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Invited Review

Systems pharmacology modeling: an approach to improving drug safety†

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ABSTRACT: Advances in systems biology in conjunction with the expansion in knowledge of drug effects and diseases present an unprecedented opportunity to extend traditional pharmacokinetic and pharmacodynamic modeling/analysis to conduct systems pharmacology modeling. Many drugs that cause liver injury and myopathies have been studied extensively. Mitochondrion-centric systems pharmacology modeling is important since drug toxicity across a large number of pharmacological classes converges to mitochondrial injury and death. Approaches to systems pharmacology modeling of drug effects need to consider drug exposure, organelle and cellular phenotypes across all key cell types of human organs, organ-specific clinical biomarkers/phenotypes, gene–drug interaction and immune responses. Systems modeling approaches, that leverage the knowledge base constructed from curating a selected list of drugs across a wide range of pharmacological classes, will provide a critically needed blueprint for making informed decisions to reduce the rate of attrition for drugs in development and increase the number of drugs with an acceptable benefit/risk ratio. Copyright © 2013 John Wiley & Sons, Ltd.

Key words: systems biology; molecular pathways; molecular networks; mitochondria; gene drug interaction; biomarkers/clinical phenotype; organ injury; cellular phenotype

Introduction

In addition to the designed on-target actions, medications can inadvertently cause adverse drug reactions (ADRs) through (1) on-target effects in off-target organs and (2) off-target effects mediated through complex biological pathways/networks. Off-targets include transcription factors, enzymes, receptors, RNAs and DNAs. Since multiple organs are simultaneously exposed to a drug following administration, one challenge is to better understand why a drug can be more toxic or even sometimes cause irreversible injury to a specific cell type and leave others unaffected [1]. This gap is expected to be bridged as sciences advance in the areas of imaging mass spectrometry [2], panels of organ-specific blood biomarkers [3], as well as pharmacokinetic modeling of absorption, distribution, metabolism and elimination (ADME) of drugs. Predicting rare ADRs that occur in only 1 in 1000 to 1 in 1000000

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users through traditional preclinical studies has proven very challenging. Advanced technologies now enable simultaneous multiplex measurements of transcriptomic, proteomic and imaging readouts, as well as measurements of organelle and cellular phenotypes [4]. Using these technologies to reverse engineer the mechanisms for failure of drugs in clinical trials may produce a useful knowledge base wherein the respective molecular pathways leading to specific organelle/cellular phenotypes can be matched to a particular clinical phenotype.

In the era of informatics-accelerated health care sciences, it is hoped that integration of systems pharmacology-based modeling into drug development could turn this notion into reality. Such efforts will hopefully minimize drug attrition throughout the life cycle of drug development. Systems approaches to companion diagnostics for more personalized medicine will also hopefully help more precisely to target drugs to the subpopulations that are likely to experience the greatest clinical benefit and the lowest rate of ADRs [3]. This review paper will focus on understanding and assessing drug effects from a systems pharmacology perspective with the hope of providing a reference framework for efforts on improving drug safety.

Systems Pharmacology Network of Drug Linked to Clinical Phenotype/Biomarker

A drug can cause both on-target and off-target perturbations at transcriptional [5], translational [6] and post-translational levels – inciting its toxicological networks in off-target organs (extended pharmacological networks) [7] across the body and inadvertently causing clinical presentations of ADRs. From the knowledge accumulated with both approved and withdrawn drugs, these networks are associated with the clinical phenotypes and biomarkers manifested during therapy.

Systems pharmacology modeling has been used for rare ADRs such as hypomagnesemia encountered with chronic use of proton pump inhibitors (PPIs) [1,8]. Magnesium homeostasis is maintained by intestinal absorption and renal excretion. Clinical biomarkers of PPI-induced hypomagnesemia include the elevation of serum Mg^{2+} concentration and a decrease of 24 h urinary secretion of Mg^{+} respectively, following de-challenge and re-challenge with a PPI [8]. With these two markers as the guide, an absorption-centric model was constructed to simulate the adverse impact of PPIs on oral absorption and serum Mg^{2+} concentration [9]. In this model, the reported off-target effect of PPIs leading to reduced intestinal pH values in human subjects was referenced [10], along with a corresponding reduction in the availability of the TRPM6 binding sites for Mg^{2+} as a result of intestinal pH reduction, and Mg^{2+} solubility in various intestinal pH values. Such absorption-centric modeling reflected how a minute daily reduction in oral absorption (1% reduction) could lead to a substantial reduction in the total body Mg^{2+} stores with resultant hypomagnesemia, following PPI use for 1 year or longer. Both the intestinal and renal reabsorption of magnesium involve paracellular absorption facilitated by claudins [11], and active transport mediated predominantly by transient receptor potential melastin 6 (*TRPM 6*) [12]. The serum Mg^{2+} concentration and urinary secretion of Mg^{2+} were associated with *TRPM6* [12] and *claudin 16* [13] expression levels. Both serum concentration and urinary secretion of Mg^{2+} increased and reduced, respectively, in response to dietary load and restriction and were correlated with down-regulation and up-regulation of both *TRPM6* and *claudin 16* mRNAs. Such a regulated, synchronized interaction between clinical biomarkers and organ transport proteins (markers) in the intestine and kidneys is an ideal case for an integrative systems pharmacology modeling, where incorporation of mechanistic renal elimination into the current absorption-centric model to reflect, at the system level, how these two organs work in concert to maintain magnesium homeostasis.

