J. Indian Inst. Sci.
A Multidisciplinary Reviews Journal
ISSN: 0970-4140 Coden-JIISAD

REVIEW ARTICLE

Digital Twin for Drug Discovery and Development—The Virtual Liver

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Abstract | Digital twins are defined as digital replicas of processes, systems or devices developed to foster deeper understanding and prediction. While the concept of digital twins has largely been applied in the manufacturing industry, one could conceive of a digital twin that integrates information from diverse scientific and clinical sources to represent the complex and dynamic relationships within biological networks. Such an integrative system would allow one to gain a deeper understanding of the biology and be used as a predictive framework to design better drugs. The liver is a key organ in the body that is implicated in various diseases and injuries leading to drug failures and withdrawals. The study describes the development of a digital twin of the liver by integrating the knowledge and understanding gained by studying various liver functions, diseases and the effect of drugs, using a mathematical framework based on ordinary differential equations. This twin has been shown to be effective in reproducing the normal liver function, evolution of disease and the impact of treatment. Finally, a system that couples the twin with experimental measurements has been demonstrated to provide insights into drug-induced liver injury. The approach described in this paper is fairly general and can be applied to other organs and biological systems to develop drugs more efficiently and safely.

1 Introduction

The process of developing a drug is extremely research-intensive spanning efforts in biology, chemistry and manufacturing, while being characterised by a low probability of success. It is estimated that 50,000 hits are tested to achieve a successful drug. The odds of a drug molecule eventually reaching patients are so low that only one in 12 drug molecules that are tested on humans in clinical trials make it successfully to the market. Toxicity and lack of efficacy account for greater than 60% of all drug failures[1]. The traditional approach to drug discovery is akin to a pipeline with targets, leads and molecules each needing to meet pre-set success criteria to progress towards clinical development and ultimately the market. In such a brute force approach, making the right decision about which targets, hits, leads and molecules to take forward is extremely critical for the successful launch of a drug in the market. However, the decision-making is based upon in vitro and in vivo systems which themselves have a questionable concordance with outcomes in the clinic[2]. An ideal decision support system for drug discovery would provide answers to the following questions:

- What is the extent of impact any target has on the clinical outcome of interest?
- Does the lead/candidate molecule modulate the target sufficiently to impact clinical outcomes?
- Is the molecule specific enough and does not have any side effects or toxic outcomes?
- Is the observed lack of efficacy due the inability of the drug to reach its target?

in vivo: experiments in animals.

in vitro Experimental system in a test-tube or petri dish.

hits: Trial versions of chemicals that are further modified to eventually develop a drug

efficacy: The ability of a drug to provide allieviate a disease process and provide relief to a patient.

Toxicity: Harmful side-effects of a drug.

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- Has the right dose and dosing regimen been selected in the trial?
- Are there any bio- or surrogate markers predictive of the success or failure of a drug?
- Have the right patients been selected in the trial?
- Is there anyway of identifying hyper- and hypo-responders at the start of the trial?

bio- or surrogate markers: An easily measured parameter in a human (like cholesterol) that acts as a representative for the underlying disease process.

pharmacokinetics (PK): the study of how a drug molecule distributes throughout the body.

2 Digital Twins and In Silico Research

Given the multifactorial nature of drug development based upon the questions above, drug failures are common and difficult to address. This problem needs to be attacked by integrating data and insights from different points in the drug-discovery pipeline and creating a predictive system that can forecast the result of an experiment or the impact of a chemical change on a drug molecule. To this end, a systems approach to modelling and simulation of biological processes is a research frontier that is likely to yield understanding of the mysteries of life at a qualitatively different level[3]. Modelling of bioprocesses at the genomic (genetic networks) and cellular (virtual cell) level has been actively supported by funding agencies like DARPA, NSF, DOE in the US and has resulted in high visibility projects like BioSPICE[4]. While pre-clinical and clinical development programmes that incorporate systems-modelling approaches are not common today, systems approaches are likely to play an important role in the future in improving the efficacies of therapeutics and diagnostics. Systems biology efforts are being used to "jumpstart" therapeutic areas that have remained stagnant without significant breakthroughs[5,6].

screening assays: high throughput experimental systems that can study the effect of thousands of drug molecules all at once.

biologics: drug molecules that are not chemical in nature but are based upon proteins or nucleic acids.

