suggested a more important role of MAPK and PI3K over JAK/STAT signaling for DMET regulation. Mathematical modeling using fuzzy-logics and extensive time-resolved data from five liver donors suggested a central role of heterodimeric RXRα/nuclear receptor complexes to coordinately downregulate most DMET genes, which was confirmed by siRNA-mediated knock-down in PHH\textsuperscript{21}. Zanger's laboratory also observed a significant downregulation of RXRα protein but not its transcript in response to IL-6 and therefore investigated a possible contribution of microRNAs. They identified numerous miRNAs strongly elevated in cholestatic liver and during inflammation, of which mir-130b was previously shown to downregulate various cytochromes P450 enzyme activities and to directly target CYP2C9\textsuperscript{24}. Preliminary data indicate that mir-130b also directly targets RXRα. Taken together, the data indicate that coordinated downregulation of DMET genes in hepatocytes in inflammatory conditions involves IL-6 activated MAPK and PI3K signaling and inactivation or down-regulation of RXRα/nuclear receptor complexes. MiR-130b appears to play a role in downregulating RXRα and certain cytochrome P450s.

### 3.4. Regulation of sulfotransferase by local and systemic liver injuries, Wen Xie from University of Pittsburgh, USA

Sulfotransferases are phase II drug-metabolizing enzymes (DMEs) that play critical roles in maintaining the chemical and functional homeostasis of xenobiotics and endobiotics. In the case of estrogen homeostasis, estrogens can be sulfated and deactivated by the estrogen sulfotransferase (EST, or SULT1E1), because estrogen sulfates cannot bind to the estrogen receptor and thus are hormonally inactive\textsuperscript{28}. Accumulating evidence suggests that many hepatic and systemic diseases can affect drug metabolism and disposition by regulating the hepatic expression and/or activity of DMEs and transporters, including the sulfotransferases\textsuperscript{29}. This presentation focused on the recent progress in describing and understanding the hepatic injury and sepsis responsive regulation of sulfotransferases in animal models. Liver ischemia and reperfusion (I/R) were used as a typical model of hepatic injury, whereas LPS and cecal ligation and puncture (CLP) were used as the sepsis models. Dr. Xie’s laboratory showed that the hepatic expression and activity of EST was markedly induced in a mouse model of I/R, which was associated with a higher level of estrone sulfate and decreased expression of estrogen responsive genes in the liver in a EST-dependent manner\textsuperscript{27}. The up-regulation of EST in the liver may have played a pathogenic role in I/R injury, because EST ablation in female mice attenuated I/R responsive liver injury. Interestingly, the effect of EST ablation was sex specific, because the EST−/− male mice exhibited heightened I/R injury. The gender specific role of EST in I/R injury remains to be better understood. In an independent study, they showed that sepsis induced the expression of EST and compromised the activity of estrogen in the liver\textsuperscript{28}. The sepsis responsive induction of EST in the liver may have played a protective role, because EST ablation sensitized mice to sepsis-induced death, which was recapitulated in wild-type mice pre-treated with triclosan, a pharmacological inhibitor of EST\textsuperscript{28}. Mechanistically, Xie’s laboratory showed that EST ablation attenuated sepsis-induced inflammatory responses due to compromised estrogen deactivation, leading to an increased sepsis lethality. It is hoped that understanding the disease effect on drug metabolism will facilitate the efficient and safe use of drugs in the clinic. In the meantime, DMEs such as sulfotransferases may be therapeutic targets that can affect the outcome of the diseases\textsuperscript{29}.