A quite comprehensive systems pharmacology model for predicting drug-induced liver injury is DILIsym™ published by Howell *et al*. [14]. This multi-scale model includes (1) biology systems at the molecular (drug ADME processes, GSH dynamics, bile salt homeostasis), organelle (mitochondria), cellular (energy balance and life cycle) and system (clinical biomarkers) levels; (2) mechanistic output of oxidative stress and GSH dynamics, ATP dynamics, drug and bile salt concentrations, liver mass dynamics; (3) clinical output of drug, GSH and bile concentrations in blood, plasma concentrations of ALT, AST, bilirubin, keratin 18,

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HMGB1, and prothrombin time and INR. The model focuses on hepatic GSH depletion, mitochondrial injury caused by reactive metabolites, adverse impact on hepatic cell cycle and the release of biomarkers into the blood. Detailed data at various levels of this multi-scale model including metabolism parameters of V_{max} and K_m and blood and serum markers were curated and compiled from various sources including the literature. Such a comprehensive model is useful for the continuous improvement on the level of sophistication to increase the prediction power and for simulation at the population level if genetic mutation can be integrated.

Systems of molecular pathways and networks are intertwined with one another via coordination and regulated cross-talk, from cells to organs/tissues to the body, to maintain the homeostatic integrity of the human body. Take the mitochondrion for instance, its physiological homeostasis is maintained and regulated by its own genome [15] as well as nuclear genes [16], although its physical structure appears to be isolated from the nucleus or other organelles. These complex microscopic interactions could be determined by temporal transcriptomic analysis to reveal the molecular pathways involved. For example, statins currently on the market cause treatment-related myopathy and rhabdomyolysis in rare cases [1]. Perturbation of eicosanoid synthesis and phospholipase C pathways by high dose statins was observed in patients' skeletal muscles, and was linked to changes in patients' plasma lipidemic profiles [17]. Integration of systems biology and genomics has gradually advanced our understanding of the complexity of cellular networks in relation to human diseases [18], especially genetic disorders [19]. A case–control study of 188 age- and gender-matched coronary heart disease (CHD) patients and 188 healthy subjects identified multi-tissue drivers (Spi-B transcription factor, *SPIB* and tumor necrosis factor receptor superfamily, member 13C, *TNFRSF13C*) as well as tissue-specific drivers (early B-cell factor 1, *EBF1*) that were associated with the risk for developing CHD. These driver genes were derived using co-expression network analysis as well as leveraging the previously published gene networks and protein–protein interaction. These reports illustrate that the pathway signatures underlying the effect of drug could be associated statistically with relevant plasma and/or urinary biomarkers. So could the gene signatures be found for a clinically defined disease phenotype via systems pharmacology analysis.

There are several publicly accessible transcriptomic databases [20,21] available for hypothesis-generating analytics of the extended pharmacological network, and for delineating the pathways perturbed by a drug within a specific cellular context. Post-translational modifications, however, further increase the complexity of biological networks throughout the life cycle of the cell. A protein can exist in more than one isoform *in vivo*, with one particular isoform expressed in a specific organ. Ryanodine receptor 2 (*Ryr2*), for example, is preferentially expressed in cardiac myocytes and was reportedly the off-target of daunorubicin cardiotoxicity [22], whereas *Ryr3*, a skeletal muscle isoform, was linked to statin-induced myopathy [23] and up-regulated in cerivastatin-induced rhabdomyolysis [24]. A precise mapping of the specific protein isoform(s) in each organ is critically important for defining the multi-organ or organ-specific systems pharmacology network of a drug for proper linkage to respective biomarkers.

Framework for Systems Pharmacology Modeling

The accumulated knowledge and data from approved [1] and withdrawn drugs may prove useful for bridging the gap between preclinical and clinical outcomes for new drug candidates. One can leverage the publicly accessible, curated databases and knowledge-bases of pathways [25,26], cellular transcriptomic signatures [20], chemogenomic signatures of FDA-approved drugs and other chemical entities on a genome-wide scale in several cell lines [20], gene–drug interactions [1,27] and gene–disease association [19] studies to conduct integrated systems pharmacology modeling [7]. The caveat, though, is that inconsistency exists among pathway and interactome databases [26]. Hence, one would need to apply prior knowledge when applying these databases for hypothesis generation. Multiplex measurements of transcriptomic, imaging and cellular phenotypic readouts are available to depict the beneficial or toxic effect of a drug from cellular pathways to organelles to cells in a compendium of immortalized cell lines, primary cell

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types, human pluripotent stem cells and induced pluripotent stem cells. In a systems-pharmacology model, the cellular pathways perturbed by a drug and its treatment-related benefits (on-target) or adverse effects (off-target) can conceivably be bridged by an array of molecular signatures, organelle phenotypes, cellular phenotypes and clinical biomarkers. With proper experimental set-ups, advances in next generation sequencing of clinical samples [28] and imaging of cellular phenotypes/biomarkers [29] are expected to provide the much needed data to bridge the preclinical–clinical gap for systems pharmacology modeling. Bordbar *et al*. studied intercellular interaction by leveraging a genome-scale metabolic network for adipocytes, hepatocytes and myocytes each [30]. A blood compartment was added to connect the three tissues to form a multi-tissue model, and was used to understand perturbation of metabolic activity in the context of obesity. With the metabolomic results accumulated in the literature, modeling metabolic perturbation using a genome-scale metabolic network will facilitate our understanding of diseases and the prediction of drug toxicity.

