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Use of a systems model of drug-induced liver injury (DILIsym®) to elucidate the mechanistic differences between acetaminophen and its less-toxic isomer, AMAP, in mice

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ABSTRACT

Acetaminophen (APAP) has been used as a probe drug to investigate drug-induced liver injury (DILI). In mice, 3′-hydroxyacetanilide (AMAP), a less-toxic isomer of APAP, has also been studied as a negative control. Various mechanisms for the divergence in toxicological response between the two isomers have been proposed. This work utilized a mechanistic, mathematical model of DILI to test the plausibility of four mechanistic hypotheses. Simulation results were compared to an array of measured endpoints in mice treated with APAP or AMAP. The four hypotheses included: (1) quantitative differences in drug metabolism profiles as a result of different affinities for the relevant enzymes; (2) differences in the amount of reactive metabolites produced due to cytochrome P450 (CYP450) inhibition by the AMAP reactive metabolites; (3) differences in the rate of conjugation between the reactive metabolites and proteins; (4) differences in the downstream effects or potencies of the reactive metabolites on vital components within hepatocytes. The simulations did not support hypotheses 3 or 4 as the most likely hypotheses underlying the difference in hepatoxic potential of APAP and AMAP. Rather, the simulations supported hypotheses 1 and 2 (less reactive metabolite produced per mole of AMAP relative to APAP). Within the simulations, the difference in reactive metabolite formation was equally likely to have occurred from differential affinities for the relevant drug metabolism enzymes or from direct CYP450 inhibition by the AMAP reactive metabolite. The demonstrated method of using simulation tools to probe the importance of possible contributors to toxicological observations is generally applicable across species.

1. Introduction

Drug induced liver injury (DILI) is a significant healthcare problem (Bleibel et al., 2007; Corsini et al., 2012; Hayashi and Watkins, 2009; Kaplowitz and DeLeve, 2013; Lee, 2003; Watkins, 2005). This is true for clinicians and patients, as many cases of liver injury, acute liver failure, liver transplant, and death are caused each year worldwide from DILI (Larson et al., 2005; Lee, 2008, 2003). DILI is equally problematic for drug developers, who commonly abandon promising drugs in the midst of development due to unexpected signs of liver injury, or worse, have drugs removed from the market after a few patients experience, rare, idiosyncratic DILI (Senior, 2007; Watkins, 2011). In an effort to resolve these issues, many researchers have studied DILI with the goal of identifying key attributes of DILI-causing agents. No exemplar hepatotoxicant has been studied more often or more thoroughly than acetaminophen (APAP). As the dominant cause of DILI cases seen clinically, including liver failure (Lee, 2008), APAP is an important drug to study for obvious reasons. APAP is also used as a probe drug to understand mechanistic linch-pins in the DILI process. This is commonly done in mice, which show a greater sensitivity to APAP-induced liver injury than rats (McGill et al., 2012).

In the midst of studying APAP in mice, an analogue of APAP, 3′-hydroxyacetanilide (AMAP), has been utilized as a comparator to APAP due to its apparent lack of liver toxicity in mice. AMAP was originally patented as a possible alternative to APAP (Nelson, 1980a). While it appears to be non-toxic in mice, AMAP has been examined in human and rat liver slices, and shown to potentially be more toxic in these species (Hadi et al., 2012). Over the past 30 years, many hypotheses describing the mechanistic underpinnings of the divergence in liver toxicity seen between APAP and AMAP in mice have been proposed by various researchers. However, one could argue that definitive conclusions have not

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yet been reached. The goal of this work was to employ a mechanistic, mathematical model of DILI (DILIsym® v1A) to test the plausibility of several of these hypotheses. The DILIsym® model allowed for comparisons between simulation results based on different hypotheses and published results on APAP/AMAP responses within a quantitative, mechanistically focused framework. While this investigation focused entirely on APAP and AMAP in mice, it represents a generally applicable approach that can be applied elsewhere in toxicology, including to human DILI events. In addition, DILIsym® can be applied to cross-species hepatotoxicity investigations, where the focus is understanding why different species respond differently to the same drug (Howell et al., 2012).