More recently, large consortia have been initiated to study organ systems in detail such as the German Virtual Liver Network that intends to create an integrated multi-scale model of the liver[7] or The Physiome Project, a multi-scale modelling framework that allows models of different parts of the human body to be combined and linked in a hierarchical fashion[8]. While we expect that the benefits from these large-scale "bottom-up" initiatives will bear fruit over the next decade as multiple models are refined and integrated, a more top-down approach to model building or models that are more limited in scope and address a specific need in drug development are being produced and applied by consulting or contract research organizations such as Applied Biomath LLC[9], Certara L.P.[10], RES Group[11], Simulations Plus[12], Syngene International[13], Vantage Research[14] and others. These organizations have demonstrated that smaller "bespoke models" designed to address very specific questions can have a huge impact by driving efficiencies in drug development. A survey conducted by Abbvie revealed that 62% of pharmaceutical organizations consider quantitative systems pharmacology to be an integral part of their research process and agree that its impact has been substantial[15,16].

Digital twins are defined as digital replicas of processes, systems or devices used for understanding study or prediction[17]. While the concept of digital twins has largely been applied in the manufacturing industry, in the pharmaceutical context, one could conceive of a digital twin that behaves in a manner akin to a process, an organ system or an individual. Creating such a system requires the integration of information from diverse scientific and clinical sources to represent the complex and dynamic relationships within biological networks. This would allow the prediction methodology to be applied in various phases of drug discovery and research such as target selection and validation, lead optimization and candidate selection, biomarker identification, assay and screen development and clinical trial optimization. For example, simulation results from target validation studies could be used to validate clinically relevant targets and identify screening assays. Optimal binding, pharmacokinetics (PK), and mechanism-of-action profiles can then be defined for lead compounds and biologics. Simultaneously, biomarkers can be identified to characterize the pathophysiologic basis of patient subtypes or used as surrogate markers for patient response. By incorporating such simulation results into clinical trial design, researchers can identify key parameters that predict therapeutic responses, including dosing, dose regimen, and inclusion/exclusion criteria. At each point in the drug development process, this approach can help focus efforts to fully exploit the product opportunities for a therapeutic-area franchise.

3 The "Top-Down" Digital Twin

Ideally one would want to represent all the complexity inherent in a biological system at a multi-scale level, starting from genetic changes all the way to phenotypic ones. However, such an endeavour is challenging requiring a very deep understanding of each component of a biological system and how various components interact with one another in a quantitative manner. A more practical approach is to work "top-down" by identifying and focusing only on those

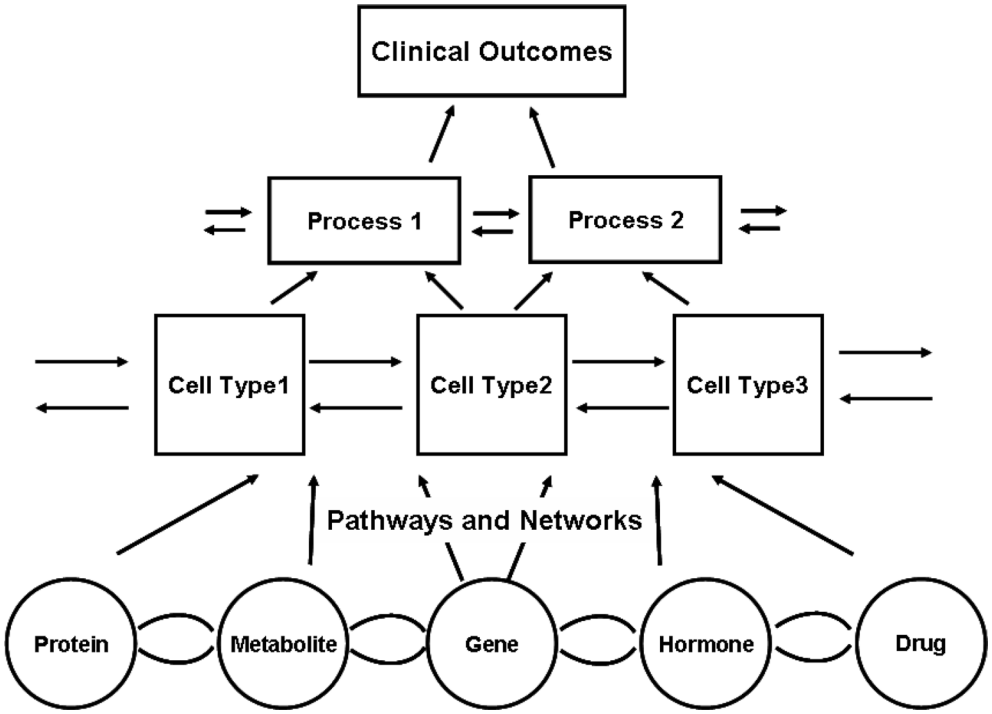

Figure 1: A top-down approach to developing a digital twin of a biological system.

components relevant to the disease/process under consideration, starting with the major organ systems, and working down to relevant tissues, cells, proteins, and genes. This approach is illustrated in Fig. 1. Using this approach, only those systems relevant and important to the disease are detailed. This process does not however, keep us from investigating targets that are not yet explicitly represented. Because higher-order activities are represented, we can reproduce modulatory effects indirectly, allowing its application to a wide number of research needs. In addition this approach allows us to marry dynamic simulations with alternative approaches such as clustering or classification in the same platform. Since the focus remains on the biology, the resultant "twin" is not tailored to a specific disease or chemical class.

