HHS Public Access

Author manuscript

Expert Opin Drug Metab Toxicol. Author manuscript; available in PMC 2015 December 21.

Published in final edited form as:

Expert Opin Drug Metab Toxicol. 2014 September ; 10(9): 1209–1219. doi: 10.1517/17425255.2014.936378.

Drug Disposition Alterations in Liver Disease: Extra-hepatic Effects in Cholestasis and Nonalcoholic Steatohepatitis

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Abstract

Introduction—The pharmacokinetics of drugs and xenobiotics, namely pharmaceuticals, is influenced by a host of factors that include genetics, physiological factors, and environmental stressors. The importance of disease on the disposition of xenobiotics has been increasingly recognized among medical professionals for alterations in key enzymes and membrane transporters that influence drug disposition and contribute to the development of adverse drug reactions.

Areas Covered—This review will survey pertinent literature of how liver disease alters the pharmacokinetics of drugs and other xenobiotics. The focus will be on nonalcoholic steatohepatitis as well as cholestatic liver diseases. A review of basic pharmacokinetic principles, with a special emphasis on xenobiotic metabolizing enzymes and membrane transporters will be provided. Specifically, examples of how genetic alterations affect metabolism and excretion, respectively, will be highlighted. Lastly, the idea of "extra-hepatic" regulation will be explored, citing examples of how disease manifestation in the liver may affect drug disposition in distal sites, such as the kidney.

Expert Opinion—An expert opinion will be provided highlighting the definite need for data in understanding extra-hepatic regulation of membrane transporters in the presence of liver disease and its potential to dramatically alter the pharmacokinetic and toxicokinetic profile of numerous drugs and xenobiotics.

1. Introduction

The World Health Organization defines an adverse drug reaction (ADR) as "any response to a drug which is noxious and unintended, and which occurs at doses normally used in humans…" [1]. Over the past several decades, the increased development and dependency on pharmacological therapy has led to a concomitant increase in the prevalence and incidence of ADRs, making them a significant cause for morbidity and mortality across the world. Current estimates suggest that in the United States alone, ADRs are directly responsible for over 2 million hospitalizations and 100 thousand deaths per year and account for 4.2–30% of hospital admissions [2;3], landing them as a top-ten cause for death in the country [4]. Coupling to its impact on public health, ADRs are also a significant economic

Declaration of Interest: All authors declare no conflict of interest.

burden and it's estimated that up to $30 billion in healthcare costs are spent yearly on managing ADRs [5].

It is clear that understanding the sources that contribute to the development of ADRs is pivotal in providing safer pharmacological practices among patients that rely on therapeutic intervention for underlying illnesses. Astonishingly, it is estimated that up to 70% of ADR cases can be preventable with the utilization of improved medication administration and proper pharmacovigilant practices within the healthcare industry [4;6;7]. Factors such as medication errors and patient compliance are undoubtedly significant sources that account for many ADR cases; however, inherent differences between individuals' ability to properly metabolize and excrete drugs are also important influences to consider and represent a greater challenge to detect due to the near limitless combination of phenotypes present across human populations.

Research into identifying sources of inter-individual variation in drug response has exploded in the past several years. The most widely investigated contributor to inter-individual variability has been the identification and characterization of genetic polymorphisms within genes that mediate the metabolism and/or the distribution and excretion of drugs. Several clinically important examples illustrating the significance of genetic influences on drug response will be highlighted and discussed in this review. However, genetic polymorphisms represent a mere portion of the total contribution to inter-individual variability and other factors such as environmental influences have been increasingly recognized as being additional, important mediators of drug response.

The idea that the environment may influence the expression and function of genes is a well-accepted phenomenon. These "genotype-environment interactions" have been elegantly described in a recent report by Baye *et al.*, who discern these interactions as they pertain to drug metabolism and disposition. In this report, the authors illustrate the vast complexity that arises when a particular genotype is influenced by multiple environmental stimuli. Each interaction yields a distinct phenotype, which in turn may influence a variety of outcomes such as pharmacological responses to therapy as well as disease onset and progression. However, it is important to stress the fact that although a particular genotype remains relatively constant throughout an individual's lifetime, exposure to environmental stressors/factors will continually change in type and frequency of exposure. Therefore, the given phenotype an individual will experience will be largely dictated by environmental cues such as diet, exposure to environmental toxicants, and to particular disease states that modify normal tissue function [8]. Given the near endless possible phenotypes, the environmental influences on drug metabolism and disposition are arguably the most influential determinant of therapeutic drug responses.

