An Analysis of N-Acetylcysteine Treatment for Acetaminophen Overdose Using a Systems Model of Drug-Induced Liver Injury


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ABSTRACT

N-acetylcysteine (NAC) is the treatment of choice for acetaminophen poisoning; standard 72-h oral or 21-h intravenous protocols are most frequently used. There is controversy regarding which protocol is optimal and whether the full treatment course is always necessary. It would be challenging to address these questions in a clinical trial. We used DILIsym, a mechanistic simulation of drug-induced liver injury, to investigate optimal NAC treatment after a single acetaminophen overdose for an average patient and a sample population (n = 957). For patients presenting within 24 h of ingestion, we found that the oral NAC protocol preserves more hepatocytes than the 21-h intravenous protocol. In various modeled scenarios, we found that the 21-h NAC infusion is often too short, whereas the full 72-h oral course is often unnecessary. We found that there is generally a good correlation between the time taken to reach peak serum alanine aminotransferase (ALT) and the time taken to clear N-acetyl-p-benzoquinone imine (NAPQI) from the liver. We also found that the most frequently used treatment nomograms underestimate the risk for patients presenting within 8 h of overdose ingestion. \( V_{\text{max}} \) for acetaminophen bioactivation to NAPQI was the most important variable in the model in determining interpatient differences in susceptibility. In conclusion, DILIsym predicts that the oral NAC treatment protocol, or an intravenous protocol with identical dosing, is superior to the 21-h intravenous protocol and ALT is the optimal available biomarker for discontinuation of the therapy. The modeling also suggests that modification of the current treatment nomograms should be considered.

Introduction

Acetaminophen (APAP) toxicity is a leading cause of liver injury, accounting for 40 to 50% of all cases of acute liver failure in the United States (Ostapowicz et al., 2002; Larson et al., 2005). APAP poisoning is the result of a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which is formed via CYP450. In therapeutic doses, this reactive metabolite is conjugated by glutathione (GSH). However, in overdose conditions, the reactive metabolite depletes cellular GSH stores, which leads to oxidative stress, mitochondrial dysfunction, and cell death (Bajt et al., 2004).

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TREATMENT WITH N-ACETYLCYSTEINE (NAC) has been the standard therapy for APAP overdose since the 1970s (Rumack et al., 1981; Heard, 2008). NAC is actively transported into hepatocytes, where it serves as a precursor to GSH (Yang et al., 2009). Effective treatment with NAC provides sufficient GSH to neutralize NAPQI and avert oxidative stress and cell death. Clinically, there are two main treatment courses used for patients with APAP overdose, as well as an alternate intravenous bolus protocol that has been proposed (Smilkstein et al., 1991) but not widely adopted. These protocols are summarized in Table 1. Historically, the oral treatment has been used in the United States, whereas the intravenous route is typically used elsewhere. Both routes of administration have been demonstrated to be effective for most patients (Whyte et al., 2007). Some clinicians believe the optimal route depends on the gap between drug ingestion and patient presentation; the intravenous route is believed to be better soon after overdose, whereas the oral route has been pro-

ABBRVIATIONS: APAP, acetaminophen; NAPQI, N-acetyl-p-benzoquinone imine; GSH, glutathione; NAC, N-acetylcysteine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SimPops, simulated sample population; RNS-ROS, reactive nitrogen species-reactive oxygen species; INR, international normalized ratio.
posed to be better for longer delays between dose and presentation (Yarema et al., 2009). Others believe that the oral protocol should always be better, because more NAC is given over the course of the treatment (Gupta et al., 2009). Still others believe there is no difference in outcomes between the two protocols, but prefer one to the other because of practical issues with administration of treatment (Hayes et al., 2008; Buckley et al., 2010).

Modifications of the standard oral and intravenous protocols have also been proposed, including shortening the oral NAC course (Kociancic and Reed, 2003) and tailoring the duration of treatment to biomarkers of liver injury (Betten et al., 2009) or APAP blood level (Woo et al., 2000; Tsai et al., 2005). Shorter treatment protocols may be advantageous, because some research suggests that NAC may impede recovery if taken beyond its period of usefulness. For example, NAC has been found to impede hepatocyte regeneration in mice (Yang et al., 2009), and in vitro data also support this effect (Athuraliya and Jones, 2009). Furthermore, there are potential side effects to NAC treatment, as well as the cost of a prolonged hospital stay (Woo et al., 2000). It is therefore important to investigate when a shorter course of NAC treatment might be sufficient. A direct comparison of the effectiveness of different NAC protocols would be difficult to carry out in a clinical setting, because it probably would require very large numbers of subjects given varied doses of APAP, varied NAC treatment delays, varied NAC treatment lengths, and interindividual differences in susceptibility to APAP liver injury.

In this study we used DILIsym (from The Hamner Institutes, http://www.thehamner.org/dili-sim), a mechanistic computer simulation model of drug-induced liver injury, to predict the optimal NAC treatment for an average patient and also for a more varied in silico population sample. We simulated mild, moderate, and severe overdoses of APAP and intervention with three advocated protocols of NAC treatment to compare the outcomes. We also simulated variations on the duration of the treatment protocols to determine the shortest duration of treatment that preserved efficacy. We then compared treatment protocols across a simulated sample population (SimPops) to determine which treatment protocols produce better results when interpatient variability is taken into account. Finally, we investigated which parameters had the greatest impact on individual susceptibility to APAP liver injury and response to NAC treatment.

