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Research

An *in silico* Approach to Predict Susceptibility to DILI in Metabolic Syndrome: An Alternative to Animal Experiments

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Abstract

Metabolic disorders, such as type 2 diabetes and atherosclerosis form a cluster of different conditions caused and mediated by complex multi-molecular interactions. The presence of metabolic syndrome (MetS) is reported to predispose an individual to drug-induced liver injury (DILI), from drugs such as acetaminophen, halothane, statin. However, the molecular mechanisms behind this are not yet understood. We have previously reported on the development of a cellular dynamic systems model of integrated carbohydrate, glutathione and fat metabolism in the rat liver. We have now modified the network with altered signaling and flux distributions of processes involved in carbohydrate and fat metabolism to create the liver physiology observed in metabolic syndrome. Triglyceride content in MetS liver is predicted to be 3- fold higher than normal individual. Our aim is to compare the impact of known drugs in the normal and metabolic syndrome liver by computationally perturbing appropriate processes, representing the effect of a drug to varying degrees. We have observed that a set of perturbations seemed to make the MetS liver more susceptible to certain forms of DILI. TMX treatment is predicted to increase cellular triglyceride by 6-fold in MetS individual. From this observation we generated a set of hypotheses for increased susceptibility to DILI under the influence of metabolic syndrome that can subsequently be experimentally proved. Thus, our approach allows one to identify susceptible patient groups in metabolic syndrome along with drug targets leading to an increased risk for DILI.

Key Words: Modeling; Metabolic Syndrome; Liver; DILI; Homeostasis; *In silico*

Introduction

The incidence of metabolic syndrome (MetS) in the developed world has been increasing over the last 10-15 years [1]. The Third Report of National Cholesterol Education Program (Adult Treatment Panel III; ATPIII) provides a working definition of MetS, a complex multifactorial disease, [2] based on a combination of 5 categorical risk factors: central obesity, hypertension, hypertriglyceridemia low levels of HDL cholesterol, and hyperglycemia. The processes that lead to MetS have not been clearly understood at a molecular level. It has been envisaged in published literature that dysfunctions in processes in hepatic fat and carbohydrate metabolism can culminate in MetS. The dysfunction can result from either altered nutritional states (environmental effect) or differences in genetic susceptibility leading to the five risk factors that are hallmarks of MetS.

The liver is the major organ involved in fat and carbohydrate metabolisms. Precursor elements from an individual's diet enter the liver from the intestine in the form of either free fatty acids or chylomicrons. After being metabolized inside the liver, fat and cholesterol are transported into the circulation as lipoproteins. Lipoprotein turnover by the respective plasma lipases forms the fat precursors delivered to various peripheral tissues including the liver. Dietary carbohydrate enters the liver in the form of precursor molecules such as glucose and fructose. Carbohydrate is stored in the form of glycogen (glycogenesis) and fat is stored in the form of triglyceride (TG) inside the liver. During starvation or any other adverse condition blocking glucose entry into the system, the liver supplies glucose to the whole body by glycogen break down (glycogenolysis) and by gluconeogenesis.

The liver is the primary organ dealing with xenobiotic load in the body. Any foreign chemical is metabolized in the liver primarily by a family of cytochrome P450 enzymes and made ready for excretion. As a result of this metabolism, it is the primary organ affected by drug or drug-metabolite mediated toxicity [3,4]. DILI prediction is still a major challenge for the pharmaceutical industry

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making it a major cause of drug withdrawal from the market. Among the three major forms of DILI, namely cholestasis, steatosis and cytotoxicity, steatosis resembles the MetS phenotype due to the abnormal processing of hepatic fat and carbohydrate leading to TG accumulation and fatty liver formation. Many processes linked to altered lipid metabolism are associated with the MetS condition, like cardiovascular disease, obesity and insulin resistance [5]. Hence, an increasing concern is whether MetS individuals are more prone to some forms of DILI compared to normal individuals [6]. Published literature reports the association of liver toxicity induced by drugs like tamoxifen (TMX) [7], halothane [8,9], acetaminophen [10,11] with obesity. An integrated analysis of fat and carbohydrate metabolism may offer a better understanding of the nature of compounds as well as their targets in causing steatosis in normal and MetS individuals.