Human diseases vary significantly in their complexity and can elicit a wide-range of effects on the expression and regulation of genetic information. This, in turn, can have significant consequences to routine physiological functions such as drug metabolism and excretion, which may ultimately contribute to the development of ADRs. More importantly, pharmacological intervention is common practice among individuals with underlying illnesses, further exemplifying the need for a better understanding of disease-mediated

modifications to drug response and/or toxicity. This review will highlight the impact of disease-mediated disturbances in drug response and in particular, hepatic diseases such as nonalcoholic steatohepatitis and cholestatic liver disease will be the focus of attention. Additionally, the impact of liver-specific pathologies on extra-hepatic xenobiotic disposition will also be explored as an expert opinion.

2. Xenobiotic Metabolism and Disposition: The Role of Metabolic Enzymes and Membrane Transporters

2.1 Pharmacokinetics of Xenobiotics

As Hodgson simply stated- “A chemical cannot be a drug, no matter how active nor how specific its action, unless it is also taken appropriately into the body (absorption), distributed to the right parts of the body, metabolized in a way that does not instantly remove its activity, and eliminated in a suitable manner- a compound must get in, move about, hang around, and then get out” [9]. These four processes, collectively termed ADME (absorption, distribution, metabolism and excretion), represent the core of what dictates the pharmacokinetics of xenobiotics, including drugs and toxicants and although these processes can be described and studied independently, they work in concert to facilitate the entry, movement, and excretion of compounds in/from the body.

ADME is what ultimately determines the concentration of xenobiotic that reaches the molecular site of action to elicit an effect. This effect can either be beneficial (pharmacologic response) or detrimental (toxicity) and any abnormality in ADME processes can result in unwanted side-effects and/or decreased therapeutic efficacy. Several factors mediate the absorption, distribution, metabolism and excretion of xenobiotics. The physiochemical properties of the compound, for example (liphophilicity, size, ionization, etc.), largely dictate its ability to passively pass through biological membranes [10]; however, the majority of xenobiotic disposition is mediated by functional mechanisms that utilize active transport and metabolism to absorb, metabolize and excrete compounds. The inter-individual variability in drug-response and toxicity is therefore largely dictated by differential regulation/activity of membrane transporters and xenobiotic drug metabolizing enzymes.

2.2 Cytochrome P450 Enzymes

By far the most intensely studied and well characterized system of drug metabolizing enzymes are the cytochrome P-450 (CYP) family of enzymes, which catalyze the oxidative biotransformation of many xenobiotics and endogenous molecules [11]. A total of 57 functional CYP genes, spread across 18 families and 44 sub-families, have been identified in humans; however, only seven (CYP3A4/5, CYP2D6, CYP2C9, CYP2C19, CYP2B6, CYP1A2, and CYP2A6) genes, belonging to families 1–3, contribute to metabolizing nearly 90% of all drugs in clinical use [11].

The functional significance of CYPs in xenobiotic PK has been well documented over the years and several factors, both physiological and genetic, have been shown to impact CYP activity and/or expression. Physiological influences such as sex [12;13] and age [14;15] on

CYP activity are well known; however, heritable genetic variations in the CYPs represent a more significant clinical concern in modern day medicine due to both the abundance of polymorphic CYP genes as well as the frequency in which these alleles occur in human populations. All seven CYP genes involved in xenobiotic metabolism are polymorphic and can lead to either gain or loss of function phenotypes.