### Materials and Methods

Simulations were performed by using DILIsym, an ordinary differential equation-based model of drug-induced liver injury implemented in the MATLAB computing platform (The MathWorks, Inc., Natick, MA). The model contains 282 differential equations and consists of the following submodels: 1) a physiologically based pharmacokinetic model of APAP, NAC, and the major APAP metabolites (glucuronide, sulfate, and NAPQI); 2) a model of GSH depletion and synthesis; 3) a model of mitochondrial dysfunction based on production of reactive nitrogen and oxygen species caused by NAPQI; 4) a model of the hepatocyte life cycle and the effects of ATP depletion and mitochondrial dysfunction on cell death; 5) a model of proinflammatory, anti-inflammatory, and prregerative mediator production and effects; 6) a model of injury propagation; and 7) a model of the dynamics of serum biomarkers such as bilirubin and ALT. Further information on the model is included in the supplemental materials.

The human data used to calibrate and validate the model are pulled from several cohort studies in the literature (Gazzard et al., 1975; Portmann et al., 1975; Davidson et al., 1976; Davis et al., 1976; Schidt et al., 2001). In almost all of those studies, there were more females than males present, and age varied widely within all study groups, although all patients in the studies were adults. No data on ethnicity or other physical characteristics were reported in those studies. A more specific breakdown of the study populations used in this model is in Supplemental Table S5.

In our model, NAC alleviates acetaminophen toxicity by serving as a precursor to GSH. It does not conjugate directly with the reactive metabolite. This is consistent with the findings of Lauterburg et al. (1983), who saw no significant binding of NAC to the reactive metabolite in rats, and with currently accepted theories on the mechanism of NAC protection (Jones, 1998; Yuan and Kaplowitz, 2009; Liang et al., 2010). Other researchers have posited that NAC serves other functions, such as increasing interleukin-6 production (Masubuchi et al., 2011), serving as a scavenger of reactive oxidants (González et al., 2009), and improving liver blood flow (Jones, 1998). There is, however, insufficient data available in the literature to adequately model these hypotheses, and including these hypotheses was not necessary to construct a model that is consistent with published data on the effect of NAC treatment. Data from validation and calibration simulations, as well as a discussion of the possible effect of alternative NAC mechanisms on the model, are included in the supplemental materials.

To understand the population effects of NAC treatment for acute APAP poisoning, we created an in silico population sample (SimPops) of 957 individuals by varying 11 distinct characteristics (model parameters). The list of parameters varied and the ranges over which they were varied are listed in Table 2. The SimPops was generated by the use of a genetic algorithm, which randomly selects values of variables within specified bounds and optimizes these parameters based on the model's fit to a specified fitness function. This method is explained in depth in the supplemental materials. The comparison of our population to the results reported by Davis et al. (1976) is in Supplemental Fig. S1.

To compare our population sample response with the treatment nomograms, we ran simulations on each simulated patient at four different APAP doses (7.5, 10, 15, and 20 g) with no NAC treatment.

### Table 1

Description of NAC treatment protocols simulated in this study

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Administration Route</th>
<th>Description</th>
<th>Suggested Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Oral</td>
<td>Loading dose of 140 mg/kg followed by 70 mg/kg doses every 4 h (Yarema et al., 2009)</td>
<td>72 h</td>
</tr>
<tr>
<td>B</td>
<td>Intravenous infusion</td>
<td>Loading infusion of 150 mg/kg over 1 h, followed by 50 mg/kg over 4 h, followed by 100 mg/kg over 16 h (Stravitz et al., 2007)</td>
<td>21 h</td>
</tr>
<tr>
<td>C</td>
<td>Intravenous bolus</td>
<td>Loading dose of 140 mg/kg over 1 h followed by 70 mg/kg doses over 1 h administered every 4 h (Smilkstein et al., 1991)</td>
<td>48 h; extended to 72 h for some simulations</td>
</tr>
<tr>
<td>D</td>
<td>Intravenous infusion</td>
<td>Loading infusion of 150 mg/kg over 1 h, followed by 50 mg/kg over 4 h, followed by 418.75 mg/kg over 67 h</td>
<td>72 h</td>
</tr>
</tbody>
</table>
to identify individuals with a hepatotoxic response. Clinically, APAP hepatotoxicity is generally characterized by an ALT value of more than 100 U/liter (Rumack et al., 1981; Sivilotti et al., 2005; Green et al., 2010). In our model after a single overdose of APAP a peak ALT value of 100 U/liter corresponds to a loss of 18% of hepatocytes, so we defined as hepatotoxic any individual with less than 82% of hepatocytes viable. Furthermore, we classified any simulated individual with less than 45% remaining viable hepatocytes as having as life-threatening (severe) hepatotoxicity, because patients with this fraction of viable hepatocytes have been shown to have high mortality (Portmann et al., 1975; James et al., 2008). We then compared the predicted blood APAP concentration at 1-h intervals with the time since APAP ingestion to determine the location of each hepatotoxic, life-threatening hepatotoxic, and nonhepatotoxic simulated patient on the treatment nomograms.