This following sections describe the top-down approach used to develop a digital twin for the liver—"Virtual Liver"[18] and its application in understanding liver disease and predicting drug toxicity, one of the major causes for drug failures in the clinic.

4 Model Development

The systems approach to the liver digital twin is based on the principle that if liver homeostasis can be modelled, any liver disease or toxicity can be considered as perturbation of this normal system.

The model development was started by compiling a list of the diseases that affect the liver and a performing a survey of the literature to identify mechanisms by which drugs injure the liver[19,20]. Some paradigm examples of the former are diseases such as metabolic syndrome or diabetes while examples of the latter are drugs such as chlorpromazine, which causes cholestasis and acetaminophen that causes necrosis. The underlying biochemical pathways that are impaired upon action of these drugs were then compiled. Some of the pathways include glutathione metabolism, bile salt and bilirubin metabolism and energy homeostasis including pathways of nutrient metabolism. These pathways were then represented by a system of ordinary differential equations of the form:

Rate of change of metabolitemetabolite concentration = rate of synthesis of the metabolite – rate of utilization of the metabolite[21,22].

The approach produced a system of differential equations with 112 states and a couple of hundred parameters. While the system was indeed large, one could apply multiple constraints on the behaviour of the entire system such as the liver's role in feeding or fasting, its diurnal variation and its behaviour under normal versus diabetic conditions. In addition the

necrosis: cell and tissue death resulting from a lack of nutrients or energy

glutathione: main antioxidant in the body.

metabolite: any chemical in the body that is formed or broken down.

homeostasis: the normal or steady state of a biological system.

Table 1: Comparison of the simulations of metabolite concentrations and fluxes with their experimental values.

Metabolite	Simulated value	Experimental value	References
GSH (cytosolic)	7.96 mM	5–10 mM	33
ATP (cytosolic)	2.95 mM	2.76 mM	34
Phosphate (cytosolic)	3.38 mM	3.34 mM	34
ATP from glycolysis	33%	38%	35
ATP from oxidative phosphorylation	66%	57%	35
Fraction of fatty acids influx in oxidation (Fed)	28%	34%	36
Fraction of fatty acids influx in oxidation (fasted)	70%	70%	36

various sub-modules had their own constraints such as the bile turnover during the day or the amount of ATP needed and produced by the liver among others. Hence we could recast the problem as a constrained parameter optimization problem and the parameter space was considerably narrowed. Adding a further constraint that the parameters needed to be in physiological ranges allowed us to further narrow their value and a suitable parameter set could be identified that allowed the system to reproduce the liver homeostasis. The model was then perturbed to represent disease, treatment and drug toxicity as described in the sections below.

5 Model in action

Homeostasis: Table 1 shows the comparison between the predicted and experimentally observed values for a selected set of metabolites from the virtual liver. A good concordance is observed between the values of metabolites as well as fluxes indicating that the model represents the biology of liver with fidelity. The energy production (ATP) from mitochondrial and non-mitochondrial sources predicted by the model is very close to experimentally measured values. The model is also able to represent the transition of liver physiology from the fed state to the fasted state and captures the impact of fatty acid metabolism under glycolytic and gluconeogenic conditions.

5.1 Representation of Disease

Bile acids are produced in the liver and stored in the gall bladder. When food arrives in the intestine, bile salts are secreted into the small intestine where they help in the digestion of fats. At conclusion of digestion, 95% of the bile salts are reabsorbed back into the liver and sent back to the gall bladder where they are stored until the next meal. 5% of the bile salts lost through faeces is supplemented by de novo synthesis in the liver. This process of bile-salt movement from the liver to the gall bladder, then to the intestine and back is called the enterohepatic circulation of bile. Transporters play a critical role in maintaining the enterohepatic circulation and bile acid homeostasis in the liver and the intestine[23]. Specifically, the secretion of bile acids from the liver to the gall bladder is mediated by the bile-salt export pump (BSEP), which sits on the cell membrane in hepatocytes. Any block of this pump would result in bile acids building up in the hepatocyte and eventually being refluxed into the blood leading to jaundice. Defects in BSEP are responsible for inherited forms of liver disease, one of them being progressive familial intrahepatic cholestasis type 2 (PFIC2), associated with low bile acid secretion, failure to thrive, intractable pruritus, progressive cholestasis, and a significantly increased risk for hepatobiliary malignancy[24]. In patients with PFIC2, BSEP synthesis, cellular trafficking, or stability is significantly impaired[25] and they exhibit jaundice characterized by very high levels of bile acids in the blood and the urine. Many of the children with this disease will die at young age unless they can obtain a liver transplant.