Following administration, a drug and its major metabolites will interact with multiple levels throughout the hierarchical structure of a human body, from molecular networks to cells and to organs, as illustrated in Figure 1. At the microscopic cell and pathway levels, a drug can perturb biochemical and physiological homeostasis, leading to clinical manifestations of its treatment-associated adverse drug reactions, including symptoms, and serum as well as urinary biomarkers. There could be similar intracellular perturbations caused by a drug across multiple organs due to the same toxic mechanisms, resulting in an array of adverse drug reactions associated with individual organs or systems. There also could be different intracellular perturbations due to different toxic mechanisms as a result of specific protein isoforms in some individual organs. For any statistical linkage with the observed clinical phenotypes in the form of symptoms and/or measureable biomarkers, the statistical rigor imposed on systems pharmacology-based modeling and linkage would reach the highest, as compared with other linkages between any two lower levels in the human body hierarchy.

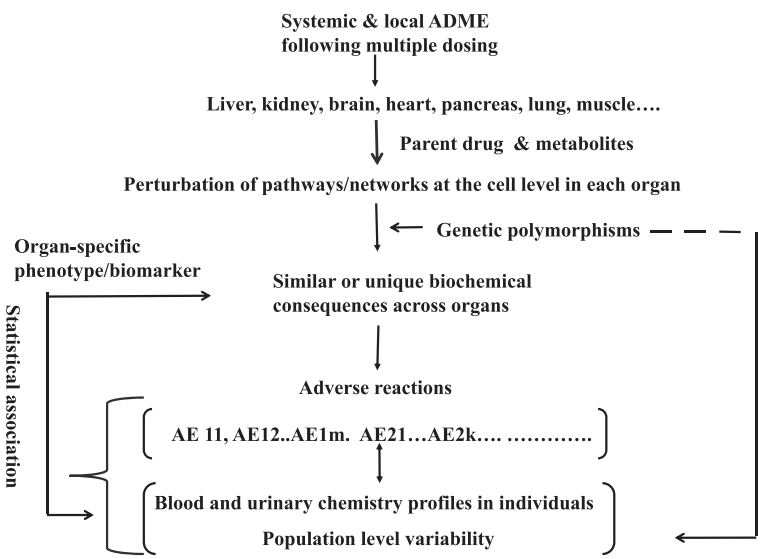

Figure 1. Following administration, a drug can cause treatment-related adverse reactions that are detected by systemic phenotypes and biomarkers. In systems pharmacology analysis/modeling, a multi-scale approach is needed where a drug and its major metabolites produced in the liver are circulated to multiple organs depending on their unique ADME profiles, and cause perturbation of physiological and biochemical homeostasis of the cell that is genetically unique resulting from inherited genetic polymorphisms. The drug and/or its major metabolites can cause similar biochemical perturbations across multiple organs or unique biochemical perturbations in some organs, which are manifested in a range of clinical phenotypes of mild or severe symptoms associated with measurable blood and urinary biomarkers. A highest statistical rigor is needed to link each organ phenotype along with relevant biomarkers at the systemic level to a specific biochemical consequence resulting from perturbation of an intracellular network

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Systems pharmacology-based prediction and assessment can leverage accumulated pharmacokinetic and physiologically based pharmacokinetic modeling technologies, curated data and knowledge for a multi-scale modeling from pathway to clinical phenotype and biomarker.

Associating Clinical Phenotype of Organ Toxicity with Cellular/Organelle Phenotype

Drug-induced mitochondrial hepatotoxicity

The cell consists of organelles that communicate with one another to form a tightly controlled community; intracellular organelles include mitochondria, endosomes, lysosomes, ribosomes, nucleus and golgi apparatus. Mitochondria are the powerhouse of the cell, and the number of mitochondria depends on the cell type and its energy requirement. Mitochondria are the most abundant organelles in hepatocytes accounting for 20% of the hepatocyte volume. An individual hepatocyte contains nearly 1000 individual mitochondria whose dominant role is oxidative phosphorylation and maintaining energy balance [31]. The intracellular ATP generated by mitochondria is essential for various cellular functions including bile secretion, gluconeogenesis, protein synthesis and ureagenesis. When hepatic mitochondrial function is impaired clinical manifestations may include lactic acidosis, hypoglycemia, hypoprothrombinemia and hyperammonemia. Drugs with known or suspected mitochondrial mechanisms of hepatotoxicity are shown in Table 1. ATP depletion can lead to liver cell necrosis or apoptosis via the release of cytochrome c into the hepatocyte cytosol. With rapid inhibition of the respiratory chain, there may be inadequate time for hepatic steatosis to develop as is seen with acetaminophen hepatotoxicity. Acetaminophen can cause mitochondrial permeability transition (MPT). Opening of the permeability transition pores is an irreversible devastating event, leading to mitochondrial depolarization and cell death. The toxicological network underlying acetaminophen hepatoxicity includes inhibition of pro-survival pathways of NF-kappaB and activation of tumor necrosis factor, causing MPT, mitochondrial injury, loss of cell integrity, apoptosis or necrosis.