Death in our model occurs when the simulated individual loses 85% of his/her viable hepatocytes. This is consistent with estimates made from analyses of liver biopsies obtained in patients with fatal and nonfatal liver injuries (Gazzard et al., 1975; Portmann et al., 1975).

Statistical analysis was performed by using JMP 9 from SAS Institute (Cary, NC). Correlation coefficients were measured by using a standard multivariate linear regression model. Statistical significance was determined by using Student’s t test. Because of the high number of data points for each of the simulated sets, SimPops results all were judged to be statistically significantly different (p < 0.05); statistical significance is therefore not presented with the SimPops data.

Results

NAC Treatment in an Average Individual. To address which NAC protocol (listed in Table 1) is most effective for the treatment of acute APAP overdose, we ran simulations of the DILIsym model for an average 70-kg human. Figure 1 shows the results of our simulation for a predicted lethal 60-g acute overdose of APAP followed by treatment with the three different NAC protocols. Each treatment was started after a delay ranging from 4 to 44 h from time of overdose. The nadir in viable hepatocyte fraction always occurred between 36 and 72 h regardless of treatment protocol or latency in starting treatment. The minimal return of hepatocyte fraction at 240 h is consistent with the data on human hepatocyte turnover, which is much slower than that observed in rodents (Portmann et al., 1975). As Fig. 1 shows, we found that the 72-h oral protocol (protocol A in Table 1) and the 48-h intravenous protocol (protocol C in Table 1) produce remarkably similar results. However, treatment with the 21-h standard intravenous protocol (protocol B in Table 1) leads to a lower fraction of viable hepatocytes after short treatment delays compared with protocols A and C. For example, when administered 4 h after overdose in our model, both protocols A and C preserved 70% of hepatocytes compared with 62% for protocol B. However, this difference diminished as the NAC treatment delay increased; if administered 34 h after overdose protocol A preserved 32% of hepatocytes, whereas protocol B preserved 31%. All three protocols failed to prevent death in the simulated average patient if administered more than 34 h after overdose.

In Fig. 1d, we show the results of our simulation for an intravenous infusion protocol similar to the 21-h intravenous protocol but where the third-stage infusion (6.25 mg/h) was extended to 67 h for a total duration of 72 h (protocol D in Table 1). Although this protocol was an improvement over protocol B at short treatment delay lengths, it remained worse than the oral protocol; at 4 h after overdose the 72-h intravenous infusion preserved 66.8% of hepatocytes versus 70% for the oral protocol.

When the APAP dose was increased to 85 g (Fig. 2) the difference between oral and 21-h intravenous NAC became more pronounced. After a 9-h delay in treatment, protocol A preserved 46.7% of hepatocytes, whereas protocol B preserved only 35.2% of hepatocytes. After 24 h protocol A preserved 32.0% of hepatocytes, whereas protocol B preserved 28.8%. Neither NAC course saved the patient if administered more than 24 h after overdose.

When we decreased the APAP dose to 30 g (Fig. 3) the difference between oral and 21-h intravenous NAC was less apparent. After a 9-h delay protocol A preserved 83.0% of hepatocytes, whereas protocol B preserved 80.1%. At 24 h after overdose both the oral and intravenous courses preserved 68.5% of hepatocytes. In addition, both treatment protocols had some positive effect on the patient even after a 44-h delay, although the effect was clearly diminished at that late stage. We see that at this dose protocol B administered with a 4-h delay was slightly less effective than the same treatment offered with a 9-h delay.

With an 85-g overdose the difference between the oral protocol A and the 21-h intravenous protocol B became more
pronounced (Table 3). Protocol B failed to save the average simulated patient if administered 4 h after the 85-g overdose (less than 15% of hepatocytes preserved; Fig. 2; Table 3). However, an interesting observation is that protocol B saved the patient if offered 9, 14, 19, or even 24 h after overdose (Fig. 3; Table 3). This suggests that the 21-h intravenous infusion course is too short when administered early to effectively treat extreme overdoses, as has been suggested by others (Doyon and Klein-Schwartz, 2009). This idea was supported by the observation that after the 85-g overdose the 72-h intravenous infusion (protocol D) saved the patient if administered 4 h after an 85-g overdose (Fig. 2d; Table 3), although it was still inferior to the oral and intravenous bolus protocols (Table 3). When administered 4 h after over-

Fig. 1. Fraction of hepatocytes viable versus time for a simulated 60-g acute overdose of APAP followed by NAC treatment given by the 72-h oral (a), standard 21-h intravenous (b), 48-h intravenous bolus (c), and 72-h intravenous (d) infusion protocols. The treatment was initiated between 4 and 44 h after overdose. A surviving hepatocyte fraction less than 15% was considered lethal (see Materials and Methods).

Fig. 2. Fraction of hepatocytes viable versus time for a simulated 85-g acute overdose of APAP followed by NAC treatment given by the 72-h oral (a), standard 21-h intravenous (b), 48-h intravenous bolus (c), and 72-h intravenous (d) infusion protocols. The treatment was initiated between 4 and 44 h after overdose.
dose protocol D preserved 39.3% of hepatocytes, and at 9 h after overdose it preserved 38.4% of hepatocytes. This was superior to protocol B, but still well below the percentage preserved by protocols A and C (Table 3).