To represent this disease in the virtual liver, a virtual knockdown of the BSEP transporter was performed. Figure 2 shows the predicted levels of total bile acids (TBA) in the plasma that results from different levels of BSEP malfunction. It is observed that there is a transient increase in TBA that resolves over several days finally settling at a new homeostatic value that is 30–50 times higher than normal representing stable disease. Thus, starting from a model of normal liver, one can generate a disease phenotype by representing the genetic disease cause by a parameter change in the model. Two of the scenarios, 90% and 95%

enterohepatic: pertaining to the liver and the intestines.

Transporters: a small pump on the surface of a cell that selectively transports ions and metabolites in and out of the cell.

hepatocytes: a type of cell found in the liver

pruritus: itch.

hepatobiliary malignancy: specific type of liver cancer.

fluxes: the rate of metabolite change.

knockdown: a partial disruption of function due to genetic changes.

glycolytic: The fed state of the body when the liver plays a role is nutrient storage.

gluconeogenic: Fasted state of the body when the liver produces glucose for the rest of the body.

phenotype: observable characteristics of an individual

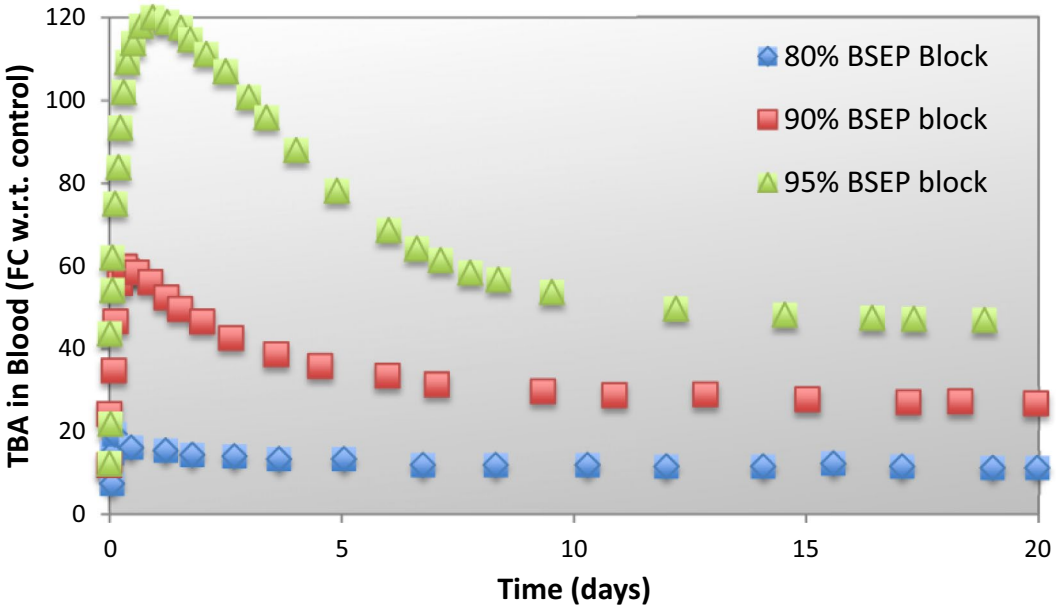

Figure 2: Total bile acid (TBA) levels in the blood upon virtual knockdown of the BSEP transporter.

BSEP block resulting in 25.3 and 46 fold increase of plasma TBA were characterized as moderate and severe disease respectively and taken forward as "Virtual patient: A digital twin representation of a diseased individual" for further examination.