Other drugs disrupt the mitochondrial function by inhibiting mitochondrial DNA transcription or replication as is seen with the dideoxynucleoside analogues. Normally, DNA polymerases do not allow

Table 1. Hepatotoxic drugs associated with mitochondrial injury

Drug (ref)	Phenotype	Clinical presentation
Tetracyline [35]	Microvesicular steatosis with minimal inflammation	High doses given i.v. or i.m. for 3 to 10 days in late pregnancy, early post-partum and renal failure patients
Aspirin [80]	Reye's syndrome; microvesicular steatosis and absence of glycogen on biopsy; frequently fatal with cerebral edema, minimal bilirubin elevation	Antecedent viral illness in children such as influenza or varicella with fever and anorexia
Valproate [33,81]	Microvesicular steatosis with necrosis; progressive fibrosis with cholestasis seen in some patients	More common in infants and individuals with inherited mitochondrial disorders; frequently within 1 to 3 months of drug initiation
Fialuridine [82]	Direct inhibition of mitochondrial DNA replication	Presented after 2 months of daily use in chronic HBV patients
Stavudine (d4T) [36,83]	Microvesicular (early) or macrovesicular (later) steatosis with minimal inflammation; rapid progression to liver failure	2 to 5 months after initiation in HIV+; can also present with cirrhosis/non-cirrhotic portal HTN; risk factors include pre-existing liver disease, female gender, obesity and alcohol
Didanosine (ddI) [38,84]	Microvesicular steatosis with minimal inflammation; may also cause acute hepatocellular necrosis with jaundice	Risk factors include pre-existing liver disease, obesity and alcohol; may present as non-cirrhotic portal HTN
Linezolid [85]	Inhibits mitochondrial protein synthesis and can decrease protein function; lactic acidosis with neuropathy	Antibiotic to treat resistant Gram-positive cocci
Tamoxifen [37]	Concentrated in mitochondrial matrix and inhibits mitochondrial ß-oxidation and respiration. Macrovesicular steatosis, steatohepatitis, and cirrhosis; acute liver injury very infrequent	Anti-estrogenic used as adjunctive therapy for breast cancer; up to 30% develop NAFLD/ NASH with prolonged treatment

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these drugs to be incorporated into nuclear DNA. However, the DNA polymerase gamma (γ) does not prevent these drugs from being incorporated into mitochondrial DNA and over time these chain terminators can lead to a depletion of mtDNA. Zalcitabine and lamivudine inhibit mitochondrial DNA polymerase gamma activity, and consequently reduce mitochondrial DNA mass [32]. These drugs also cause peripheral neuropathy, lipodystrophy, myopathy and lactic acidosis [1]. For unclear reasons, the nucleoside analogues have differential effects on mtDNA replication with zalcitabine (ddC), didanosine (DDI) and stavudine (d4T) being more likely to lead to mtDNA depletion than zidovudine (AZT), lamivudine (3TC) and abacavir (ABC) [32]. Individuals with inherited or acquired mitochondrial defects may be more susceptible to liver injury from certain drugs as has been reported with valproate hepatotoxicity [33]. In addition, malnutrition and certain disease states including viral infections may lead to circumstances that increase the risk of drug-induced mitochondrial toxicity.

Severe impairment of fatty acid oxidation leads to hepatic steatosis when accumulated free fatty acids that are taken up or synthesized by the liver become esterified into triglycerides in the hepatocyte cytoplasm. Acute impairment of fatty-acid ß-oxidation leads to the formation of microvesicular steatosis wherein numerous tiny lipid vesicles are formed around the nucleus and have a 'foamy' appearance. In contrast, when ß-oxidation impairment is more chronic, the small vesicles can coalesce into larger fat droplets that then become eccentric in the hepatocyte. Therefore, incorporation of cellular toxicity kinetics would facilitate informed systems pharmacology modeling. The choice of an appropriate cell model will, however, be very critical if a specific biochemical or molecular pathway is to be properly linked to specific cellular and clinical phenotypes. The HepG1 and HuH7 cell lines, for example, have low capacity to oxidize long-chain fatty acids and will not be suitable to study how stavudine and valproic acid cause intracellular lipid accumulations leading to fatty liver [34].

Clinical phenotypes

Acute and severe impairment of mitochondrial function can lead to acute fatty liver with lactic acidosis which is a distinctive and frequently dramatic clinical syndrome associated with a high rate of morbidity and mortality if not promptly recognized and treated. The hallmark of this syndrome is small droplets of fat (microvesicular) that accumulate in the hepatocyte cytosol from impaired ß-oxidation of fatty acids as reported with tetracycline and valproic acid [33,35]. In contrast, drugs, toxins and disease states that lead to partial but chronic depletion of mitochondrial function can lead to the accumulation of large fat droplets (macrovesicular) that are often eccentrically located in the cell. Drugs associated with the latter phenotype include tamoxifen and the dideoxynucleoside analogues used to treat HIV infection [36–38].

