COMMENTARY

Improving Interpretation of New and Old Serum Biomarkers of Drug-Induced Liver Injury Through Mechanistic Modeling

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The study by Mason et al. in this issue used mechanistic modeling and simulation to address how both the dose of acetaminophen consumed and the time since ingestion can be estimated from biomarkers measured in a single serum sample in mice. Translation into the clinic would potentially be an advance in the treatment of acetaminophen poisoning. Importantly, this approach could transform the evaluation of liver safety in clinical trials of new drug candidates. CPT Pharmacometrics Syst. Pharmacol. (2018) 7, 357–359; doi:10.1002/psp4.12303; published online 26 April 2018.

Acetaminophen overdose is the most common cause of acute liver failure in the United States and this often occurs when a person intent on doing self-harm impulsively swallows a hand-full or bottle full of acetaminophen tablets. Fortunately, many of these individuals have second thoughts and seek out medical help before the toxicity fully manifests itself and in time to receive the effective antidote (N-acetyl cysteine). The physician faced with such a patient must decide whether the patient is at high risk of developing serious liver injury without antidote treatment. This decision is currently largely based on nomograms that plot serum acetaminophen level as a function of time since the overdose ingestion, and define ranges of those two variables that are associated with high likelihood of liver injury. However, when patients do not know the exact time when they ingested the overdose, worst case scenarios must be assumed, leading to overtreatment. This is not desirable because the treatment carries some risks and involves hospitalization and its associated costs. The most obvious relevant data for determining the need for treatment is the amount of acetaminophen ingested and the time that has expired since that ingestion. However, in addition to not knowing the exact time of ingestion, many affected individuals do not know the exact dose of acetaminophen they consumed.

In this issue, Mason et al. have used modeling and simulation of data obtained in mice to address how both the dose of acetaminophen consumed and the time since ingestion can be estimated from biomarkers measured in a single serum sample. The modeling was possible because of a prior published study in which mice were administered a single toxic dose of acetaminophen and then euthanized at various times up to 24 hours. At each time point, liver histology was assessed for the extent of cell death and liver levels of glutathione were determined. The traditional serum biomarker of liver injury, alanine aminotransferase (ALT), was measured at each time point, but also measured were experimental serum biomarkers of cell necrosis (K18 and HMGB1) and apoptosis (K18 caspase cleaved fragment).

Using these mouse data and an understanding of the mechanism underlying acetaminophen toxicity (production of a reactive metabolite in excess of available glutathione), Mason et al. used a variety of approaches to generate models in which the values of the measured biomarkers considered together provided estimations of both the dose consumed and time since dose ingestion. To validate the model, they treated mice with varying doses of acetaminophen and measured the biomarkers and liver glutathione at a single time point and found values similar to those predicted by their model. Finally, they found that HMGB1 was the serum biomarker most predictive of liver injury as assessed histologically – if you estimate dose consumed and time since consumption from the models involving all the biomarkers, knowing the HMGB1 level provided the best estimate of the probability of liver injury.

The hope would now be to translate this approach to humans who are victims of acetaminophen overdose, but this may not be straightforward. For example, a sensitivity analysis revealed that the baseline glutathione level in the liver was the most influential variable in the models and, whereas this was measured directly in the mice, this will not be known and likely to vary in patients. In addition, the time course of liver injury in humans is quite different than in mice. Humans consuming toxic doses of acetaminophen generally do not develop clinical or biochemical evidence of liver injury for up to 24 hours postdose, whereas the mice showed histological evidence of hepatocyte death at 3 hours. It is encouraging that, in patients who have consumed an overdose of acetaminophen, HMGB1 seems to rise in serum prior to ALT and seems to be a better predictor of progression to severe liver injury.