5.2 Target Validation and Drug Treatment

A potential use of a digital twin in pharmaceutical R&D is to examine targets and their impact on clinical phenotypes and treatment outcomes. In the case of PFIC2 illustrated in the section above, it was hypothesized that inhibiting bile-salt reabsorption in the intestine would lead to greater bile-salt excretion from the faeces eventually leading to lower levels of bile acids in the blood and consequently in the liver thus ameliorating disease severity. This hypothesis was tested in the virtual liver by retarding intestinal bile-reabsorption mediated by another transporter pump called the ileal bile acid transporter (IBAT), in the virtual patients described in the previous section. IBAT was blocked to varying degrees to simulate different doses of a drug. The levels of bile salts in the blood and the dynamics of change were studied along with the amounts of bile excreted in the faeces. A comparison of the simulations with the results of a clinical trial of A4250, a potent and selective inhibitor of the IBAT transporter was performed. Under normal conditions, 95% of bile salts are reabsorbed from the intestine into the liver and only 5% excreted. Of the reabsorbed bile salts ~ 58% is absorbed "actively", via IBAT and the rest passively via diffusion across the gut. As the inhibition of IBAT function increases, the actively absorbed component of the bile salts is blocked, resulting in a greater elimination through the faeces (Table 2). When 50% and 80% IBAT block is applied to the "virtual patients" representing a moderate and severe phenotype (red and blue curves in Fig. 2) 50–75% reduction in serum bile acid level and 350–500% increase in faecal excretion is predicted over a period of 2 weeks (Figs. 3, 4). These results compare well with clinical studies that showed a 300–600% increase in faecal excretion and 50–75% reduction in TBA levels[26]. This is an example of how a digital twin can be used to represent a disease situation; where a target can be studied for its feasibility in changing disease course and consequentially, the implications of a therapeutic intervention can be predicted.

ileal bile acid transporter (IBAT): a transporter on the intestinal cells that helps in absorbing bile acids from the gut into the liver.

Drug-induced liver injury

Mitochondria are cell organelles that generate most of the chemical energy needed to power the cell's biochemical reactions. This energy is stored in the form of a molecule called adenosine triphosphate (ATP). However, it has been observed that mitochondria are commonly involved in the toxicity of many drugs and xenobiotics that leads to liver injury. One of the ways injuries occur is by the inhibition of mitochondrial complex I, that depletes hepatocellular

organelles: a small structure inside a cell that performs a specific function

xenobiotics: synthetic chemical that can harm the body.

complex I: an enzyme in the mitochondria that is involved in energy generation

Table 2: % Increase in bile excreted by IBAT inhibition of active bile salt reabsorption.

Total bile (%)	IBAT inhibition (%)	% Bile reabsorbed			Excreted (%)	Elimination (fold change)
		Active	Passive	Total		
100	None	55	40	95	5	1
	50	27.5	40	67.5	32.5	6.5
	80	11	40	51	49	9.8