Clinical features at presentation

Patients with acute fatty liver typically have a systemic lactic acidosis due to impaired mitochondrial function as well as stupor, coma, encephalopathy, hyperammonemia and coagulopathy. Drugs that directly inhibit mitochondrial function or protein synthesis typically present within 1 month of drug initiation. Initial clinical symptoms are frequently non-specific and vague such as nausea, anorexia and abdominal pain and typically proceed the liver injury by 1 to 4 weeks. With lactic acidosis, some patients may present with shortness of breath, muscle weakness or impaired mental status. Jaundice is rarely present at drug-induced liver injury (DILI) onset and is usually mild. Serum aminotransferase concentrations are usually only minimally elevated (< 5 to $10 \times$ ULN) at DILI onset, despite the presence of concomitant hypoalbuminemia, hypoprothrombinemia, hyperammonemia and hypoglycemia. Because the mitochondrial dysfunction is usually not isolated to the liver, many patients may have myopathy, neuropathy or pancreatitis prior to or after the liver injury onset. Patients with sub-acute or chronic drug-induced mitochondrial liver injury are more likely to present with mild to moderate transaminase elevations over many months without prominent lactic acidosis, hypoprothrombinemia or jaundice. Clinical symptoms are also likely to be vague and non-specific. As a result, a liver biopsy may be needed to establish a diagnosis wherein mixed or macrovesicular steatosis with variable degrees of inflammation, cholestasis and fibrosis may be

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seen. The major alternative disease processes to consider in patients with either an acute or chronic drug-induced mitochondrial hepatotoxicity syndrome are alcoholic and non-alcoholic fatty liver disease or sudden weight gain due to corticosteroid use. So, careful assessment and ascertainment of drug-induced liver toxicity is as important for systems pharmacology modeling as the quality of network and pathway data.

Other drug-induced organ toxicities

Valproic acid can also cause intracellular accumulation of lipid droplets in skeletal muscles that may result in rhabdomyolysis [39,40]. Cyclosporine, an immunosuppressant used in organ transplant recipients, is nephrotoxic, and reportedly causes autophagic vacuoles in rat kidney cells [41]. Cyclosporine therapy increases BUN and serum creatine concentrations, causes renal tubular structural damage and globally reduces renal function in patients [1]. Statins are associated with a functional decline and muscle cramping in patients with amyotrophic lateral sclerosis (ALS) [42]. Fuvastatin depletes spinal cord motor neurons and causes the degeneration of neurons [43]. This observation is in agreement with that observed in ALS patients on statins, although further studies are needed to establish the mechanistic linkage. In patients who developed hyperlactatemia during treatment with linezolid, decreased mitochondria mass and protein content were observed in their peripheral blood mononuclear cells, although mitochondrial membrane potential or intact cell oxidative capacity did not change significantly [44]. Mitochondrial permeability transition (MPT) has also been associated with cardiotoxicity of doxorubicin [45] and renal toxicity of non-steroid anti-inflammatory drugs [46]. Cerivastatin was withdrawn from the market due to its treatment-related rhabdomyolysis, kidney failure and deaths [47]. Subsequently, various statins were shown to reduce the mitochondrial ATP concentration in myoblasts [4]. Elevated plasma creatine phosphokinase (CPK) levels were observed in patients who experience myopathy or myalgia or rhabdomyolysis with the highest elevation in patients with statin-induced rhabdomyolysis [1]. Apparently, statin induced mitochondrial toxicity was felt to be responsible for these adverse muscle reactions.

Summary

Cellular phenotypes can be useful pharmacodynamic endpoints for bridging cell/organ and clinical phenotypes. A mixed array of common and different molecular events can lead to mitochondrial toxicity, causing irreversible MPT, cell death and devastating injury to various organs [45,46,48]. A library of drugs that can cause mitochondrial toxicity, along with their individual molecular signatures in relevant cell lines/tissues and clinical biomarkers, would be highly useful for modeling and establishing a predictive model for developing safer new drugs.

Consideration of Immune Surveillance

The innate immune system plays a key role in drug-induced organ injury. Accumulated evidence has incriminated toll-like receptor 4 in acetaminophen-induced acute liver and lung injury [49]. Damaged mitochondria are usually digested in the lysosomal-autophagy system. Lysosomes can become overwhelmed, when extensive mitochondrial damage occurs, resulting in an accumulation of mitochondrial DNAs that could eventually escape lysosomal digestion. Mitochondrial DNAs that escape digestion by lysosome-autophagy processing reportedly trigger toll-like receptor 9-mediated inflammatory responses in mouse cardiomyocytes, resulting in myocarditis and cardiomyopathy [50]. Transforming growth factor-β controls mitochondrial metabolism via receptors on the plasma membrane; its signaling pathways are involved in renal and liver diseases [51] and play a role in chemical-induced toxicity [52]. Human macrophage-lung epithelial cell co-cultures were used for evaluating the effects of environmental toxins [53], and such a co-culture system could conceivably be useful for evaluating drug toxicity. In short, the cross-talk among circulating immune cells, their secreted cytokines and individual organ resident cells contribute to clinical symptoms and biomarkers in the context of drug toxicity. Consideration of immune participation in modeling drug safety is important, and such modeling could be greatly aided by curated pathways reflective of immune participation (Figure 2), which can be readily adopted [25], if confirmed to be adequate, and incorporated into the model.