We have studied the drug-induced hepatotoxicity problem, by analyzing perturbations of the metabolic network in the liver mathematically. The model network encompasses fat, carbohydrate, glutathione and bile acid metabolism. We have previously described the ability of the model to predict experimentally observed DILI [12]. In the present study we have extended the model to describe the altered homeostasis of the liver under metabolic syndrome. We have simulated the impact of drugs on the normal and MetS livers and compared our predictions with experimental outcomes. We have also been able to predict that the liver under metabolic syndrome is more prone to drug-induced steatosis compared to a normal liver. Simulations also led us to develop hypotheses for types of idiosyncratic toxicity that may be observed in a MetS individual.

Methods and Model

Software

The model building and simulations were executed using the software tool Syngene SysBio™.

Model Structure

The aim of this study is to create a version of the model that enables us to represent the phenotype of the liver (in the context of key metabolites relevant to the present study) observed in metabolic syndrome and use this adapted version to characterize the impact of drugs on the liver in MetS individuals. To do so, we modify the existing liver model that represents basal homeostasis to represent qualitative and quantitative phenotypic changes observed in MetS. This is followed by simulations to represent the effect of drugs in both the normal and metabolic syndrome model and comparing the results. Finally, we have generated hypotheses that may explain forms of idiosyncratic toxicity observed in MetS individuals.

Model Description

A detailed description of the normal liver model including the differential equations and the associated parameter values is provided in our earlier publication [12]. In addition to glycolysis, gluconeogenesis, TCA cycle, oxidative phosphorylation (partially), *de novo* lipogenesis, esterification and betaoxidation of fatty acids that were part of the earlier version of the model, some additional processes like cholesterol metabolism, TG metabolism, VLDL assembly and secretion into the plasma, plasma lipoprotein turnover (VLDL, LDL and HDL) are modeled as well in the present version (Figure 1).

For each of the metabolites associated with the processes described in Figure 1, we created differential equations that quantitatively describe their rates of formation and consumption. We then linked them to the earlier model to create a more advanced version including additional features of liver biology.

Converting A Normal Liver To Represent MetS:

JCR:LA-corpulent rat is the experimental model that is typically used to study the altered biology behind metabolic syndrome [13, 14].

In this animal model, the following processes have been observed to be altered:

- Transporter mediated fatty acid uptake by liver [15]
- *De novo* lipogenesis [16,17]
- Turnover of plasma lipoproteins [18]
- Re-uptake of plasma lipoprotein by liver [18]
- Pentose phosphate pathway [19]
- Oxidative phosphorylation and oxidative stress [20-22]

Based on the above-mentioned observations, we altered the respective processes in our homeostasis model. These changes were implemented by altering enzyme activity levels as shown in Table 1 to bring about the corresponding changes in the observed processes.