Several examples and evidence of polymorphic CYP genes and their impact on xenobiotic metabolism and disposition can be found in the literature; however, for the purpose of this review, examples will be limited and the reader is encouraged to read several, excellent reviews on the topic [11;16]. The impact of genotype on CYP function, and ultimately xenobiotic metabolism and disposition, could not be highlighted more elegantly than examining the case of CYP2C19 and clopidogrel. Clopidogrel is an anti-platelet aggregating agent that requires metabolic activation by primarily CYP2C19 for full pharmacological activity. Numerous clinical studies have shown that patients harboring the CYP2C19*2 allele, which creates a splicing defect resulting in null-expression of the CYP2C19 gene, have significantly decreased anti-coagulation effects by the drug and are associated with an increased risk in developing serious adverse cardiovascular events [17–20]. Other reports, however, drew inconsistent results and found no association with CYP2C19 loss-of-function genotypes and cardiovascular events in clopidogrel therapy [21;22]. Nonetheless, the accumulated amount evidence has resulted in the addition of a *Black Box* warning for clopidogrel alerting health care professionals of the decreased responsiveness to the drug in patients with CYP2C19 loss-of-function genotypes [11]. Additionally, it was recently shown that a specific polymorphism (18492T>C) occurring in the CYP2B6 gene is associated with decreased plasma levels of efavirnez in two separate patient populations infected with HIV [23;24]. It is interesting to note that the frequency of heterozygosity of this polymorphism was 42%, resulting in nearly a two-fold decrease in efavirnez plasma levels [23]. The CYP2D6*10 polymorphism was found to have a frequency of 51% in a Chinese population and patients homozygous for this allele had a significant reduction in tramadol clearance leading to an increased half-life and AUC [25]. CYP2C9 is also highly polymorphic and a recent report showed a 2.7-fold increase in the half-life of celecoxib in patients harboring the CYP2C9*3 polymorphism [26]. Taken together, these examples highlight the importance of CYP function in regulating the metabolism of xenobiotics and in particular, pharmaceuticals. Furthermore, the high frequency of these polymorphic alleles in certain racial populations is concerning and should be taken into consideration.

2.3 Membrane Transporters

Much of the focus in identifying inter-individual variability in xenobiotic disposition has been on xenobiotic metabolizing enzymes; however, membrane transporters have become increasingly recognized as additional contributors in altered drug response. Membrane transporters are also important mediators of ADME and like CYPs, many members are polymorphic and are therefore of particular interest in their contribution to inter-individual variability in drug response. Two large superfamilies of transporters exist; the ATP-binding Cassette (ABC) and solute carrier (SLC) superfamily [27;28]. Members of the ABC superfamily are primarily efflux pumps, utilizing the power of ATP-hydrolysis to actively transport substrates against concentration gradients. SLC transporters, on the other hand, are

primarily uptake transporters that utilize ion gradients to drive the active transport of substrates [28].

Several genetic polymorphisms have been identified in clinically relevant members of the ABC and SLC families. The efflux transporter ABCC2 (multidrug resistance-associated protein 2) is important for the biliary and renal efflux of a variety of drugs and other xenobiotics. A recent study shows a high degree of association with a specific ABCC2 polymorphism (−24C>T) with higher risks of methotrexate toxicities in children receiving high-dose treatment for acute lymphoblastic leukemia [29]. These observations were correlated with higher plasma retention of methotrexate after 48 hours of drug administration suggesting that this polymorphism functionally alters the excretion of substrates. Other members of the ABC family with confirmed, functional polymorphisms include ABCG2 (breast cancer resistance protein) and ABCB1 (P-glycoprotein). In a study conducted among 20 patients with cancer, the ABCG2 421 C>A polymorphism was found to cause a nearly 3 fold increase in plasma diflomotecan levels [30]. Furthermore, this increase was not associated with other polymorphisms in other transporter and CYP genes. A more recent study investigating the functional consequence of the ABCB1 1191G>A polymorphism demonstrated decreased activity exemplified by increased tacrolimus levels in cells over-expressing the ABCB1 variant [31]. Clearly, polymorphic membrane transporter genes contribute to the inter-individual variability in xenobiotic pharmacokinetics and their importance cannot be understated.

The uptake of xenobiotics from systemic blood into tissues is an important step that facilitates their metabolism and excretion. Genetic polymorphisms in uptake transporters, most notably the organic anion transporting polypeptide family (OATP; SLCO), can have a significant impact on xenobiotic disposition. The OATP family of transporters is a large family of organic anion transporters with broad-substrate specificity. They are expressed ubiquitously in various tissues including the liver, kidney, and small intestine [28]. OATP substrates include bile acids, bilirubin, hormones, and drugs such as statins [28]. The OATP family is highly polymorphic and has been extensively reviewed elsewhere [32]. By far the most intensely studied class of drugs in respect to OATP polymorphisms are the statins. Several functional OATP1B1 (liver specific OATP) polymorphisms have been associated with altered statin disposition, primarily leading to increased statin exposure [32]. It must be noted, however, that although little information or concern is raised regarding OATP1B1 polymorphisms and statin response, caution is warranted with the association of some polymorphisms and statin-induced myopathy; a rare adverse event associated with statin therapy [32].