We next investigated whether the entire 72-h oral course of NAC is always required for optimal treatment. We ran simulations for a 60-g APAP overdose with oral NAC treatment starting between 4 and 34 h after overdose and varied the total number of NAC doses administered in the treatment. These results are displayed in Fig. 4, which plots the minimum fraction of viable hepatocytes against the number of doses for different treatment delay lengths. When started 4 h after overdose, the effectiveness of NAC treatment in preserving hepatocyte viability was limited to the first 10 doses. When we began oral NAC treatment at 14 h after APAP overdose hepatocyte viability did not increase appreciably for any treatment course longer than eight doses. When treatment was started at 24 h after APAP overdose the number of useful doses was even fewer; only six doses had a beneficial effect. In each of these cases, the final dose with a therapeutic effect was the dose taken between 44 and 48 h after APAP overdose.

Table 3

<table>
<thead>
<tr>
<th>Dose (g)</th>
<th>4 h</th>
<th>14 h</th>
<th>24 h</th>
<th>34 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 g</td>
<td>0.855</td>
<td>0.801</td>
<td>0.854</td>
<td>0.809</td>
</tr>
<tr>
<td>60 g</td>
<td>0.702</td>
<td>0.626</td>
<td>0.699</td>
<td>0.668</td>
</tr>
<tr>
<td>85 g</td>
<td>0.534</td>
<td>D</td>
<td>0.527</td>
<td>0.353</td>
</tr>
</tbody>
</table>

At 85 g D indicates that the simulated patient died despite treatment (i.e. reached a fraction of viable hepatocytes of ≤0.15).

Fig. 3. Fraction of hepatocytes viable versus time for a simulated 35-g acute overdose of APAP followed by NAC treatment given by the 72-h oral (a), standard 21-h intravenous (b), 48-h intravenous bolus (c), and 72-h intravenous (d) infusion protocols. The treatment was initiated between 4 and 44 h after overdose.

Fig. 4. Minimum fraction of hepatocytes viable after oral NAC treatment with a varying number of doses. Simulated APAP doses given were 60 g (a) and 85 g (b). NAC treatment was delayed by 4, 14, and 24 h.
If we increase the APAP dose to 85 g we see a larger number of NAC doses having a therapeutic effect. When oral NAC was started 4 h after an 85-g overdose 14 doses increased the minimum fraction of viable hepatocytes (Fig. 4b). If oral NAC was started 14 h after overdose 12 doses were useful; at 24 h after overdose only 10 doses were useful. In each of these cases, the final dose with a therapeutic effect was taken between 60 and 64 h after overdose.

The intravenous bolus protocol (protocol C) had the same number of effective doses as the oral course for all delay lengths investigated (data not shown). Because the intravenous bolus protocol administers the same amount of NAC as the oral protocol at each dose, this indicates that the route of administration of NAC does not matter. It is noteworthy that with continuous intravenous infusion protocols (protocols B and D) the maximum effective treatment duration was longer than the maximum effective length of the oral NAC or bolus intravenous courses (Fig. 5). If started 4 h after an 85-g overdose intravenous infusion treatment was useful up to 73 h after overdose, and if started 14 h after overdose it was useful up to 71 h after overdose. At 24 h after overdose the treatment was useful until 73 h after overdose.

We also investigated the length of time the reactive metabolite NAPQI was simulated to remain in the liver as well as the amount of time required for GSH levels to return to baseline. In cases where protocol A was administered between 4 and 34 h after an 85-g overdose the predicted NAPQI concentration in the liver fell to zero at 60 h (Supplemental Fig. S2), and the GSH was replenished between 28 h (for the 4-h delay) and 44 h (for the 34-h delay) before rising above baseline levels. For the 60-g overdose NAPQI was eliminated from the liver at 48 h after overdose for all lengths of NAC delay, and the GSH was replenished between 22.5 h (for the 4-h delay) and 40 h (for the 34-h delay). The NAPQI residence time in the liver varied with the infusion length when intravenous NAC was administered; however, for the longest NAC infusions NAPQI was cleared from the liver at 48 h after overdose (Supplemental Fig. S3).

**Population Effects.** Reaction to APAP overdose and NAC treatment is likely to highly depend on variability within the human population. As such, it is important when considering a clinical treatment to look not only at the effects of that treatment on the average human but also on the most and least susceptible members of a large population.

We first sought to compare our results to the treatment nomograms that are often used in the clinic to determine whether a patient is at risk for hepatotoxicity and thus needs NAC treatment. Treatment nomograms involve plotting the plasma acetaminophen concentration of the patient versus the estimated time since overdose and comparing where this point falls on the plot relative to a “treatment line.” The most well-known of these nomograms is the Rumack-Matthew nomogram, where the treatment line begins at a blood APAP concentration of 200 μg/ml (at 4 h after APAP ingestion); at the Food and Drug Administration’s request, this line was later lowered to begin at 150 μg/ml (Rumack et al., 1981). Modifications to the Rumack-Matthew nomogram are fairly common in clinical practice; a survey of Australasian hospitals by Reid and Hazell (2003) showed that the treatment line varied among hospitals, with some choosing a 100 μg/ml line, some the 150 line, and others the 200 line.