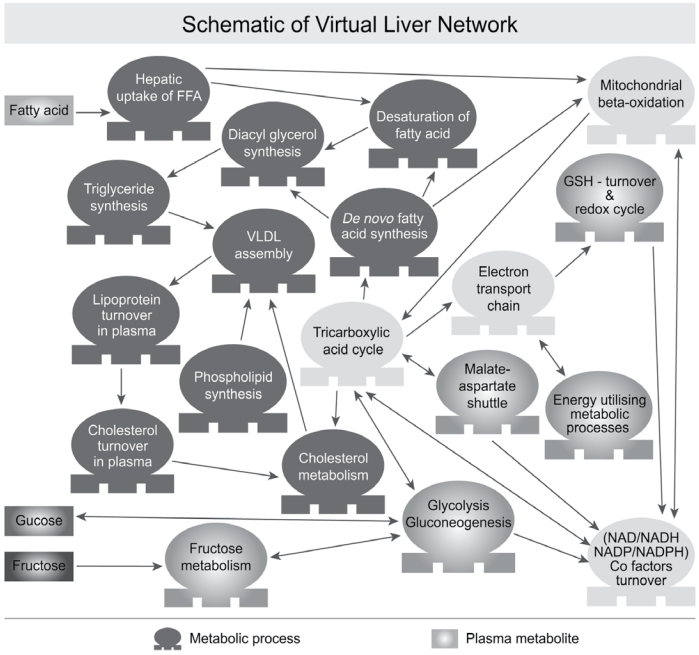

Figure1: Biological processes connected quantitatively in the metabolic network *in silico*.

Simulations were performed using the altered parameter values to generate a new homeostasis that represents the liver metabolism in metabolic syndrome. Simulated concentration of key metabolites and processes were compared with literature observations.

Mimicking the Effect of Tamoxifen:

We represented the biological effects of tamoxifen in the liver [7,23,24] by the alterations in the enzymes fluxes listed in Table 2.

The impact of tamoxifen on the alterations of key metabolites is presented in the results section.

Idiosyncratic DILI in MetS:

Alterations in mitochondrial function are predicted to be associated with susceptibility to idiosyncratic DILI [25]. Idiosyncratic abnormalities in the expression of superoxide dismutase (SOD) are linked to hepatotoxicity induced by the antidiabetic drug troglitazone [26-28]. Idiosyncratic toxicity associated with reduced expression of complex I has been reported for many diseases [29,30].

As a case study of idiosyncrasy, we have selected complex I of the electron transport chain as a target and reduced its activity over a range (starting with normal [0%] to 50% inhibition) to simulate varying drug effects on complex I efficiency leading to differing outcomes of mitochondrial damage. The effect of perturbed complex I activity has been simulated in the background of both the normal liver as well as the MetS liver. The impact on cytotoxicity is compared between the normal and the MetS individual and assessments are made on the propensity of the normal versus the MetS individual to mitochondrial mediated cytotoxic damage.

Results

The Liver Phenotype Under Metabolic Syndrome

Table 3 compares the model-simulated changes in the TG metabolism under MetS condition with experimental observations. The model-simulated homeostatic value for cellular TG under MetS compares well with what has been reported in the literature [16,31]. Simulations predict approximately a three-fold increase in cellular TG under MetS condition, which is similar to what has been observed experimentally [16]. Our predictions indicate that extracellular accumulation of fatty acids (high plasma fatty acid concentration) under metabolic syndrome can be attributed to enhanced intrahepatic TG content partially and eventually to the enhanced rate of TG secretion via VLDL. The model-simulated increase in TG secretion also correlates well with experimental evidence [31]. Increased TG synthesized by the hepatocytes due to the abnormal fat metabolism associated with MetS did not only accumulate inside the cell but was also secreted out in the form of VLDL.

Simulations to Predict Susceptibility to DILI:

Case Study I – Effect Of Tamoxifen: Anticancer drug tamoxifen (TMX) treatment is associated with increased risk of developing fatty liver [32,33]. Our model simulation suggests a possible mechanism behind the enhanced risk of TMX induced fatty liver on overweight and obese animals with metabolic syndrome. TMX is reported to alter many enzymes (listed in Table 2) involved in the metabolism of fat and carbohydrate in the liver [7,23,34].

We have simulated the effect of TMX on the normal and MetS liver using the altered parameter values listed in Table 2. Figures 2 and 3 show the results of these simulations. Our simulations predict that TMX causes approximately a 6-fold increase in intracellular TG in MetS individuals compared to ~2- fold increase in normal (Figure 2). The abnormal fat metabolism leading to enhanced TG formation in MetS individuals is exacerbated by the inhibition of TG secretion by TMX (Figure 3). The combination of increased production and reduced secretion leads to accumulation of TG in the liver for MetS individuals due to TMX treatment.