### 4. Parallel session on “role of gut microbiota in drug metabolism and toxicity”, chaired by Drs. Hyunyoung Jeong and Edward T. Morgan

#### 4.1. The microbial pharmacists within us, Peter J. Turnbaugh from University of California at San Francisco, USA

Large inter-individual variability in drug response has been a major limiting factor for achieving optimal drug therapy. The important role of the intestinal microbiota in drug metabolism has been known since the discovery by Domagk of the antibacterial activity of prontosil, and the subsequent determination of its activation to sulfanilamide\textsuperscript{30}. However, the roles of the micro-biome in modern pharmacology, drug metabolism and toxicity are underappreciated. Dr. Turnbaugh's laboratory is pursuing a more comprehensive view of pharmacology that includes the structure and activity of the resident microbial communities in the human gut and a deeper understanding of their interactions.
with each other, with their host habitat, and with the nutritional milieu of the gastrointestinal (GI) tract. More than 50 drugs are known to be metabolized by the microbiome, mostly by reductions and hydrolyses\textsuperscript{31}. Many of the hydrolytic reactions provide the bacteria with nutritional substrates. One important such drug is digoxin, which is reduced by the Actinobacteria *Eggerthella lenta* to pharmacologically inactive dihydridigoxin. About 10\% of individuals taking digoxin excrete large amounts of dihydridigoxin. The enzymes responsible have been identified as products of the bacterial cardiac glycoside reductase *Cgr* operon. *Cgr*\textsubscript{1} and *Cgr*\textsubscript{2}\textsuperscript{31}. Only a subset of *E. lenta* strains encode the *Cgr* operon and are thus able to metabolize digoxin. The abundance of the *Cgr* operon in the human gut microbiota was shown to be predictive of the inter-individual differences in digoxin inactivation using an *ex vivo* assay. *Cgr* expression and function are inhibited by arginine. Studies in germ-free mice colonized with wild-type *E. lenta* suggested that dietary protein reduced the inactivation of digoxin, *via* increasing the luminal concentration of arginine, resulting in a significant increase in digoxin bioavailability. These results demonstrate the interplay between strain-level differences in the gut microbiota and dietary intake in modulating drug disposition and thus drug response. Dr. Turnbaugh's laboratory is continuing to study the molecular mechanisms responsible for digoxin reduction and using this proof-of-principle to inform the study of other drugs that are metabolized by gut microbes.

4.2. RNA-Seq quantification of hepatic drug-processing genes in germ-free mice, Curtis D. Klaassen from University of Washington, USA

Dr. Klaassen's presentation focused on the effect of intestinal bacteria on the expression of hepatic drug-processing genes in the host. RNA sequencing (RNA-Seq) was used to profile drug metabolizing enzymes and transporters in germ-free (GF) and conventional (CONV) mice, as well as in GF mice treated with probiotics (*i.e.*, *bifidobacterium* and *lactobacillus*, VSL3\textsuperscript{33,34}). In the livers of GF mice, a number of cytochrome P450 mRNAs were increased as compared to those in CONV mice, including the livers of GF mice, a number of cytochrome P450 mRNAs were increased as compared to those in CONV mice, including the livers of GF mice, a number of cytochrome P450 mRNAs were increased as compared to those in CONV mice, including the livers of GF mice, a number of cytochrome P450 mRNAs were increased as compared to those in CONV mice, including the livers of GF mice, a number of cytochrome P450 mRNAs were increased as compared to those in CONV mice, including the livers of GF mice, a number of cytochrome P450 mRNAs were increased as compared to those in CONV mice, including the livers of GF mice, a number of cytochrome P450 mRNAs were increased as compared to those in CONV mice, including the livers of GF mice. Among the increases in the intestinal bacteria that have yet to be determined. In general, smaller effects were seen in the intestines than in livers of GF mice, although *Cyp3a* subfamily transcripts were down-regulated in the intestine. Administration of probiotics to GF mice had little effect, whereas housing GF mice with CONV mice reversed the observed effects. Overall, it is clear that intestinal bacteria can affect drug metabolism and transport, and are likely to be responsible for some individual differences in drug responses.