We administered four doses of APAP (7.5, 10, 15, and 20 g) to our SimPops virtual population of 957 individuals. At 7.5 g 1.04% of the population developed hepatotoxicity (i.e., a viable hepatocyte fraction of ≤82%), and at 20 g 56.6% of the population developed hepatotoxicity. We plotted the APAP concentration of each patient at 1-h intervals from 4 to 24 h after overdose. Over all four doses investigated, we found that 9.31% of individuals whose blood APAP concentrations fell below the 150 line developed hepatotoxicity, whereas 3.19% of those falling below the 100 line developed hepatotoxicity. However, there is a difference in risk between patients presenting at 4 h and patients presenting at 24 h. At 4 h after overdose 17.16% of individuals falling below the 150 line developed hepatotoxicity, whereas 5.21% of individuals falling below the 150 line at 24 h after overdose developed hepatotoxicity. A similar disparity was present for the 100 line; 6.28% of individuals below the 100 line at 4 h after overdose developed hepatotoxicity, whereas only 2.33% of individuals below the 100 line at 24 h developed hepatotoxicity. The risk of hepatotoxicity below the 150 line for our simulated patients declined to 7.77% at 12 h after overdose.

To explain the increase in hepatotoxic risk for individuals falling below the nomogram line at early presentation, we compared the APAP pharmacokinetics of the minimum hepatotoxic dose to the modeled average human (17 g; peak ALT = 1000 U/liter) to the nomogram lines (Fig. 6). The time course of APAP concentration has a flatter trajectory than the nomogram treatment lines until approximately 12 h after overdose; in fact, the average model human concentration-time curve crosses over the 200 line at approximately 8 h after overdose. This demonstrates that the slope of the current nomogram lines does not reflect the pharmacokinetics of APAP in the blood, and because of this, an early presenting patient whose blood APAP concentration is initially below the nomogram lines and would not receive NAC may actually
have a blood APAP concentration in the at-risk region of the nomogram at a later time. The validation of our model APAP pharmacokinetics is shown in the supplemental materials.

**NAC Treatment on a Sample Population.** We modeled the response of the simulated population to 30- and 60-g acute overdoses of APAP with 1) no NAC treatment, 2) oral NAC treatment using protocol A, 3) intravenous infusion NAC treatment using protocol D, or 4) intravenous bolus NAC treatment using protocol C extended to 72 h. The effects of these treatment courses on the simulated population are described in Supplemental Table S1. As with the average patient, protocols A and C performed better than protocol D when administered to the simulated population soon after overdose. After a 30-g overdose and a 4-h treatment delay 11.1% of the population developed hepatotoxicity after protocol A compared with 11.5% after protocol C and 22.9% after protocol D. However, when administered with a 14-h delay the difference among the treatment protocols shrank substantially; 37.1% of the simulated population developed hepatotoxicity after treatment with protocol A compared with 37.7% of those who received protocol C and 38.7% of those who received protocol D.

After a 60-g overdose, the oral and intravenous bolus courses outperformed the intravenous infusion course at delay lengths of 4 and 14 h. After a 14-h delay 13.8% of the simulated population that received protocol A developed severe hepatotoxicity compared with 14.3% after protocol C and 18.6% after protocol D. For comparison, we also simulated protocol B; after a 60-g overdose and a 14-h treatment delay as expected from our simulations in the average individual, this protocol was the least efficacious because 22.4% of the simulated population developed severe hepatotoxicity. In addition, we investigated the effect of using a shorter oral NAC course on the population; we found that there was little difference between a 12-dose course and the standard 18-dose course when treatment began 4 or 24 h after either a 30- or 60-g overdose.

**Biomarker Analysis.** Woo et al. (2000) suggested the use of serum APAP concentration as a biomarker to guide the termination of NAC treatment. Others have suggested that treatment may be safely stopped once serum ALT or AST have reached their peak and are decreasing or below a certain value (Betten et al., 2009). Based on the assumptions in the DILIsym model, NAC is no longer effective once the reactive metabolite NAPQI has cleared from the liver; as such, we examined the correlation between liver NAPQI and serum concentrations of APAP and aminotransferases in our virtual patient population as a function of dose (60 and 30 g) and treatment latency (4, 14, 24, 34, and 44 h). We then compared the time required for liver NAPQI levels to reach 1% of its peak value to the time required for blood APAP concentration to fall to 5 μg/ml or when the peak ALT/AST concentration was reached. When the time required for NAPQI to reach 1% of its peak value is less than the time required for the biomarker to reach its critical value guiding therapy by this biomarker should overtreat the patient; when the time required for the biomarker to reach its critical value is shorter the patient should be undertreated. The comparison of the time required for NAPQI to drop below 1% of its peak value with the time required for blood APAP concentration to drop below 5 μg/ml is in Fig. 7a; statistics for the biomarker analysis can be found in Supplemental Table S2. Only 0.16% of patients would be undertreated by stopping NAC treatment when blood APAP is below 5 μg/ml (and none would be undertreated more than 4 h, meaning a missed oral or intravenous bolus dose). The maximum overtreatment is 48 h, and the median overtreatment is 14 h. At a 24-h delay length, however, 4.60% of patients were undertreated (0.21% more than 4 h), and at a 44-h delay length 73.9% of patients were undertreated (55.38% more than 4 h). Because APAP is often undetectable in the bloodstream so long after overdose, it is not surprising that this biomarker is a weak one at long delay times.