3. Xenobiotic Metabolism and Disposition in Disease: Beyond Genetics

As mentioned previously, inter-individual variability in drug response is not completely attributable to genetics. In fact, factors such as an individual’s diet and overall health status may be equally impactful and constitute a greater challenge to predict due to the vast cultural diversities and practices across human populations. In particular, the manifestation of disease is gaining an increased appreciation for its ability to alter normal physiological processes, such as drug metabolism and disposition. Specifically, diseases that manifest in

the liver as well as the kidneys are of particular clinical concern given their importance in mediating the metabolism and elimination of drugs and other xenobiotics. Moreover, given the common medical practice to treat diseases with pharmaceutical intervention, patients with disease that results in hepatic or renal impairment may have a significant impact on the efficacy and/or toxicity of xenobiotics. Several cases from the recent literature highlight the negative impact of renal and/or hepatic impairment on the pharmacokinetics of several drugs, leading to variable exposure levels that may affect drug toxicity [33–36]. More importantly, it is noteworthy to mention the fact that although clinical markers may be useful in predicting tissue function (creatinine as a measure for glomerular filtration rate, for example) they do not necessarily always predict the pharmacokinetic outcome of xenobiotics. For example, the elimination rate of cimetidine is 2.8 times higher by active renal secretion (transporter-mediated) than by glomerular filtration [37]. Therefore, it is important to further study the effects of altered tissue function on endogenous transport and metabolism mechanisms to better predict the pharmacokinetic outcome of numerous xenobiotics.

The following sections will review current knowledge of disease-mediated effects on drug disposition and toxicity. A special focus on liver diseases will be explored; however, an accumulating amount of evidence supports the notion of extra-hepatic compensatory mechanisms existing in distal sites such as the kidney, which may further influence the pharmacokinetic outcome of xenobiotics.

3.1 Drug Disposition Alterations in Cholestasis

Cholestasis is a condition in which there is decreased or impaired bile flow. The etiology is extensive and can vary from physical obstruction of the common bile duct (gallstones), termed extra-hepatic cholestasis, to congenital and autoimmune disorders such as primary biliary cirrhosis (intra-hepatic cholestasis). In addition, cholestasis can occur secondary to various liver disorders. The resulting reduction in bile flow leads to an accumulation of bile acids (BA) and other biliary constituents within the liver ultimately causing toxicity if not ameliorated.

In response to the increased intra-hepatic BA levels, several liver-specific adaptations occur which serve to further limit hepatic exposure to BAs. Repression of hepatic CYP7A1, for example, serves as an important defense mechanism that limits BA production during cholestatic liver diseases [38;39]. Decreased expression and function of several BA hepatic uptake transporters including the Na(+)-dependent taurocholate cotransporting polypeptide [40;41] and OATP family members [41–43] serve to limit further hepatic exposure by decreasing the entry of BAs into hepatocytes. Additionally, induction of hepatic efflux transporters during cholestasis and in particular, members of the multidrug-associated resistance proteins (MRP) such as MRP3 and MRP4, enhances the excretion of BAs from hepatocytes into circulating blood for renal elimination [39;42]. Together, the cumulative effects of these adaptive changes in membrane transporter regulation may limit hepatic exposure to drugs and other xenobiotics during cholestasis, but increase systemic and renal exposure.

Several investigations to date have exemplified the important implications of cholestatic liver disease on xenobiotic disposition. Methotrexate disposition has been found to be altered in rat models of intra- and extra-hepatic cholestasis. Specifically, biliary efflux of methotrexate was reduced with a concomitant increase in plasma methotrexate retention [44]. These observations were at least partially attributed to the respective disturbances in biliary and sinusoidal efflux transporters and may have clinical implications pertaining to the nephrotoxicity of this chemotherapeutic agent. More recently, Hasegawa *et al.* studied the impact of cholestasis on the disposition of morphine and its glucuronide metabolite, morphine-3-glucuronide (M3G), in a rat model of extra-hepatic cholestasis. The group showed that bile duct ligation in rats caused a significant increase in M3G exposure, presumably due to an induction of hepatic Mrp3 and Ugt2b1 protein expression, resulting in increased efflux of M3G into systemic circulation and morphine metabolism, respectively [45]. The clinical implications of these findings are quite profound. Hepatic glucuronidation of morphine in humans yields a secondary metabolite, M6G, which is not present in rodents and represents 15% of metabolized morphine in humans [45]. M6G is a more potent analgesic than morphine itself and is also a substrate for MRP3 transport [46]. Similarly to rodents, human MRP3 protein has also been found to be induced in patients with obstructive cholestasis [39], suggesting that M6G exposure after morphine administration may be elevated in a similar fashion. Due to the increased biochemical activity of M6G, increased exposure may lead to significant perturbations in pharmacological response and/or toxicity following morphine administration.