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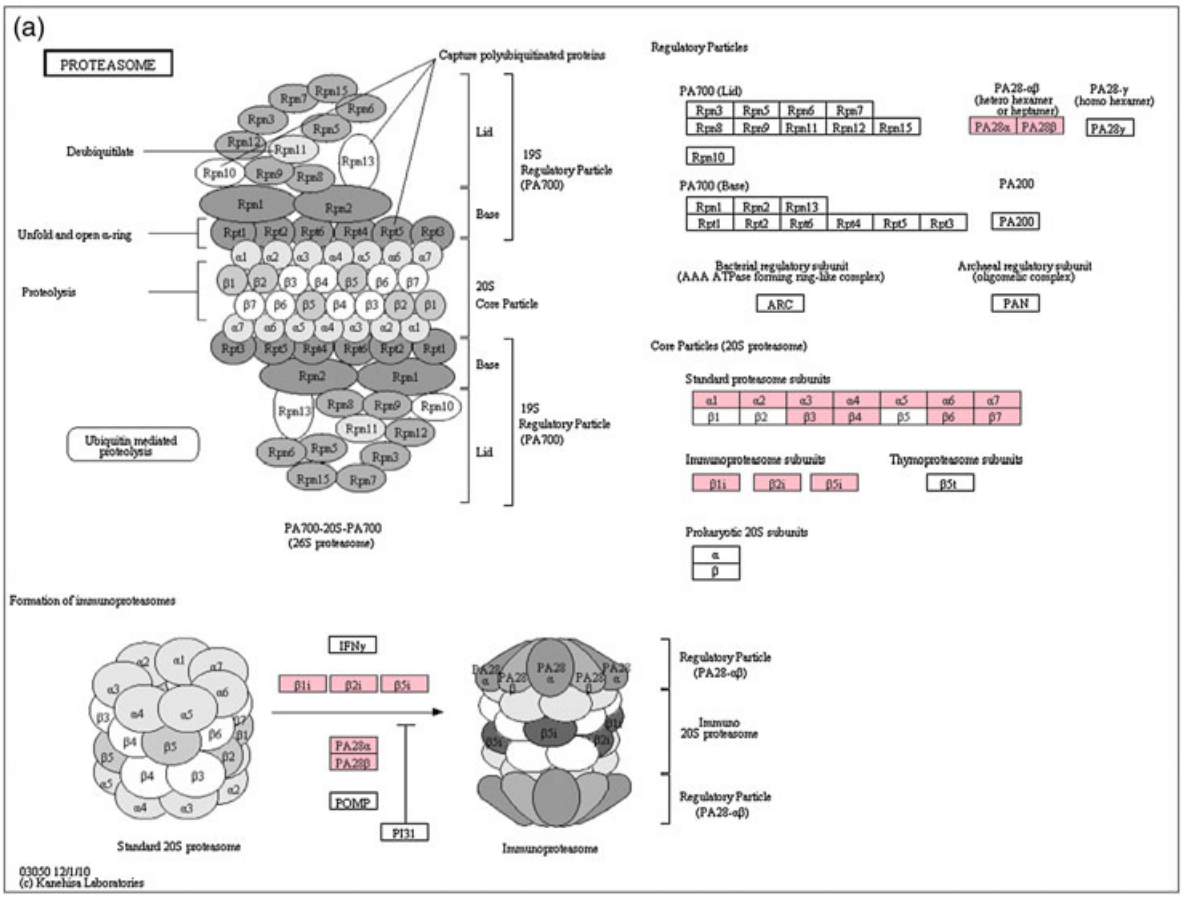

Figure 2. Participation of immune components should be included when modeling or analysing drug toxicity. There are curated immune pathways available for reference. In the process of immune responses to abnormal proteins generated due to a drug's toxicity, proteasomes (a) are involved in degrading abnormal proteins and generating antigenic peptides. The antigenic peptides can cause a cascade of immune responses through toll-like receptor signaling pathway (b) and/or (c) cytokine signaling pathway. Pathways adopted from http://www.genome.jp/kegg/kegg1.htm

Consideration of Genetic Polymorphisms in Perturbed Biochemical Networks

Genetic polymorphisms of ADME enzymes and transporters [27] contribute to the extent of variability in exposure–response relationship of drug and to drug–drug interaction [1]. There are ethnicity-related differences in drug exposure and responses resulting from ADME enzymes and transporters, as well as in HLA allele related adverse reactions [27,54]. Somatic mutations and epigenetic changes could further alter an individual's phenotypic characteristics and result in diseases [55]. Epigenetic mutations could conceivably contribute to inter-subject variability in the efficacy and safety of a drug; however, their impact is not well understood. Mutations that result in partially reduced activities of multiple components in the pathways involved in intracellular physiological homeostasis could lead to the occurrence of disease [56]. This notion could be applied to understanding the effect of drug action since a drug can inadvertently jeopardize the functions of genetically impaired proteins in patients who carry those mutations. The networks of genes and proteins involved in individual genetic diseases with similar clinical phenotypes appear to be associated among one another [16], further expanding the universe of specific disease-related genes [19] and proteins [57].