Serum ALT performed better than blood APAP concentration at the 30-g APAP dose. Using peak ALT (Fig. 7b) undertreated the fewest patients (maximum of 1.88% at 4 h after overdose), but median overtreatment time using ALT was 15 to 20 h, which is the worst of the four biomarkers. Using peak AST also undertreated 1.88% of patients at 4 h after overdose, but only had a median overtreatment time of 16.6 h at 4 h after overdose and 7.15 h at 44 h after overdose (Fig. 7c; Supplemental Table S2). It is noteworthy that using blood bile acid concentration (Fig. 7d) undertreated 7.84% of patients after a 4-h delay (although only 0.10% for more than 4 h), but the median overtreatment time was between 2 and 3 h for all treatment delays, making it the best of the bio-
markers by that measure. We also tracked peak international normalized ratio (INR), which is a commonly used clinical biomarker for the cessation of NAC treatment (Fig. 7e). We found that using INR only undertreated 0.10% of the population; however, the median overtreatment with peak INR was between 56 and 59 h, which is far greater than any other biomarker examined. Further discussion of INR as it relates to NAC treatment can be found in the supplemental materials.

After a 60-g overdose, however, the results were different. Stopping treatment at serum APAP concentration of 5 μg/ml (Fig. 8a) undertreated only 7.21% of the population after a 44-h treatment delay, and using peak ALT (Fig. 8b) undertreated no one at any delay length. Conversely, using peak AST (Fig. 8c) undertreated between 6 and 19% of the population, whereas using peak blood bile acids (Fig. 8d) undertreated between 5 and 10% of the population (Supplemental Table S2). Like ALT, using peak INR (Fig. 8e) did not undertreat anyone; however, the mean and median overtreatment with INR was approximately twice the mean and median overtreatment for peak ALT (Supplemental Table S2).

We also measured the ideal treatment length for each patient, defined as the length of time between the initiation of NAC treatment and clearance of NAPQI from the liver (data not shown). Even for a massive overdose, the ideal treatment time does not extend far beyond the current clinical recommendations; after a 100-g overdose, the longest ideal treatment time in our simulated population was 81 h for NAC administered after a 4-h delay and 63 h for NAC administered after a 24-h delay. According to our model, the current 72-h clinically recommended oral course would undertreat 1.57% of the population when administered 4 h after a 100-g APAP overdose.

Parameter Correlation Comparison. To determine which variables in the population model had the greatest effect on the hepatocyte fraction, we performed a multiple regression analysis both without NAC and with oral NAC treatment with simulated patient characteristics (Table 4). We found that the 10 parameters that varied continuously (excluding sex, which is binary) over the population varied in terms of correlation with hepatocyte fraction. With no NAC treatment the most important variables were those directly related to toxic metabolite production and effects: NAPQI $V_{max}$ (CYP2E1 activity) > or $\sim$ RNS-ROS generation $V_{max}$ > ATP decrement $V_{max}$ > injury propagation trigger point. Other less important factors were: body mass $\sim$ GSH baseline level $\sim$ GSH transport $V_{max}$. Variation in sulfation $V_{max}$ and APAP uptake rate had no effect on hepatotoxicity susceptibility.

With oral NAC dosing (protocol A) the effects of the variables were similar, but glucuronidation $V_{max}$, baseline GSH concentration, and GSH transport $V_{max}$ were generally less important. The length of treatment delay changed the level of correlation between some variables and hepatocyte fraction. The correlation coefficient for the injury propagation trigger point was higher after a 34-h NAC treatment delay ($r = 0.3500$; third) than after a 4-h delay ($r = 0.1243$; fifth). It is also interesting to note that the GSH precursor transport $V_{max}$ correlation coefficient was essentially not correlated with hepatocyte fraction after a 4-h treatment delay ($p = 0.1261$), but was the fifth most important variable after a 34-h treatment delay ($r = 0.1843; p < 0.0001$). The relative
importance of the correlation coefficients and the differences among coefficients at different lengths of treatment delay were similar after a 60-g overdose.

**Discussion**

There is controversy about whether oral or intravenous NAC provides the best protection against APAP toxicity and when completing the full protocol is unnecessary. We addressed these questions by using DILIsym, a computer model based on current knowledge about the mechanisms underlying APAP toxicity. Computer modeling can simulate experiments and measurements that would be impossible on human subjects; as such, potentially important insights can be gained from such a mechanistic computer model.

Our principal conclusion is that the 72-h oral course (protocol A) is superior to the 21-h intravenous course (protocol B) when treatment is started within 24 h of overdose. There is little difference between protocol A and the intravenous bolus protocol C in the outcome of the simulated patient for all APAP doses and treatment latencies. Because these two protocols administer NAC at similar rates, the difference in effectiveness between standard oral and intravenous NAC courses is not method of delivery but rather the duration and structure of the treatment. As Fig. 9 shows, there is a significant difference in the total amount of NAC administered by the standard oral and intravenous infusion protocols beyond 8 h after starting treatment, and this may become more important the earlier NAC is administered.

It is noteworthy that the opposite conclusion concerning the merits of oral versus intravenous NAC was reached based on a prospective analysis of 4048 patients (Yarema et al., 2009). Those investigators found that, for patients treated <12 h postingestion, the incidence of hepatotoxicity was lower in those receiving intravenous treatment.