A wide variety of studies on APAP versus AMAP have been conducted. Many have focused on the in vivo metabolism of one or both of the molecules (Dai et al., 2006; Hamilton and Kissinger, 1986; Lee et al., 2009; McGill et al., 2013; Rashed et al., 1990; Vaccarino et al., 2007). Others have taken an in vitro approach to understanding metabolism and potency differences using microsomes or hepatocytes (Bauman et al., 2009; Holme et al., 1991; Rashed et al., 1989; Streeter et al., 1984). Covalent binding studies have also been a prevailing theme (Rashed et al., 1990; Roberts et al., 1990). In vivo toxicity endpoints, such as glutathione (GSH) and liver injury biomarkers, have commonly been measured (Nelson, 1980b; Priyadarsiny et al., 2008; Salminen et al., 1997; Tirmenstein and Nelson, 1989). Mechanistic investigations have also focused on drug metabolism, where enzyme inhibition studies have shown AMAP to inhibit its own reactive metabolite production (Halmes et al., 1998). More recently, investigations of downstream perturbations of liver homeostasis, such as gene expression and critical pathway analysis, have been done (Priyadarsiny et al., 2008; Salminen et al., 1997; Stamper et al., 2010). The totality of the literature was reviewed for distinct mechanistic hypotheses that could explain the divergence in toxicological response between APAP and AMAP. The various reports were narrowed to four primary hypotheses that offered explanations for the observed differences. Of the various hypotheses listed above, the four below were deemed most supported by published data and most comprehensive:

- Hypothesis 1 – the structure of AMAP lends itself to a quantitatively different drug metabolism profile than APAP (and less reactive metabolite produced as a result);
- Hypothesis 2 – the default metabolism parameters describing the conversion from parent compound to glucuronide, sulfate, and reactive metabolite conjugates are the same for APAP and AMAP, but the AMAP reactive metabolite inhibits its own production through mechanism-based inhibition of CYP2E1;
- Hypothesis 3 – the AMAP reactive metabolite binds to cellular proteins at a higher rate than the APAP reactive metabolite (and thus depletes less GSH);
- Hypothesis 4 – AMAP reactive metabolites cause injury or disrupt cellular processes in different (and less potent) ways on an equimolar basis than APAP reactive metabolites.

Hypotheses 1 and 2 can be viewed as 'upstream' or drug metabolism hypotheses, while Hypotheses 3 and 4 relate more to the action or properties of the reactive metabolites generated by APAP and AMAP.

2. Materials and methods

A mechanistic, mathematical model of drug-induced liver injury was the primary means used to accomplish the goal of this work: elucidating the most likely mechanistic explanation for why APAP and AMAP show divergent toxicological responses in mice. Accordingly, some general information about the model (DILIsym® v1A) is discussed below. The general process of simulating exposure to APAP and AMAP in mice is summarized. Next, the four hypotheses tested with the model in the baseline mouse are described in detail, including which parameters in the model were adjusted to simulate each scenario.

2.1. The DILIsym® model, version 1A

The DILI-sim Initiative is a joint effort between the Hamner Institutes for Health Sciences and the pharmaceutical industry. The goals of the DILI-sim Initiative include developing DILIsym®, a predictive, mechanistic, mathematical model of drug induced liver injury (DILI), and advancing the knowledge of DILI for all parties involved (see www.DILIsym.com for more information on the goals and scope of DILI-sim). The method for model design is best described as 'middle out.' The 'middle out' approach involves starting at the organ level (liver in this case), and working down to the molecular level or up to the organism level when necessary (Michelson et al., 2006; Shoda et al., 2010). As a result, this is a multi-scale approach, where models based on different scales are connected through scaling factors. The model is organized into various smaller sub-models, but all sub-models are mathematically integrated to simulate an organism level response. DILIsym® version 1A primarily focuses on C57Bl6 mice, Sprague Dawley rats, and humans (the mouse representation was used for this analysis). Exemplar hepatotoxic compounds were sequentially used to add mechanistic detail to the model. APAP was the first exemplar represented. DILIsym® v1A is therefore primarily a model of oxidative stress-induced hepatotoxicity.