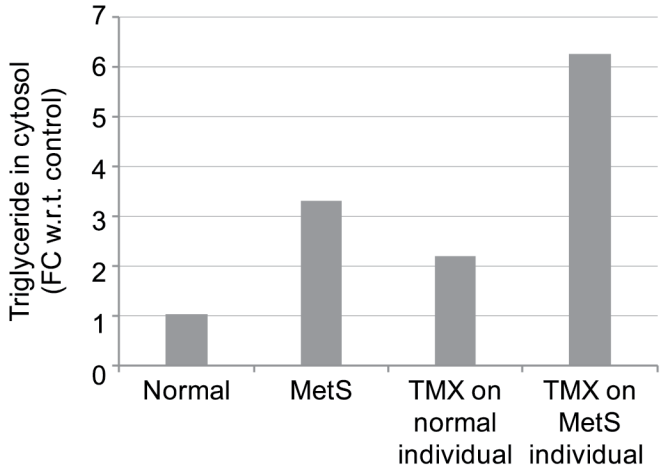

Figure 2: Simulated TG level due to TMX treatment in normal and MetS individual.

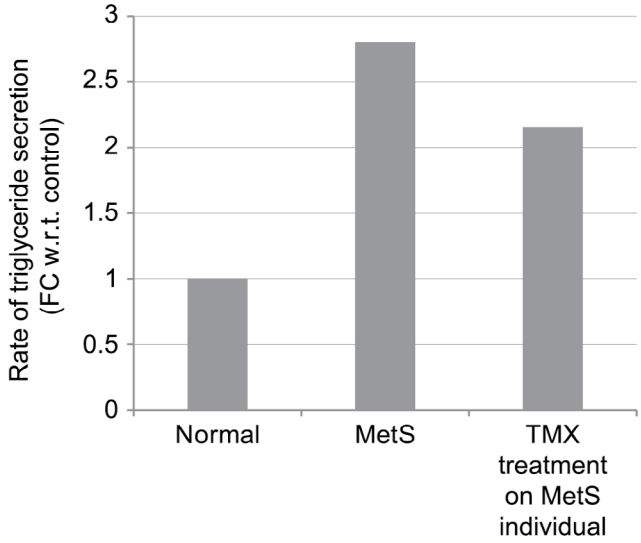

Figure 3: Simulation predicted reduction in VLDL-TG secretion from MetS liver when treated with TMX.

Case Study II – The Role Of Mitochondria In Idiosyncratic DILI: Mitochondria are commonly involved in the toxicity of many drugs and xenobiotics. It has been estimated that more than 50 million adults in the US suffer from mitochondrial dysfunction induced disease [25]. One of the causes of cytotoxic liver damage can be attributed to drug-induced inhibition of mitochondrial function

leading to cellular ATP depletion. Reduced complex I activity can enhance depletion of cellular ATP when the mitochondria become a toxic drug target leading to necrotic cell death. Individuals with an inherently compromised complex I activity may be more susceptible to drugs that target mitochondrial functions [25].

Due to the inhibition of oxidative phosphorylation, disruption in mitochondrial ATP generation appears to be a common cause of lethal cytotoxic cell injury [35]. In the MetS liver, complex I activity is already compromised (Table 1). Simulations (Figure 4) show that the extent of cytotoxic damage due to mitochondrial complex I inhibition (by the same magnitude) is more pronounced for a MetS liver than a normal liver in causing depletion of cellular ATP.

While a 50% inhibition in complex I activity in normal individuals leads to only a 7% change in intracellular ATP, under MetS condition, the reduction in ATP level is >75% (Figure 4). This implies that the impact of ATP depletion and the associated cytotoxic damage is more pronounced for a liver under metabolic syndrome. Cytotoxic damage due to ATP depletion is predicted to be ~7 fold higher for MetS liver and largely unchanged for a normal liver.