4.3. Drugging the microbiome, Aadra P. Bhatt and Mathew R. Redinbo from University of North Carolina at Chapel Hill, USA

Glucuronide metabolites of drugs are hydrolyzed by intestinal \(\beta\)-glucuronidase (GUS) enzymes, with the glucuronide moiety feeding in to the microbial citric acid cycle. This can lead to long half-lives of drugs due to enterohepatic circulation, and in the case of the anticancer drug irinotecan and non-steroidal anti-inflammatory drug (NSAIDs), to gastrointestinal toxicity in the form of severe diarrhea or ulcerations, respectively. Irinotecan is converted to bioactive SN-38 by carboxylesterases in the body, which is subsequently glucuronidated and excreted to the small intestine *via* bile. Hydrolysis of the nontoxic SN-38 glucuronide metabolite by the microbiota GUS enzymes leads to the release of SN-38 in the gut and subsequent related toxicity. These bacterial enzymes thus present a therapeutic target to reduce irinotecan toxicity *via* inhibition of SN-38 glucuronide hydrolysis. Dr. Redinbo's laboratory used crystal structures of bacterial GUS enzymes to elucidate the enzymatic mechanism, and identified potent and bacteria-selective inhibitors of these enzymes by high-throughput screening\textsuperscript{35}. In a mouse xenograft model, one such inhibitor did not alter the efficacy of irinotecan in reducing tumor growth, but did prevent irinotecan-induced toxicity (*e.g.*, weight loss) in the mice. GUS inhibition also reduced the formation of intestinal ulcers in mice treated with diclofenac, which is also due to hydrolysis of its glucuronide in the intestine\textsuperscript{36}. The current inhibitors have been developed with a focus on the *Escherichia coli* enzyme, but Dr. Redinbo's laboratory is examining the diversity of GUS enzymes in the GI microbiota. While this illustrates the challenges of developing drugs to related microbial targets whose representation may differ greatly among individuals, the study underscores the enzymes in the GI microbiota as potential drug targets that can be manipulated to achieve optimal drug responses.

5. Parallel session on “roles of long non-coding RNAs in liver development, functions, and diseases”, chaired by Drs. Xiao-bo Zhong and Li Wang

5.1. Non-coding RNAs and hepatic responses to drugs, environmental chemicals, and endogenous hormones, Pengying Hao, Tisha Melia, Nicholas J. Lodato, and David J. Waxman from Boston University, USA

The liver responds to both xenobiotic and hormonal stimulation with dynamic changes in gene expression and the epigenetic landscape. Some of these responses may involve the action of long non-coding RNAs (lncRNAs), which are increasingly recognized as potential chromatin regulators, as well as microRNAs, which are important post-transcriptional regulators. In work presented by David Waxman's laboratory, non-coding RNA dynamics in liver was examined under diverse conditions, including: (1) exposure to TCPOBOP, an agonist ligand of the nuclear receptor CAR, representing short-term liver responses to environmental chemical exposure; and (2) stimulation of the liver by plasma growth hormone, whose sex-differential pituitary secretion pattern imparts widespread hepatic sex-differences\textsuperscript{37}. First, they used a stringent computational discovery pipeline\textsuperscript{38} to identify 15,558 mouse liver-expressed lncRNAs, based on an analysis of a diverse set of 186 mouse liver RNA-seq datasets, representing 30 biological
5.2. LncRNAs in liver metabolic functions, Li Wang from University of Connecticut, USA