Cholestasis may also occur secondary to high estrogen exposure in susceptible pregnant women, as well as women taking replacement hormonal therapy or oral contraceptives. Jin *et al.* investigated the effects of ethynlyestradiol (EE)-induced cholestasis in rats, a common estrogen-induced model of cholestasis, on the disposition and pharmacodynamics of metformin. Interestingly, hepatic uptake of metformin was reduced in EE-induced cholestasis, which was attributed to a decrease in Oct1 protein expression [47]. Furthermore, the decrease in metformin uptake in the liver resulted in a decrease in metformin-stimulated glucose utilization. These results suggest that the functional disruption of metformin disposition results in a measurable decline in the therapeutic efficacy of metformin treatment. Therefore, patients relying on metformin pharmacotherapy in order to manage diabetes should be cautious while concomitantly taking estrogen-boosting therapy/ supplementation.

The mechanisms responsible for regulating membrane transporters in cholestatic diseases is not completely understood and are likely influenced by multiple factors. The development of cholestasis is associated with an increase in inflammatory markers [48]. In particular, the exposure of primary cultured hepatocytes to BAs increased the expression of various pro-inflammatory genes, including cytokines and chemokines, suggesting that direct exposure of hepatocytes to BAs is sufficient to induce an inflammatory response [49]. Moreover, the pro-inflammatory cytokines, TNFα, IL-1β, and IL-6, are up-regulated in cholestatic rodent models [50;51] whereas plasma TNFα levels were found to be increased in patients with obstructive cholestasis [39]. Interestingly, a study performed by Bohan *et al.* showed that MRP3 expression is induced in HepG2 liver cells following exposure to TNFα and IL-1β.

Specifically, the investigators determined that MRP3 induction is dependent on TNFα-dependent induction of the orphan nuclear receptor, fetoprotein transcription factor, and this response is abolished in TNF receptor-deficient mice [50]. Later studies confirmed these findings as well as implicated the activation of intra-cellular kinase cascades as a molecular mechanism for the TNFα-dependent induction of MRP3 [39].

Although these findings suggest a role for the cytokine-dependent regulation of xenobiotic transporters, regulation of membrane transporters in cholestatic liver disease is complex and may involve several mechanisms. Previous reports have demonstrated a role for the transcription factor nuclear factor-E2-related factor 2 (Nrf2) in cholestasis-mediated regulation of membrane transporters [52;53]. In particular, targeting Nrf2 via small molecule activation may provide a novel therapeutic target to help improve cholestatic liver disease pathology presumably through induction of membrane transporters, which help restore bile flow [54;55]. Other nuclear receptors that have been postulated to play a role in the regulation of membrane transporters in cholestatic liver disease include the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) [56].

In addition to disturbances in the expression and function of membrane transporters, several reports have shown alterations in the regulation of CYP enzymes in cholestatic disease. In a recent investigation using cholic acid treated mice, protein expression and enzymatic activity of several Cyp enzymes, including Cyp3a11, Cyp2b10, and 2C9, were found to be induced [57]. In a similar fashion, bile duct ligated rats demonstrated decreased hepatic Cyp1A1, Cyp1A2, Cyp2b1/2, Cyp2c11, Cyp3a1, and Cyp3a2 mRNA levels [58;59]. Moreover, decreased liver Cyp3a protein expression in an EE-induced rat model of cholestasis lead to a 21.9% decrease in the metabolism of doxorubicin [60]. Investigations into the effects of cholestatic liver diseases on CYP regulation in humans is limited; however, recent findings suggest no change in hepatic expression of CYP3A4 along with PXR and CAR in patients with primary biliary cirrhosis and biliary atresia [61;62]. Further investigations are needed, however, to elucidate the effects of cholestatic liver diseases on CYP function and the clinical consequences associated with them.