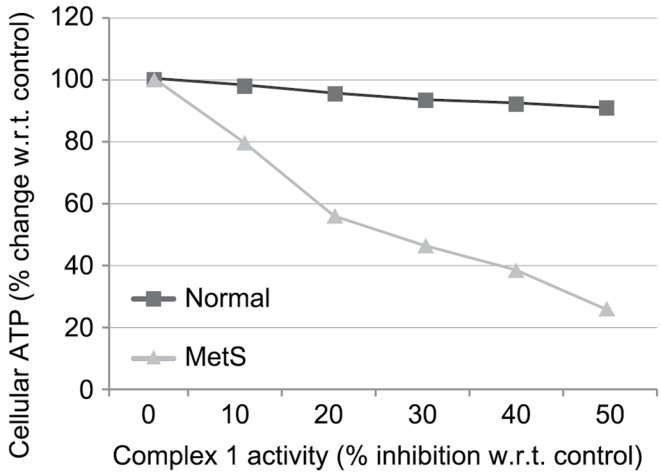

Figure 4: Reduction in cellular ATP content is more pronounced in MetS individual when mitochondrial function is perturbed due to secondary insult.

Discussion

The aim of the present study is to develop a generalized predictive platform to understand the intracellular mechanisms that drive the phenotype of a complex disease, such as MetS, and how a diseased background may have an impact on DILI predisposition.

Towards this end we have developed an *in silico* model of liver metabolism that allows researchers to perform testing using bio simulations. We started from our model of normal liver function [12] and then altered the fluxes in processes known to be affected (Table 1) in MetS to create a disease-specific model. The model-

Table 1: List of altered parameters to represent liver in metabolic syndrome

Flux/ metabolite changed	Altered parameter in the model	Fold change of altered parameter
Plasma fatty acid uptake rate	V_{max} of the fatty acid uptake transporter CD36	2
Glucose uptake rate	V_{max} of the glucose uptake transporter GLUT 2	1.4
Rate of ApoB synthesis	Rate constant	1.5
VLDL turnover to LDL	Rate constant	0.5
VLDL cholesterol uptake	Rate constant of the cholesterol uptake transporter present on cell surface	0.5
AcetylCoA carboxylase (ACC)	V_{max}	1.5
Diacylglycerol acyltransferase (DGAT)	V_{max}	1.5
Diacylglycerol cholinephosphotransferase (DCPT)	V_{max}	0.5
CTP: phosphoethanolamine cytidylyltransferase (EDT)	V_{max}	0.5
Glucose-6-phosphate dehydrogenase (G6PDH)	V_{max}	0.5
Glyceraldehyde-3-phosphate dehydrogenase (GPDH)	V_{max}	0.5
Complex 1 of ETC (electron transport chain)	Concentration of complex I	0.6
Reactive oxygen species (ROS) production	V_{max}	1.5
Glutathione reductase (GR)	V_{max}	1.5
Microsomal triglyceride transfer protein (MTTP)	V_{max}	1.5
Fatty acyl synthase (FAS)	V_{max}	1.5
Glycerol-3- phosphate acyltransferase (GPAT)	V_{max}	1.5
Plasma glucose	Concentration	1.25
Plasma palmitate	Concentration	5

Table 2: List of altered parameters to mimic effect of tamoxifen

Flux	Altered parameter	Fold change to mimic tamoxifen treatment
LDL- receptor activity	V_{max} of LDL uptake receptor	1.5
AcetylCoA carboxylase (ACC)	V_{max}	0.5
Diacylglycerol acyltransferase (DGAT)	V_{max}	2.0
Microsomal triglyceride transfer protein (MTTP)	V_{max}	0.5
Fatty acyl synthase (FAS)	V_{max}	0.6