**TABLE 4**

Correlation coefficients (R) between each varied parameter and the fraction of hepatocytes viable

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<thead>
<tr>
<th>APAP Dose</th>
<th>Treatment Delay</th>
<th>Body Mass</th>
<th>GSHo</th>
<th>APAP Uptake Rate</th>
<th>Glucuronidation V\text{\textsubscript{max}}</th>
<th>Sulfation V\text{\textsubscript{max}}</th>
<th>NAPQI V\text{\textsubscript{max}}</th>
<th>RNS-ROS V\text{\textsubscript{max}}</th>
<th>ATP Decrement V\text{\textsubscript{max}}</th>
<th>Propagation K\text{\textsubscript{m}}</th>
<th>GSH Transport V\text{\textsubscript{max}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 g 4 h</td>
<td>0.2603*** 0.0329</td>
<td>-0.0046</td>
<td>0.0040***</td>
<td>0.0117</td>
<td>-0.5427*** -0.4525***</td>
<td>-0.2780*** 0.1343***</td>
<td>0.0775***</td>
<td></td>
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</tr>
<tr>
<td>30 g 34 h</td>
<td>0.1234*** 0.1089***</td>
<td>-0.060</td>
<td>-0.0227</td>
<td>0.0331</td>
<td>-0.4469*** -0.4926***</td>
<td>-0.3099*** 0.3500***</td>
<td>0.1843***</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>60 g 4 h</td>
<td>0.1618*** 0.0555</td>
<td>-0.0159</td>
<td>-0.0266</td>
<td>0.0281</td>
<td>-0.5249*** -0.4328***</td>
<td>-0.2789*** 0.3137***</td>
<td>0.0285</td>
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</tr>
<tr>
<td>60 g 34 h</td>
<td>-0.1000 0.0800</td>
<td>-0.0333</td>
<td>-0.0282</td>
<td>0.0004</td>
<td>-0.4095*** -0.5803***</td>
<td>-0.3026*** 0.2336***</td>
<td>0.0970***</td>
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<tr>
<td>15 g No NAC</td>
<td>0.2269*** 0.1167***</td>
<td>-0.0173</td>
<td>0.0239***</td>
<td>0.0538</td>
<td>-0.5323*** -0.4385***</td>
<td>-0.2928*** 0.1168***</td>
<td>0.1812***</td>
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</tr>
<tr>
<td>20 g No NAC</td>
<td>0.2070*** 0.1316***</td>
<td>-0.0224</td>
<td>0.0099***</td>
<td>0.0591</td>
<td>-0.5087*** -0.4480***</td>
<td>-0.3147*** 0.1696***</td>
<td>0.1938***</td>
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<tr>
<td>30 g No NAC</td>
<td>0.1504*** 0.1138***</td>
<td>-0.0143</td>
<td>-0.0097***</td>
<td>0.0428</td>
<td>-0.4710*** -0.4743***</td>
<td>-0.3122*** 0.3258***</td>
<td>0.2228***</td>
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</tr>
<tr>
<td>60 g No NAC</td>
<td>-0.0152*** 0.0722</td>
<td>-0.0385</td>
<td>-0.0414</td>
<td>0.0059</td>
<td>-0.4284*** -0.5223***</td>
<td>-0.2842*** 0.1670***</td>
<td>0.1536***</td>
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</tr>
</tbody>
</table>

*** denotes a statistically significant correlation (p < 0.001).
ever, the orally and intravenously treated cohorts were different; the former was from the United States, and the latter was in Canada, and the median time from ingestion to NAC initiation differed significantly. Furthermore, the details of the intravenous protocol referenced in this paper were not provided. In preliminary modeling, we explored whether addition to our model of direct antioxidant effects of NAC independent of GSH repletion (González et al., 2009) altered our conclusions, and it did not (Supplemental Figs. S27 and S28). Although we would not at this time recommend a change in clinical practice based solely on our modeling, DILIsym provides a mechanistic, data-based, and rigorous approach to the problem, and our results should prompt reexamination of relevant clinical data.

We also examined biomarkers that might predict when treatment can be safely terminated. Others suggest that when blood APAP drops below detectable levels NAC should be stopped (Woo et al., 2000). We found that blood APAP is a reasonable biomarker of hepatic NAPQI when NAC is administered <24 h after overdose; however, if NAC is administered >24 h after overdose, APAP will often be eliminated from the bloodstream before NAPQI has completely cleared the liver. Following APAP blood levels in this instance would lead to undertreatment of many patients.

We found improved performance with serum ALT. We demonstrated that terminating treatment once peak ALT is observed will prevent undertreatment for most patients; using AST in this context presents a greater risk of undertreatment presumably because of its shorter half-life in serum. However, terminating NAC treatment after peak ALT is observed will lead to ≥20 h of unnecessary treatment for some patients. Because overtreatment is preferable to undertreatment, our results support the use of ALT to guide therapy termination. Because simulations suggest that extending NAC treatment beyond 72 h generally provides no additional benefit, terminating NAC treatment at 72 h regardless of ALT measures would help minimize overtreatment issues.