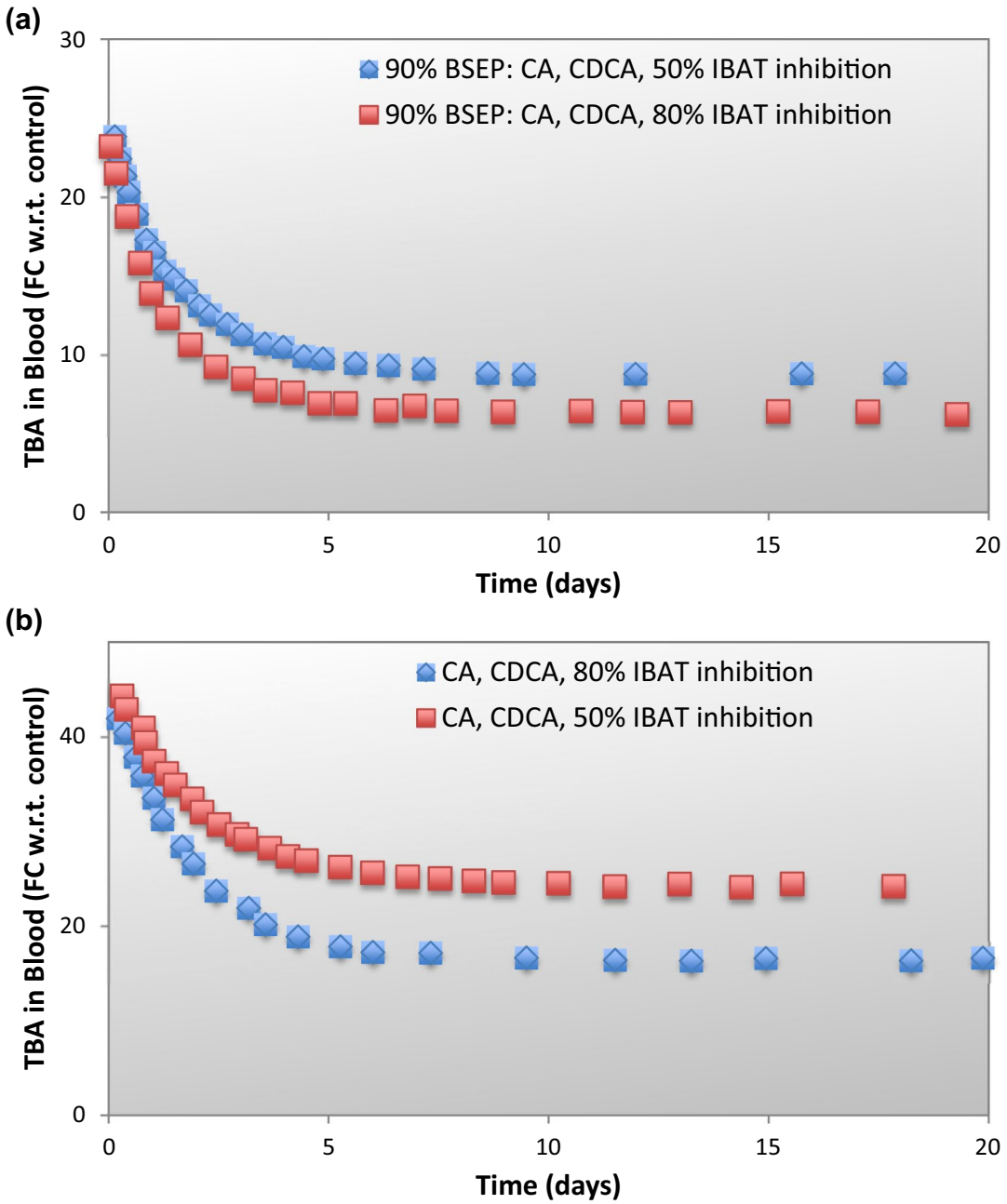

Figure 3: **a** IBAT block causes a reduction in bile acid levels in plasma of moderate patients: 49% reduction with 50% block, 66% reduction with 80% block. **b** IBAT block causes a reduction in bile acid levels in plasma of severe patients: 68% reduction with 50% block; 76% reduction with 80% block.