Table 3: Differences in homeostasis in the normal and MetS liver

Metabolites/fluxes	Simulated value	Experimental value
Change in cellular triglyceride (MetS/Normal)	3.25	3.0
Change in rate of triglyceride secretion (MetS/Normal)	~ 3	3-4

simulated intracellular metabolite concentrations for the normal and MetS liver are very similar to the reported experimental observations [7]. Our simulations indicate that an obese individual with MetS phenotype is more prone to suffer TMX-induced steatosis compared to a non-MetS counterpart. This is in concordance with clinical observations that obese cancer patients treated with TMX tend to develop steatosis at a faster rate [23]. Model simulations also reveal that reduced TG secretion due to the presence of TMX compared with the increased influx of plasma fatty acids and *de novo* lipogenesis in MetS individuals lead to a large increase in intracellular TG in these individuals compared with normal.

Our liver model has been used to hypothesize that there may be forms of DILI idiosyncratic for MetS individuals alone. Hence, this type of systems model can be used to predict the involvement of associated idiosyncratic aberrations as well. While we have focused on MetS in this study, other complex diseases like cardiovascular diseases, diabetes, NASH (non-alcoholic steatohepatitis) and how they impact DILI can also be understood using a systems modeling approach. In this context, expert opinion made by Teschke et.al [36] based on available case reports clarifies that pre-existing and non-cirrhotic chronic liver diseases may make the liver prone to DILI by some but not all drugs. Due to the unavailability of a gold standard as clinical diagnostic marker for DILI assessment, the use of RUCAM, the Roussel Uclaf Causality Assessment Method, is advised to diagnose an individual's DILI [37].

The conventional animal model for MetS is corpulent (JCR:LA-cp) rat [13,38], which is studied either at the whole animal level or *in vitro* with hepatocytes derived from the diseased animal. There are several drawbacks behind these conventional approaches, including (1) difficulties in direct extrapolation of results, and (2) long and involved experimentation etc; in addition, lack of mechanistic insight making the understanding of idiosyncratic toxicity difficult if not infeasible.

Figure 4 shows a simulation representing the impact of a drug that affects mitochondrial complex I in normal and MetS patients. It has been observed that a 30-40% inhibition of complex I in the MetS individual leads to more severe cytotoxic cell death associated with profound ATP depletion when compared with normal counterparts. It can be hypothesized that a patient whose mitochondria are compromised to begin with [39], have a greater potential for necrotic DILI when they also have a concomitant MetS condition. Thus, our computational approach allows one to test multifactorial combinations of effects, genetic variations, drug (environmental) insults and physiological status to understand the molecular basis of drug-induced liver injury.

Our systems model, with its mechanistic description of metabolic fluxes, allows us to simulate the response of the liver metabolic network to changes of environmental conditions (drugs) - and cellular processes, e.g. altered gene expression. We started with a normal liver and re-parameterized it to create one representing MetS. This approach is general enough to apply to other individual level changes representing hypotheses for idiosyncratic behavior or system level changes representing altered states of disease or health (such as diabetes). Since each biosimulation provides mechanistic insights, it is possible to use such simulations to design experiments to verify biological hypotheses. For example, possible involvement of mitochondria in idiosyncratic DILI has been demonstrated by the model. Targeted experiments can now be designed to verify whether such a mechanism is responsible for a subpopulation of idiosyncratic responders, segregated based on various features of the idiosyncratic response, such as liver enzyme levels, VLDL levels, etc.

This approach can be considered extremely useful to reduce animal experimentation. Over 100 million animals are used every year in laboratory experiments worldwide Alternatives to animal use are being considered actively in many centers [40]. However, there is an inherent challenge in how to translate their results to the *in vivo* situation. In addition, toxicity responses in animals may only poorly correlate to the human organism. Our approach provides an alternative that is less time consuming and allows one to integrate information and insights from laboratory experimentation, both *in vitro* and *in vivo* to predict toxicity and ascertain a mechanistic assessment of risk.

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