Peak serum bile acid concentration also seemed to be a promising biomarker for stopping NAC treatment, resulting in the lowest median overtreatment. These observations should be interpreted with caution. Our model of bile acid homeostasis is based on physiological values for bile acid concentrations in the blood and the liver and tracks well with dose-response results from patients with APAP overdose (James et al., 1975). However, we were unable to find reports of a time course of blood bile acids after APAP overdose, and as such we do not know whether the time course of blood bile acids in our model is an accurate representation of what is seen in the clinic. The point is moot from a clinical perspective because serum assays of bile acids are slow and not generally available in the clinic.

Some clinicians suggest using the INR as a biomarker for stopping NAC treatment (Betten et al., 2009). We found that the INR does not undertreat anyone, but is the worst biomarker for overtreatment. Furthermore, NAC acts to prolong the INR (Jepsen and Hansen 1994), and the INR can be increased in patients with APAP overdose without hepatotoxicity after NAC treatment (Schmidt et al., 2002; Lucena et al., 2005).

The DILIsym model predicted that the initiation of NAC therapy is not effective in the average human when administered >34 h after an overdose (however, some members of a sample population benefit from treatment starting as late as 44 h after overdose). This is true despite the fact that the model predicted that NAPQI has not cleared the liver by then. In addition, DILIsym indicated that NAC therapy, once started, can be beneficial up to 72 h after overdose. The explanation for these apparently discordant observations is the critical role of the injury propagation response. Limaye et al. (2003) noted that propagation effects continue in liver injury after the toxic compound has cleared the liver and probably are a contributor to lethality. Mehendale and Limaye (2005) implicate calpain as a contributor to this propagation effect; others point out that inflammation (Tukov et al., 2006) or communication through gap junctions (Patel et al., 2012) may play a role in propagation. In our model, the propagation effect is modeled by a steep Hill function that represents a “trigger” determined by the size and duration of the necrotic flux. In both the simulated fatal overdoses of 60 and 85 g the propagation effect is triggered at 48 h; after this time it is injury propagation that leads to death, not direct toxicity from NAPQI. Our model predicts that NAC treatment must begin before 34 h after overdose in the average patient to provide enough therapeutic effect to avoid the injury propagation that leads to death; however if propagation does not occur, as with nonlethal doses of APAP, less-susceptible individuals, or when NAC has been started in time, the continuation of NAC treatment can be effective until NAPQI is cleared from the liver.

The hypothesis that NAC treatment must be administered early enough to prevent injury propagation is reinforced by the increased importance of the location of the propagation trigger when NAC is administered late. In our model, after a 30-g overdose the correlation coefficient of the variable controlling the location of the trigger point increased almost 3-fold when NAC was administered at 34 h after overdose compared with a 4-h delay. This is likely because early administration of NAC allows the hepatocyte GSH to replenish fast enough to eliminate NAPQI and prevent the level of injury necessary for propagation in all but the most susceptible individuals. However, the later NAC is administered the more likely it is that the necrotic flux will reach the range over which the trigger point location varies within the population. When NAC is delayed 34 h hepatocyte injury is already well underway, and the die is cast on whether the propagation response will occur.

We also found that variation in $V_{\text{max}}$ for NAPQI formation, which should correlate chiefly with hepatic activity of the
enzyme CYP2E1 was the most significant factor underlying susceptibility to APAP toxicity. This is consistent with the observation that chronic alcoholics, who have generally higher CYP2E1 activity (Hu et al., 1995), are at increased risk of APAP hepatotoxicity (Ali et al., 2008).

We also used DILIsym to assess the adequacy of nomograms used to determine which patients receive NAC treatment. It has been established that the 150 μg/ml line popularized by Rumack and Matthew is inappropriate for highly susceptible patients (James et al., 2008; Ali et al., 2008). We found that using the 100 line to determine treatment initiation led to only 3.19% of simulated patients developing hepatotoxicity. We also noted that patients who presented early (4–8 h after overdose) and seemed on the nomogram below the 100 line (and would likely not receive NAC therapy) had a higher risk of hepatotoxicity than patients who presented below the 100 line later. In our model, this observation can be explained by the slow absorption of very large doses of acetaminophen, which delays the fall in serum APAP concentrations. This suggests that patients with early presenting APAP overdose who fall below but near either the 100 or 150 nomogram lines should be considered for NAC therapy.

In conclusion, DILIsym predicts that the standard oral NAC treatment protocol, or an intravenous protocol with identical dosing, is superior to the standard 21-h intravenous protocol in preventing hepatotoxicity after acetaminophen overdose. The modeling also indicates that because of the slow absorption of large APAP overdoses, current treatment nomograms may underestimate the risk of hepatotoxicity for some early presenting patients. Once NAC therapy is initiated, the modeling indicates that serum ALT is the optimal available biomarker to guide safe discontinuation of the therapy.

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Authorship Contributions

Participated in research design: Woodhead, Howell, Yang, Harrill, Clowes, Andersen, Silser, and Watkins.
Conducted experiments: Harrill.
Performed data analysis: Woodhead.
Wrote or contributed to the writing of the manuscript: Woodhead, Howell, Andersen, Silser, and Watkins.

References

Hu Y, Ingelman-Sundberg M, and Lindros KO (1995) Induction mechanisms of cytochrome P450 2E1 in liver: interplay between ethanol treatment and starv-...


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