Since the necessary drug absorption, distribution, metabolism, and excretion (ADME) sub-model frameworks were built within version 1A using APAP, this analysis of APAP versus AMAP was a natural extension of previous work. Additional DILI mechanisms have since been and are currently being added to future versions of the model. More information on DILIsym® version 1A is available through several previously published articles (Bhattacharya et al., 2012; Howell et al., 2012; Shoda et al., 2014; Woodhead et al., 2012). These articles include a more thorough description of the DILIsym® sub-models, data samples used for model optimization, and a comprehensive list of the data used for version 1A. The model is also directly available to industry through membership in the DILI-sim Initiative. Academic and non-profit groups may access the model by contacting the Hamner Institutes for Health Sciences or the corresponding author.

2.1.1. Comparing AMAP to APAP in mice in DILIsym®

The underlying assumption of the present work is that DILIsym® v1A adequately simulates a hepatotoxic event in mice arising from APAP exposure. The methods used to construct the model were discussed in several previous publications (Bhattacharya et al., 2012; Howell et al., 2012; Shoda et al., 2014; Woodhead et al., 2012). To briefly summarize the series of assumptions leading to APAP toxicity simulation in DILIsym® v1A, the inputs include the desired dosing quantity and route. With regard to this study, the simulated quantities of APAP and AMAP dosed, as well as the routes, were taken from the cited publications used for the comparison. Once the simulation is initiated, DILIsym® v1A accounts for the absorption and distribution of the drug. APAP (or AMAP) is then converted via one of three metabolic pathways: sulfation, glucuronidation, or CYP-mediated reactive metabolite production. The sulfate and glucuronide metabolites are represented as inert with regard to liver toxicity. The reactive metabolite is conjugated in two possible ways. GSH conjugation is represented with an extremely high reaction rate, but is only available in limited quantities (substrate limited). Protein adduction is also represented. Protein availability is not assumed to be a constraint, but the rate of reaction for reactive metabolite/protein conjugation is much lower than for GSH conjugation, based on published data used to optimize the rate constant (Howell et al., 2012; Woodhead et al., 2012). Once GSH depletion occurs, the reactive metabolite can build up in the representative liver tissue compartments until GSH recovery occurs or protein adduction occurs. The reactive metabolite concentration in the liver is the actual perpetrator of toxicity in DILIsym® v1A. A function connects the concentration of reactive metabolite in the liver to oxidative stress generation, which is subsequently connected to ATP production. As reactive metabolite accumulates, oxidative stress accumulates, ATP production and concentration decline, and necrosis ensues. Details on the equation structure and parameters dictating these processes are available in previously published articles (Bhattacharya et al., 2012; Howell et al., 2012; Woodhead et al., 2012) or in DILIsym® version 1A, which can be accessed as directed above. The level of mechanistic detail included in v1A is important to understand when considering each of the four hypotheses discussed below. For each hypothesis, any limitations or assumptions that could influence conclusions regarding APAP versus AMAP are pointed out.

To utilize DILIsym® v1A to identify the most likely mechanistic rationale for divergent toxicological responses for APAP versus AMAP in mice, the baseline APAP model for mice was first used. Baseline mice in the model represent 'average' or 'mean' responses when compared to a larger group of mice. For the purposes of this work, the APAP model in mouse was considered to be the starting point. Experimental protocols where APAP was administered to mice were initially simulated (Priyadarsiny et al., 2008; Rashed et al., 1990; Salminen et al., 1997; Tirmenstein and Nelson, 1989). Changes were then made to parameter values within the model in a step-wise fashion to mimic hypothesized mechanistic differences between APAP and AMAP hepatotoxicity (Hypotheses 1–4), and experimental protocols where AMAP was dosed to mice were also simulated (Priyadarsiny et al., 2008; Rashed et al., 1990; Salminen et al., 1997; Tirmenstein and Nelson, 1989). While the entirety