Although mechanistic modeling using traditional and newer biomarkers is clearly promising to improve assessment of risk after acetaminophen overdose, applying such modeling to predict liver safety risk of new drug candidates may have a much larger public health benefit. The most problematic form of drug-induced liver injury occurs suddenly after weeks to months on treatment and is a rare
Mechanistic Modeling of Drug-Induced Liver Injury

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Figure 1 Proposed pathogenesis for idiosyncratic drug-induced liver injury. It is currently believed that in addition to producing a neoantigen in the liver, drugs causing idiosyncratic liver injury must also produce hepatocyte stress (not necessarily cell death), which results in release of danger signals that activate innate immune cells. Biomarkers that reflect each of these steps have been proposed and together with modeling and simulation approaches hold the promise of identifying unsafe drugs early in development as well as providing precision medicine strategies for risk management. Modified from ref. 8 with permission.

event. Unlike the case with acetaminophen, the relationship between dose and these idiosyncratic injuries is complex and this liability is often not suggested by preclinical toxicology studies. Drugs with this liability typically cause asymptomatic elevations in serum ALT in a small subpopulation of treated patients, but so do some drugs that do not pose a serious liver safety risk. The only current way to tell whether treatment emergent elevations in serum ALT represent harmless “transaminitis” or portend potential for serious liver injury is to perform large and prolonged clinical trials to see if serious liver injuries occur. This is costly, delays introduction of important new drugs to the market, and places clinical trial participants at risk.

Traditional liver chemistries, which have not changed in more than half a century, do not provide mechanistic insight, and their interpretation has generally been based merely on peak serum values observed. For example, according to the current US Food and Drug Administration guidance on assessing liver safety in clinical trials, it is recommended that treatment with the study drug should be discontinued if a subject’s serum ALT rises greater than 8 times the upper limits of normal (ULN). The guidance also states that the most serious liver safety signal observed in a clinical trial is a subject who experiences a rise in serum ALT exceeding 3 times the ULN and concomitant elevation in serum total bilirubin >2 times the ULN (the “Hy’s Law Case”). The idea is that the rise in serum bilirubin must be assumed to reflect substantial reduction in global liver function due to loss of hepatocytes, and this indicates life-threatening risk. However, assessing liver safety based on peak biomarker values alone ignores production and clearance kinetics, and the mechanisms underlying these. Mechanistic modeling has recently been proposed that uses serial measurements of serum ALT to estimate net hepatocyte loss (death vs. regeneration), and to define the relationship between residual hepatocyte mass and rises in serum bilirubin due to global liver dysfunction. Because release of serum ALT is reduced in hepatocyte apoptosis vs. necrosis, measurements of the serum ratio of K18 caspase cleaved fragment/K18 (the “apoptotic index”) have been recently incorporated into simulations to improve prediction of aggregate hepatocyte death occurring in some subjects participating in a clinical trial. Serum bilirubin disposition and metabolism have also been recently incorporated into simulations and can account for drug-induced increases in serum bilirubin in the absence of global liver dysfunction. In some cases, modeling and simulations have been included in communications with regulators to refine interpretation of “Hy’s Law Cases.”

However, this is only the beginning. The delayed drug-induced liver injuries seem to generally result from an adaptive immune attack on the liver. It is believed that there exist a series of necessary but not sufficient steps that must happen in the liver before an immune attack can occur. As noted in Figure 1, this involves drug-induced stress to the hepatocyte (but not necessarily hepatocyte death), release from the hepatocyte of “danger signals,” and activation of innate immune cells in the liver. Novel biomarkers, including HMGB1, have been proposed to be danger signals that can be measured in serum, and various cytokines, microRNAs, and acetylated HMGB1 have been proposed as serum biomarkers of immune cell activation. Identification of new and mechanistic biomarkers of drug-induced liver injury has been a major focus of international research efforts, including a major new effort to start in 2019. Mechanistic modeling incorporating production and release kinetics of these new biomarkers could make it possible to identify serious liver safety liability of new drug candidates early in clinical trials and with minimum subject risk and thus reducing the current need for large clinical trials to define liver safety. Furthermore, identifying susceptible patients early during their treatment and before they would develop liver injury would provide precision medicine strategies for risk management and potentially permit successful development of important new drugs with liver safety liabilities. Finally, it is likely that the requisite steps and informative biomarkers will be relevant to drug-induced toxicities involving organs other than the liver.

The article by Mason et al. is notable because it is among the first to combine mechanistic and pharmacokinetic modeling using data from some of these newer injury biomarkers to address an important human toxicity. This should be a very fruitful area for modeling and simulation in the near future.

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