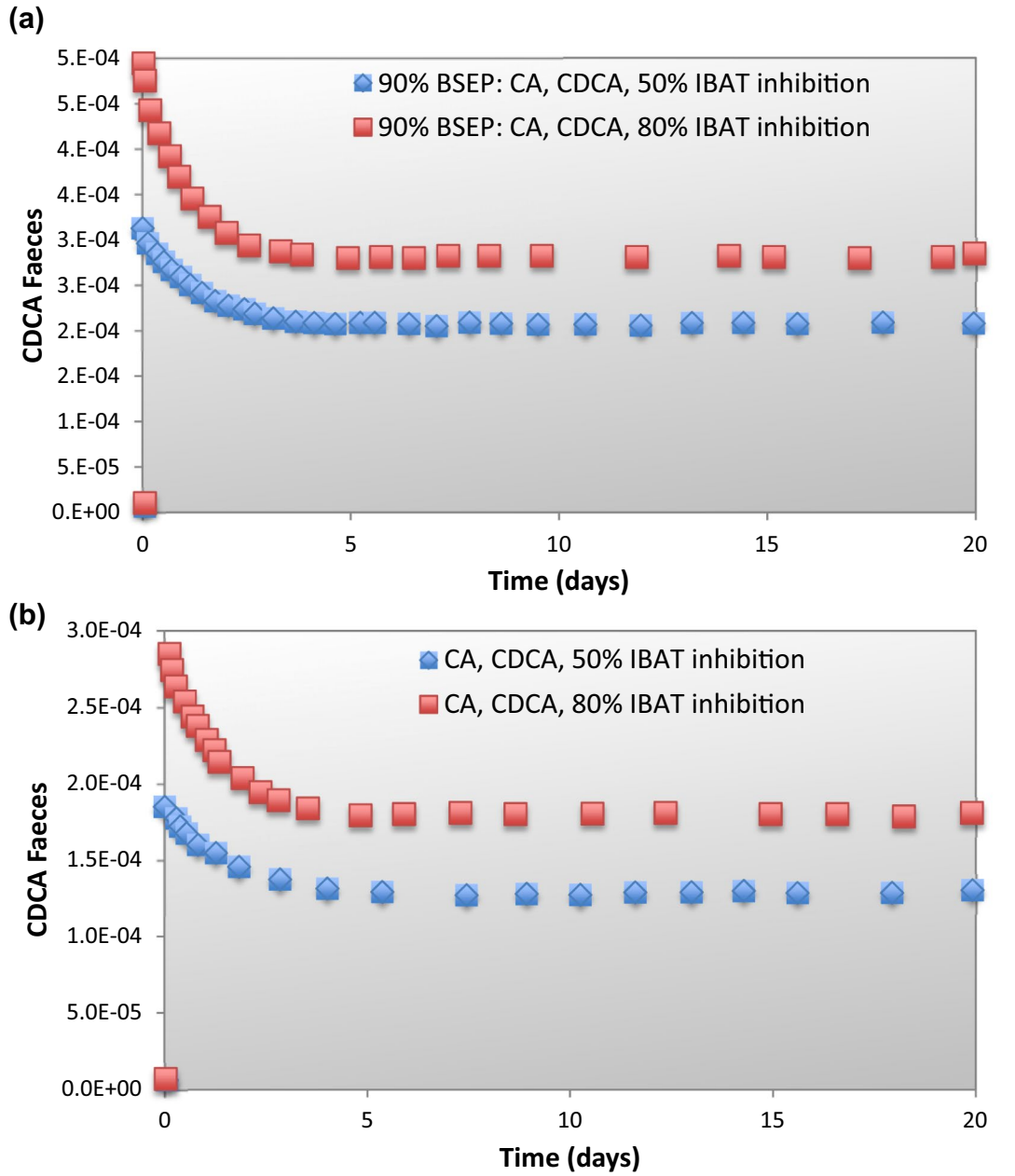

Figure 4: **a** IBAT block causes an increase in faecal elimination of bile acids in moderate patients: 343% increase with 50% block, 490% increase with 80% block. **b** IBAT block causes an increase in faecal elimination of bile acids in severe patients: 372% increase with 50% block, 527% increase with 80% block.

ATP and leads to cellular damage. Idiosyncratic liver injury has also been associated with alterations in mitochondrial function by exposing cells with partially compromised complex I activity to mitochondria targeting drugs[27]. To assess the enhanced risk of ATP depletion in specific individuals, complex I activity was reduced over a range (up to 50%) to simulate the different extent of drug effects on mitochondria. The impact of this change was compared in normal individuals versus metabolic syndrome (MetS) individuals. Metabolic syndrome represents a cluster of conditions; high blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol levels that increase the risk of heart disease, stroke, and diabetes.

Firstly, a MetS virtual patient was created by changing enzyme rates in a set of pathways that are dysregulated in the disease. The following pathways were modified from the baseline levels

Idiosyncratic: person specific.

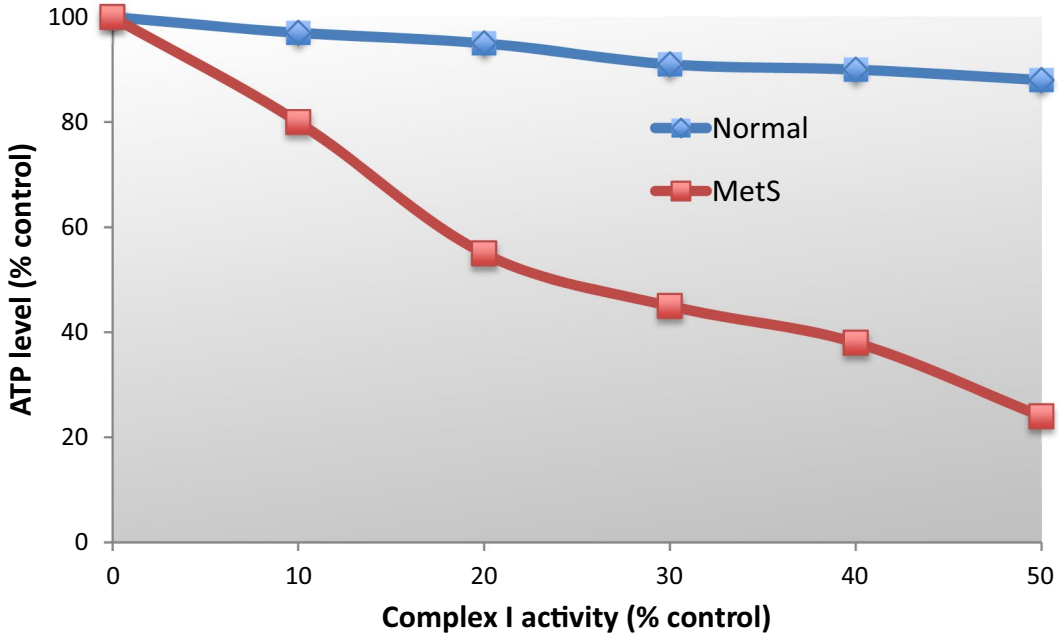

Figure 5: Impact of complex I activity reduction on cellular energetics—ATP levels drop precipitously in MetS individuals compared to normal individuals.