Adaptive changes in drug transporter expression and function in extra-hepatic sites such as the kidney in response to liver disease has been well documented. It is hypothesized that these adaptations function to facilitate the reduction of overall BA load by increasing BA secretion into urine in response to hepatic dysfunction. This, in turn, would aid in minimizing further hepatic exposure and toxicity. The mRNA expression of the uptake transporter Oatp1a1 in the kidney was reduced in a mouse model of extra-hepatic cholestasis whereas the efflux transporters Mrp1-5 were induced, suggesting a potential for increased urinary secretion of BAs [42]. Additionally, protein up-regulation of the renal organic anion transporter, Oat3, was induced in several rodent cholestatic models [63;64] along with up-regulation of Mrp3 and Mrp4 protein in the rat kidney [63]. An independent investigation also identified increased Mrp4 expression in the kidney following bile duct ligation in mice [65]. Moreover, induction of the organic cation transporter, Oct2, is induced in bile duct ligated rats, resulting in a functional increase in cimetidine renal secretion [66]. Disturbances in the renal secretion of p-aminohippurate and bromosulphopthalein in cholestatic rodent models was also found to be linked with membrane transporter expression

and function [67;68]. The mechanisms that govern these adaptive changes in renal drug transport expression in cholestasis is unknown and more information regarding compensatory changes that occur in humans with cholestatic liver disease is warranted for a better understanding of the translational effects these findings may have in the clinic.

3.2 Xenobiotic Disposition Alterations in Nonalcoholic Steatohepatitis

Nonalcoholic steatohepatitis (NASH) is a progressive form of liver disease associated with increased hepatic lipid deposition accompanied by oxidative stress, chronic liver inflammation, and varying degrees of fibrosis [69]. NASH encompasses a large set of liver pathologies collectively known as nonalcoholic fatty liver disease (NAFLD), which is estimated to affect up to 50% of the population, whereas the prevalence of NASH can be as high as 17% [70;71]. The manifestation of NASH is highly associated with metabolic disorders such as obesity and type-II diabetes. As a result of the liver damage sustained by increased oxidative stress and inflammation in NASH, global ADME gene expression profiles in the liver are altered in human NASH [72]. As a result, these perturbations may lead to disturbed xenobiotic disposition and toxicity in susceptible patients. This is especially relevant given the close association of NASH with obesity and diabetes; a population that is more likely to be treated with pharmacotherapy for complications arising from metabolic disorders such as hypertension and dyslipidemia.

Over the past several years, our understanding of how NASH alters drug disposition has grown quite considerably. The expression of xenobiotic membrane transporters in the liver is altered across several rodent models and humans with NASH. Specifically, the expression of the efflux transporters MRP3, MRP4 and MRP2 are induced, whereas uptake transporter expression (Oatp) appears to be down-regulated [73–77]. Interestingly, these observations are similar to what occur in cholestatic liver diseases and may represent a global adaptation in the liver to limit further exposure to potential toxicants in a stressed state. Investigation into the effects of NASH on xenobiotic disposition has revealed interesting findings. In a mouse model of NASH, acetaminophen and acetaminophen metabolite disposition was altered. Specifically, a shift from biliary elimination to plasma retention of acetaminophen glucuronide was observed, which was attributable to an up-regulation of the hepatic basolateral efflux transporters, Mrp3 and Mrp4[75]. These findings were later confirmed in a cohort of children with NAFLD that were found to have higher plasma levels of acetaminophen glucuronide following acetaminophen exposure [78]. Similar findings were noted with the cholesterol-uptake inhibitor, ezetimibe, where increased plasma levels and decreased biliary efflux of the ezetimibe glucuronide metabolite were observed in a rat model of NASH [79]. Interestingly, although expression of the canalicular transporter, Mrp2, increased, its trafficking to the canalicular membrane appeared to be selectively disturbed, resulting in decreased biliary efflux of ezetimibe glucuronide and possibly acetaminophen glucuronide [79]. This is of particular clinical concern since biliary efflux of ezetimibe glucuronide is required for drug delivery to the small intestine where it inhibits dietary cholesterol absorption. Therefore, inhibiting this function may impact its therapeutic efficacy. In addition, NASH disrupts the disposition of simvastatin [77], pravastatin [80], and the environmental contaminant arsenic [81]. Interestingly, a recent study investigating the combined effects of NASH and genetic polymorphisms suggests that a synergistic effect

may be present when NASH and a genetic dysfunction of xenobiotic transporters co-exist. Specifically, genetic loss of Oatp1b2 or NASH in mice alone, resulted in a modest increase in pravastatin exposure; however, the combination of Oatp1b2 genetic ablation and NASH resulted in a synergistic increase in pravastatin exposure, presumably to the NASH-dependent down-regulation of compensatory hepatic Oatp transporters [80].