Dr. Li Wang reported a novel function of lncRNA MEG3 in bile acid homeostasis and cholestatic liver injury. Bile acids play critical physiological functions in cholesterol homeostasis and deregulation of bile acid metabolism causes cholestatic liver injury. Maternally expressed gene 3 (MEG3) was recently shown as a potential tumor suppressor; however, its basic hepatic function remains elusive. Using RNA pull-down with biotin-labeled sense or anti-sense MEG3 RNA followed by mass spectrometry, Dr. Wang's laboratory identified RNA binding protein poly pyrimidine tract-binding protein 1 (PTBP1) as a MEG3 interaction protein and validated their interaction by RNA immunoprecipitation ( RIP). Bioinformatics analysis revealed putative binding sites for PTBP1 within the coding region (CDS) of small heterodimer partner (SHP); a key repressor of bile acid biosynthesis. Further, activation of the nuclear receptor CAR in mouse liver (TCPOBOP treatment for 3–27 h) was found to significantly alter the expression of 166 lncRNAs. Many of these lncRNAs are intragenic or transcribed anti-sense to CAR-regulated CYP genes and genes that encode other drug-metabolizing enzyme RNAs, suggesting their coregulation. Comparing the male and female liver transcriptome, they identified 247 lncRNAs showing strong sex bias and tight regulation by growth hormone, with significant enrichment for nearby growth hormone-regulated DNase hypersensitive sites and sex-dependent binding sites for growth hormone-regulated transcription factors such as STAT5 and HNF6. In other studies, the Waxman laboratory investigated the role of micro-RNAs in liver sex differences, and identified 13 sex-biased liver microRNAs by small RNA sequencing. Two of these microRNAs were found to be tightly regulated by the transcription factor STAT5 following its activation by growth hormone. To assess functionality, one of the male-specific miRNAs was overexpressed in female mouse liver using adenovirus, which led to widespread gene expression changes in liver. This work leverages deep sequencing and integrative data analyses to elucidate exogenous and endogenous stimuli-induced liver transcriptome dynamics.

5.3. Role of lncRNAs in postnatal liver maturation, Chad Pope and Xiao-bo Zhong from University of Connecticut, USA

The adult liver conducts critical functions in metabolism of various endogenous and exogenous compounds, including drugs. However, the functions are not mature yet in liver at neonatal and infant ages. There is a postnatal maturation process in liver to reflect a functional transition, in which the molecular mechanisms controlling the process have not been fully established. LncRNAs have been implicated to play important roles in organ development and cell proliferation. Dr. Zhong presented an initial study to establish the role of lncRNAs in postnatal liver maturation in a mouse model. In a preliminary screening, Dr. Zhong's laboratory applied RNA-Seq to examine ontogeny of all annotated lncRNAs in mouse liver during postnatal maturation from perinatal (day −2) to adult (day 60). They found nearly 2000 lncRNAs were differentially expressed in liver during liver maturation. In general, lncRNAs were expressed at a lower level than the coding mRNAs. Both coding mRNAs and lncRNAs displayed three major ontogenetic patterns with a similar proportion among the neonatal, adolescent, or adult enriched patterns. Closed neighboring pairs of coding mRNAs and lncRNAs showed the trend to exhibit highly correlated ontogenetic expression patterns, indicating that lncRNAs may share similar regulatory mechanisms with their cis-coding genes in same chromatin segments. In a comparison with the previously deciphered developmental dynamics of the mouse liver transcriptome of all coding mRNAs, gene ontology (GO) analysis revealed that some lncRNAs enriched at neonatal ages had their neighbor protein-coding genes also enriched at neonatal ages and functions of those proteins were associated with liver growth, immune activation related processes, cell proliferation, tissue organization, and hematopoiesis. Some other lncRNAs enriched at adult ages had their neighboring protein-coding genes associated with different metabolic functions.

Dr. Zhong's laboratory further identified 433 pairs of such lncRNAs and coding mRNAs, in which lncRNAs were significantly differentially expressed during postnatal liver maturation and their neighboring protein-coding genes were also expressed in the same ontogenetic patterns. Several top candidate lncRNAs in the list were selected for further validation. One of the selected lncRNAs is H19, a lncRNA located in a genomic imprinting region differentially expressed in liver regulated by a DNA methylated region on maternal and paternal chromosomes. At the end of the presentation, Dr. Zhong described an experimental design to use a genetic modified mouse strain with a deletion of H19 on the maternal chromosome to further investigate the role of H19 in liver postnatal maturation.

6. Summary

A number of talks presented at the 21st MDO conference have overviewed recent important findings on the effects on drug metabolism and toxicity by multiple factors of genetics, epigenetics, lncRNAs, gut microbiota, and diseases. The programs nicely covered both traditional and rising topics in drug metabolism and related areas. These exceptional presentations, following questions, and insightful discussions shall undoubtedly stimulate further studies on the regulatory mechanisms underlying variable drug metabolism and toxicity, which will ultimately advance the field of drug metabolism.
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