by changing the appropriate parameters in the model; transporter mediated fatty acid entry, de novo lipogenesis, plasma lipoproteins turnover and re-uptake, pentose phosphate pathway and oxidative phosphorylation and stress[28]. If one compares the impact of complex I inhibition in a MetS patient to a normal individual, at the same level of complex I inhibition there is a profound reduction in cellular ATP in MetS individuals likely leading to cytotoxic damage (Fig. 5). This example illustrates the process by which the impact of a drug can be different in different individuals. In such a scenario, a drug that targets the mitochondria may appear safe when tested in healthy volunteers but may prove problematic in diseased individuals. The digital twin allows one to create virtual patient populations the represent the biological variability seen in different individuals and assess the population level risk for side effects well before a large-scale testing.

cytotoxic damage: cell death due to an external chemical

6 The Road Ahead

In fields where complex and expensive R&D is needed to create a product, digital twins increasingly provide an attractive alternative to traditional approaches to test hypotheses in silico. With high performance computing now available ubiquitously, it is possible to create digital versions of biological systems informed by the breadth of knowledge accumulated over the last several decades, which replicate the complexity inherent in them.

in silico: on the computer using models

In this study one such digital twin of a complex organ, the liver based on an understanding of its homeostasis and perturbations by the environment, genetics and drugs has been described. Since the underlying biochemical framework of the digital twin encompasses basic metabolic processes within the liver such as antioxidant, bile, energy and nutrient metabolism, it can be applied in a comprehensive manner to describe various disease and drug related processes, allowing it to be used across the drug discovery and development pipeline as demonstrated by the examples in the previous section. All biological systems upon perturbation respond actively and adapt to resist the change. An understanding of this is only possible if one builds models using methods that allow non-linearity, feedback and dynamic analysis such as ordinary differential equations. The approach, homeostasis leading to disease evolution or drug perturbation or a combination of both is versatile and general making it applicable to other organ systems.

One of the biggest challenges in drug development is the prediction of idiosyncratic toxicity in humans; impossible to predict using existing in vitro and in vivo models. The digital twin approach defines the initial state of any simulation and can be easily modified to represent disease conditions and immunological states

allowing the creation of a new homeostasis that mimics disease or patient-specific effects. Idiosyncrasy therefore is a logical outcome of complexity driven by a combination of factors that relate to the impact of drug, disease and the patient. Hence an entire population of patients that represent varying combinations can be created and tested to predict the occurrence of what was hitherto considered as unpredictable or idiosyncratic.

Parameter sensitivity analyses can be performed to identify pathways and processes that perturb the system significantly away from the starting homeostasis. A computational "mining" of this nature will allow one to design appropriate in vitro assays that measure the impact of any perturbation on the pathways, enzymes and processes that have the greatest potential impact on the liver. Combining the effects of these perturbations via simulations provides for true integration of multiple measurements using multiple methodologies (in silico and in vitro). This approach brings with in an ethical component where by reducing animal experimentation. Approximately 200 million animals are used every year in laboratory experiments world wide[29,30]. The digital twin allows a direct translation of in vitro measurements into what could expected in vivo either in animal models or in humans.

However, one needs to be cognisant of the limitations of the approach. In our study, we focused on specific systems in the liver and were thus limited by the "biological space" that we were operating in. For example, the propensity of a drug to injure the liver is by causing cancer in liver cells cannot be predicted by the current system. Another important point to note is that while this study describes a mechanistic approach to create a digital twin, a complementary approach could take a data-driven route by linking liver related outcomes to measurements using machine learning or deep learning.

In summary, as the field continues to move away from the current state-of-art where biology is an observational discipline recording effects on animals towards one, where consequences of hypotheses can be predicted; integrative methods that combine in vitro and in silico approaches to understand the basic mechanisms is key. Over the past decade the idea of developing digital twins has grown, with multiple twins under development such as the heart[31], that combines various functional measurements with multi-scale modelling and the virtual kidney[32] that uses distributed computing to integrate geographically separated models and databases. The approach presented in this study shows the potential of being able to provide a quantitative and mechanistic assessment of disease pathology, therapeutic interventions and toxic liabilities of chemical entities in the liver.

Publisher's Note

Compliance with ethical standards

Conflict of interest

The author states that there is no conflict of interest.Ethical approval
Not applicable.Consent to participate
Not applicable.Consent for publication
Not applicable.Availability of data and material
Not applicable.Code availability
Not applicable.

Received: 9 August 2020 Accepted: 3 September 2020
Published online: 1 October 2020

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