In addition to membrane transporters, the expression and function of hepatic CYP enzymes is altered in NASH. Most of these studies were performed in rodent models and yield interesting, but conflicting, findings. Fisher *et al.* demonstrated an induction of Cyp3a11 in a mouse model of NASH, whereas a recent study using an obese guinea pig model shows Cyp3a4 expression and activity down-regulated [82;83]. In a more recent analysis using a novel rat high-fat fed diet model of NASH, total CYP content was found elevated along with reduced Cyp2c11 expression [84]. Similarly, in a separate study conducted in a high-fat diet model in mice, Cyp2a5 expression was induced, whereas Cyp2a5 induction was ablated in mice fed a high-fat diet lacking Nrf2 expression [85]. These results suggest that the activation of transcription factors are a potential mechanism regulating CYP expression in NASH. Interestingly, a study using a rat model of NASH showed that Cyp3a2 and Cyp2d2 expression was induced, which lead to an increased hepatic clearance of several cationic drugs as determined by *in situ* liver perfusion; however, the pathophysiologial changes as a result of the disease (fibrosis, fat deposition, etc.) resulted in a total decrease in the hepatic extraction ratio, suggesting that these physiological factors are also important when considering drug metabolism alterations in NASH. Investigations into human CYP regulation in NASH are limited. Fisher *et al.* conducted a thorough analysis describing a decrease in CYP1A2, 2D6, and 2E1 mRNA expression, whereas CYP2A6, 2B6, and 2C9 mRNA are induced. Moreover, it was also shown that the functional activity also decreased in CYP1A2 and 2C19 whereas microsomal activity increased for CYP2A6, and 2C9 [86]. These findings highlight the complex regulation of CYPs in NASH and a further understanding of these effects in clinical drug use is warranted.

Like cholestasis, the regulation of membrane transporters in NASH may be multi-faceted and may involve the activation of transcription factors and release of pro-inflammatory cytokines. Due to increased oxidative stress associated with NASH, Nrf2 is activated leading to the Nrf2-dependent up-regulation of downstream drug metabolizing genes including NQO1 and glutathione-s-transferase isoforms [87]. Nrf2 is known to regulate the transcription of several xenobiotic transporters, including Mrp2, Mrp3, and Mrp4 [88;89]. In addition, TNFα and IL-1β abundance increased in livers from NASH patients and may represent a mechanism for the observed alterations in membrane transporters and CYP enzymes [86].

Currently, knowledge regarding the effects of NASH on renal handling of xenobiotics is lacking. Increased urinary arsenic elimination was observed in NASH mice; however, the mechanism for this observation is unknown [81]. Conditions associated with NASH, such as type-II diabetes, demonstrate altered renal drug transporter expression, which may have implications in drug disposition. A study performed in leptin-deficient, diabetic, *ob/ob* mice, show nearly a 6-fold and 3-fold mRNA induction of Mrp4 and Mrp2, respectively, whereas renal uptake transporter expression is generally down-regulated [90]. Additionally,

streptozotocin-induced diabetic rats (type-I diabetic model) demonstrate that renal Oat3 expression is decreased, suggesting a potential disturbance in organic anion secretion in diabetic conditions [91]. Further understanding of the function of other renal Oat transporters in response to disease, such as Oat1, is warranted for a more complete understanding of organic anion secretion regulation in diabetes. Studies investigating the effects of diabetes on renal drug transporters in patients is currently lacking and necessitates exploration given not only the importance of the kidneys in xenobiotic disposition but also due to the high prevalence of diabetes and other metabolic disorders within the general population.

4. Conclusion

Xenobiotic, and in particular, drug disposition can be dramatically influenced by a gamut of factors that can range from inherent genetic polymorphisms in drug metabolizing enzymes and transporters to environmental and physiological influences such as disease and age. In addition, the interactions between genetic and environmental factors lead to a near unlimited set of phenotypes impacting xenobiotic disposition. To date, much of the clinical focus on factors impacting xenobiotic disposition has been on genetic polymorphisms. Indeed, polymorphic alleles do significantly affect drug disposition and examples of such can be found throughout the literature. However, it must be stressed that other factors, and in particular, disease states, also impact the metabolism and/or excretion of xenobiotics and two examples of liver-specific conditions, cholestasis and NASH, were reviewed herein in respect to their contribution to drug disposition. In conclusion, disease may cause global adaptive changes in gene/protein regulation across several physiological sites. In turn, these adaptive changes may cause coordinated differential responses in xenobiotic disposition that may ultimately affect xenobiotic toxicity and the occurrence of ADRs.