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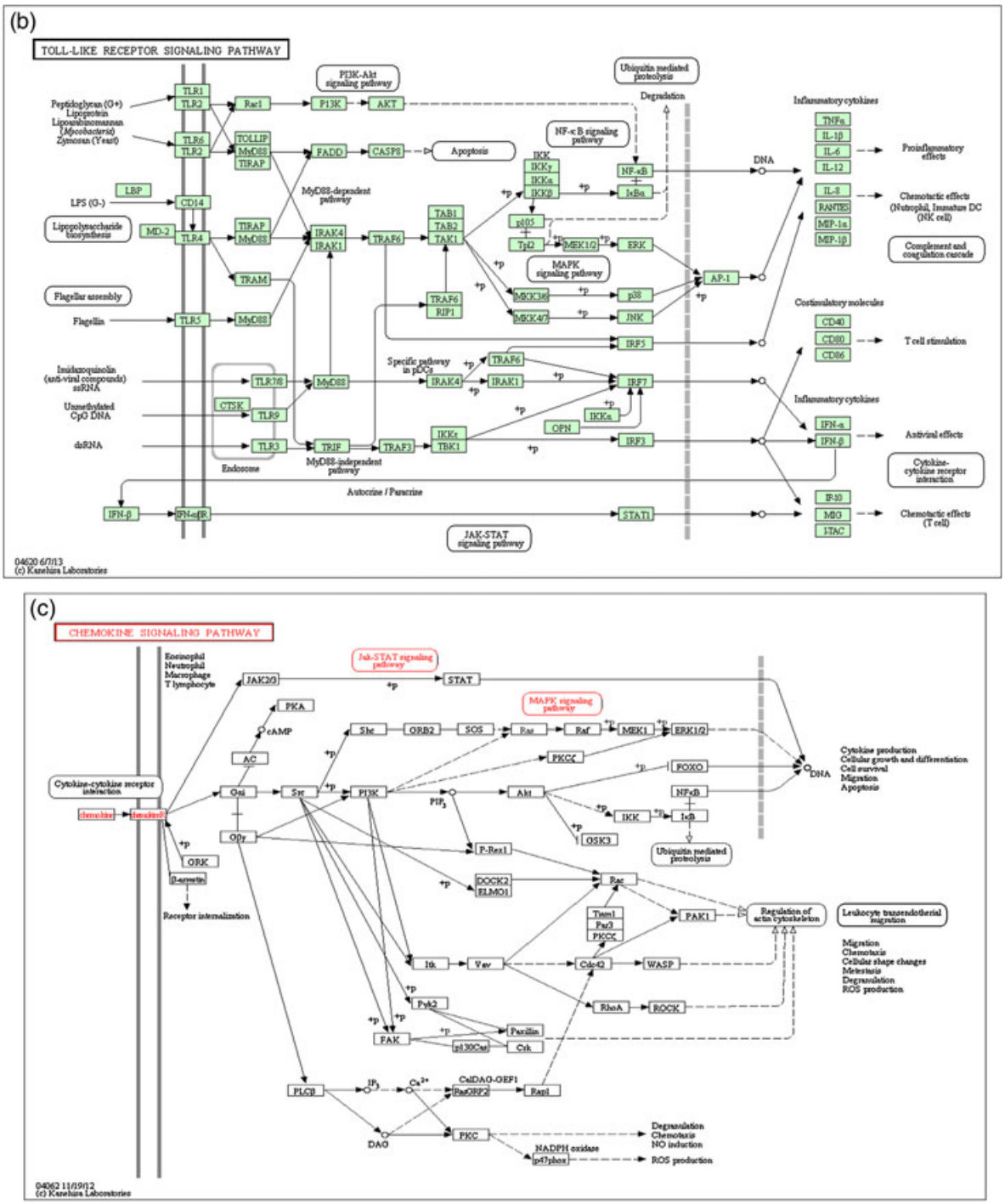

Figure 2. Continued

The clinical phenotypes associated with a specific drug toxicity could be similar, to a varying degree, to those observed in a genetic disorder [1,19,58,59]. Genetic disorders of McArdle's disease and Duchenne's muscular dystrophy can lead to rhabdomyolysis and cause patients to suffer from tea-colored

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urine and eventually renal failure. Drug-induced rhabdomyolysis can also lead to these clinical manifestations [60]. Drug treatment could exacerbate the symptoms of a certain genetic disorder or cause a silent genetic mutation to manifest clinically [61]. One such gene–drug interaction leading to drug-induced rhabdomyolysis is valproic acid and carnitine palmitoyltransferase 2 (*CPT II*) [61]. Determination of CPT II deficiency should be considered in patients who need to take drugs that would affect the biochemical network involving CPT II to prevent drug-induced myopathy and rhabdomyolysis [62]. Pathway, network and cellular perturbations caused by a drug and by a loss of function mutation of a gene could be similar, as could their respective clinical symptoms and circulating biomarkers [1,19,58]. Therefore, leveraging the known wealth of genetic polymorphisms [59] could potentially guide mapping of individual systems pharmacology networks of drug [7] to respective safety biomarkers, as shown in Figure 3. Incorporation of mutations in relevant biochemical pathway genes would increase the predictive power of systems pharmacology modeling with respect to the efficacy/risk ratio of a drug.

Mitochondrial Toxicity-Centric Modeling

Mitochondria are the epicenter of drug-induced organ toxicity. Different mitochondrial toxicity mechanisms could lead to distinct clinical adverse phenotypes in the same organ [40,48,63], while a similar mitochondrial toxicity seems to cause a similar organ toxicity [1,34,64]. Relationships exist between cellular toxicity and clinical adverse phenotypes, such as inhibition of mitochondrial fatty acid oxidation leading to intracellular accumulation of lipid droplets in hepatocytes and fatty liver [1,34,64].