5. Expert Opinion

Information regarding liver disease and its contribution to altered ADME and adverse drug reactions is becoming more and more abundant. Interestingly, although the etiology of liver disease may differ, there appears to be a coordinated effect on the regulation of ADME genes, particularly membrane transporters, independent of disease manifestation. Specifically, examples of several cholestatic liver diseases and NASH highlight a coordinated response in which sinusoidal efflux transporters are up-regulated and hepatic uptake transporters are repressed. It is hypothesized that these responses may act as a hepato-protective mechanism by limiting further exposure of potentially toxic compounds in the liver during times of stress. As a result, however, these compensatory alterations in the liver lead to functional disturbances in xenobiotic disposition. Specifically, the cumulative adaptations in hepatic transporters lead to an overall increase in systemic exposure and decreased biliary elimination of drugs and other xenobiotics, which may have significant clinical implications in drug therapy. However, it must be stressed that the effect of these perturbations on clinical drug toxicity and/or efficacy is still lacking and warrants further investigation.

When considering the impact of liver disease on drug pharmacokinetics, it may be tempting to make global predictions on the pharmacokinetic fate of drugs in diseased states based on known scientific data pertaining to the drug/xenobiotic's disposition. However, two specific challenges arise when attempting this approach and further research is warranted to clarify these concerns. First, with the rising practice of 'polypharmacy', it is possible that the increased systemic exposure of a particular drug in a patient with liver dysfunction may increase the likelihood of drug-drug interactions while taking multiple medications concomitantly. This is especially concerning when considering the whole gamut of environmental contaminants (arsenic, pesticides, air pollutants, for example) that an individual may be exposed to concomitant to receiving pharmacotherapy. Second, the secondary effects of hepatic dysfunction on extra-hepatic elimination mechanisms complicate PK predictions in liver disease and potentially increase the likelihood of developing ADRs.

The concept of 'extra-hepatic' regulation during times of hepatic stress has been exemplified by the occurrence of kidney-specific alterations in the expression of membrane transporters in diseases such as cholestatic liver disease. It is hypothesized that these changes in renal transporter expression are a result of adaptations to the increased burden of drugs and other xenobiotics the kidney is faced with during times of hepatic stress. Nonetheless, these findings have important implications in further elucidating the effects of liver disease on xenobiotic PK and toxicity. First, pharmacokinetic disturbances observed in states of hepatic dysfunction cannot be solely attributable to liver-specific alterations but rather the combined effects of liver and kidney dysfunction. This allows for further complexity in not only predicting the occurrence of altered PK in liver disease but in investigating the relative contributions of renal and hepatic disposition independently. Second, and arguably most important, further consideration and investigation into the potential for increased xenobiotic-induced renal toxicity in liver disease is needed. Interestingly, several studies have linked an association of chronic renal failure with liver diseases. Further identification of the causal nature of liver and kidney diseases is still ongoing; however, these observations suggest a link between the occurrence of liver and kidney disease and may provide potential mechanistic insights into how these two tissues coordinate gene expression and physiological function.

A major limitation to these studies is the extrapolation to the human disease. The lack of well cataloged samples of human kidney tissue from patients with well characterized staging of liver disease creates uncertainty in our ability to predict the functional outcome in human patients. In addition, our understanding of the molecular mechanisms responsible for driving these physiological adaptations is limited; however, factors such as increased oxidative stress, and the induction of pro-inflammatory cytokines are being investigated as mediators of kidney transporter regulation in liver disease. Nonetheless, further research is needed to not only identify a mechanism, but to investigate the impact of these extra-hepatic effects on xenobiotic disposition. In particular, further identification of these effects in humans afflicted with liver disease is needed to assess the overall clinical impact.

Acknowledgments

This work was supported by National Institutes of Health grants [AI083927], [ES006694], [HD062489], National Institute of Environmental Health Science Toxicology Training Grant [ES007091], and The National Science Foundation of Arizona.

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