Reactive oxygen species generated by damaged mitochondria is one particularly interesting area in drug toxicity, and one that is amenable to quantitative mechanistic modeling [65]. During normal ATP production through oxidative phosphorylation, 0.4–4% of all consumed oxygen is converted to superoxide radical [O_2^-] [66]. In normal physiology, the superoxide radical is largely reduced through a number of enzyme-catalysed steps, with the oxygen molecule ending in water; however, when this cascade of reactions is disrupted by a drug, then free radicals can accumulate and damage mitochondria, leading to sub-level functioning of mitochondria, even higher production of ROS and eventually mitophagy, which can lead to cell death as in Parkinson's and Huntington's disease [67]. This process is represented by a kinetic model simulating ROS-induced mitochondrial death in Parkinson's disease [65].

ROS also damages the proteins, lipids and genes responsible for the normal functioning of the mitochondria [68] as observed in a number of cancers. ROS removes the sulfhydryl group of pyruvate kinase isoform 2 [69] resulting in sub-level functioning

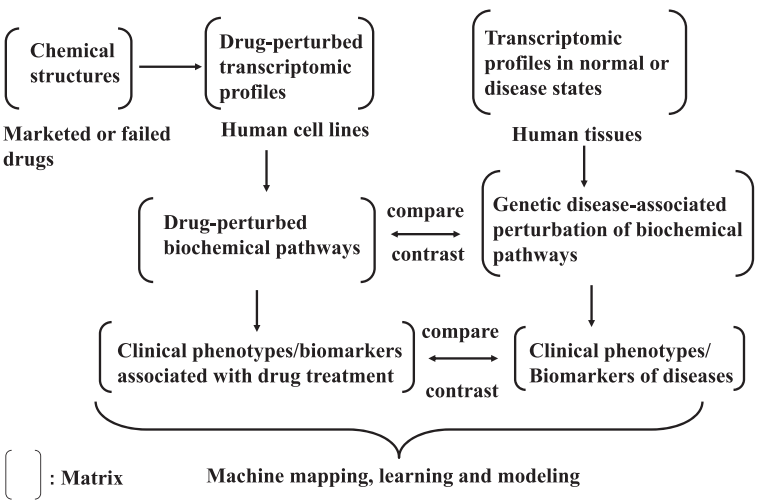

Figure 3. Leveraging the known genetic diseases and their individual clinical biomarkers and phenotypes to assess and predict the effects of drug via technologies of machine learning and modeling

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of mitochondria, leading to the production of lactate and creating an acidic microenvironment that can enhance tumor growth. ROS inflicted DNA damage and deficient repair mechanism has been associated with a risk of cancer and other diseases such as autoimmune disease [70,71]. Additionally, ROS promotes tumor growth through mitogen activated protein kinase (MAPK) mediated signaling cascades [72]. MAPK signaling modulated the expression of genes that are involved in proliferation, metabolism, programmed cell death and others [73]. Thus, drugs that affect the ability of the cell to cope with ROS can lead to a myriad of potential health problems. Higher levels of ROS can cause extensive mitochondrial damage and lead to a wide spectrum of maladies ranging from neurodegenerative to various cancers. ROS in mitochondria has been studied with a concentrated focus on disease origination over a long period of time, however, drug-induced ROS mediated mitochondrial damage has lacked attention. This presents an opportunity to leverage a knowledge of ROS and mitochondrial damage to reduce future drug attrition rates.

Future Perspectives

Leveraging systems biology to delineate the effects of a drug from its on-target(s) beneficial effect and off-target(s) adverse effects through the systems pathways and networks would aid our understanding and assessment of its safety and overall therapeutic index. Current scientific advances and accumulated knowledge of disease [19], clinical toxicity [1] and systems biology [25] have presented opportunities to extend traditional pharmacokinetic and pharmacodynamic modeling/analysis to execute systems pharmacology modeling. In such modeling pharmacological responses at the pathway/network, organelle and organ levels are integrated through incorporation of relevant bridging biomarkers [7,74]. Conceivably, systems pharmacology network modeling may not involve all intracellular networks at the same level and depth of complexity as systems biology, but instead may only require capturing the portion of intracellular networks that a drug affects. Systems pharmacology modeling of the clinical phenotypes manifested during a drug therapy would advance at a faster pace if one can leverage systems biology and cellular phenotypes in relation to perturbation of cellular networks and corresponding biochemical consequences. Modeling a specific organelle phenotype as the surrogate of a defined cellular phenotype, under a prior defined specificity and sensitivity, for the statistical linkage to a clinical phenotype could enable early prediction of the toxicity profile of a drug, and help to screen out toxic drugs prior to phase 2 and 3 clinical trials. Compiling a database of use cases with respect to a specific surrogate would surely be the beginning step to ascertain the linkage between surrogate marker(s) and a clinical phenotype. Systems-pharmacology modeling with curated individual components would be the best approach to handling the complex system of organ toxicity. Such approaches would benefit also from integration with genome-scale networks such as those that have been built for metabolism in humans generally [75,76], as well as for specific organs and cell types [77–79]. Both the molecular signature in a specific cell type of an organ and the panel of cellular signatures following exposure to a drug adequately covering all critical cell types of human organs would help to define the systems-pharmacology network of an array of pharmacodynamic responses following its administration.

In summary, systems pharmacology modeling is essential for linking systems networks that are affected by a drug to its treatment-associated clinical measurable biomarkers and phenotypes through specified statistical criteria and consideration of gene–drug interactions and immune system elements. Such approaches, if applied across all the pharmacological classes of marketed drugs, will improve the yield of future drug products with an adequate benefit/risk ratio.

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Conflict of Interest

The authors have no conflict of interest.

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