An Analysis of N-Acetylcysteine Treatment for Acetaminophen Overdose

Using a Systems Model of Drug-Induced Liver Injury

Jeffrey L. Woodhead, Brett A. Howell, Yuching Yang, Alison H. Harrill, Harvey J. Clewell III, Melvin E. Andersen, Scott Q. Siler, Paul B. Watkins

The Hamner-UNC Institute for Drug Safety Sciences
The Hamner Institutes
6 Davis Drive
Research Triangle Park, NC 27709 (JW, BH, YY, AH, HC, MA, SS, PW)
Schools of Medicine and Pharmacy, University of North Carolina, Chapel Hill, NC. 27514 (PW)
NAC treatment analysis using a systems model of the liver

Corresponding author: Paul B. Watkins
The Hamner Institutes for Health Sciences
6 Davis Drive, Box 12137
Research Triangle Park, NC 27709
Phone: (919) 226-3140
Fax: (919) 226-3150
E-mail: pwatkins@thehamner.org

Pages of text: 17
Tables: 4
Figures: 8
Abstract words: 250
Introduction words: 641
Discussion words: 1500

APAP: acetaminophen
DILI: drug-induced liver injury
NAC: N-acetyl cysteine

Section: other
Abstract

N-acetylcysteine (NAC) is the treatment of choice for acetaminophen poisoning; standard 72-hour oral or 21-hour intravenous protocols are most frequently employed. 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 employed DILIsym™, a mechanistic simulation of drug-induced liver injury, to investigate optimal NAC treatment after a single acetaminophen overdose for an average patient and for a sample population (n=957). For patients presenting within 24 hours of ingestion, we found that the oral NAC protocol preserves more hepatocytes than the 21-hour intravenous protocol. In various modeled scenarios, we found that the 21-hour NAC infusion is often too short, while the full 72-hour 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 NAPQI from the liver. We also found that the most frequently employed treatment nomograms underestimate risk for patients presenting within 8 hours of overdose ingestion. $V_{max}$ 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 hour iv protocol and that 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 40-50% of all cases of acute liver failure in the United States (Larson et al. 2005), (Ostapowicz et al. 2002). APAP poisoning is the result of a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), 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).

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 IV 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, while the IV 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; IV is believed to be better soon after overdose, while oral has been proposed to be better for longer delays between dose and presentation (Yarema et al. 2009). Others believe that the oral protocol should always be better, since 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 (Buckley et al. 2010), (Hayes et al. 2008).

Modifications of the standard oral and iv 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 to APAP blood level (Tsai et al. 2005), (Woo et al. 2000). Shorter treatment protocols may be advantageous because some recent 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, as it would probably 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 use DILIsym™, a mechanistic computer simulation model of drug-induced liver injury, to predict the optimum NAC treatment for an average patient and also for a more varied in silico population sample. We simulate mild, moderate, and severe overdoses of APAP and intervention with three advocated protocols of NAC treatment in order to compare the outcomes. We also simulate variations on the duration of the treatment protocols in order to determine the shortest duration of treatment that preserved efficacy. We then compare treatment protocols across a simulated sample population (SimPops™) in order to determine what treatment protocols produce better results when interpatient variability is taken into account. Finally, we investigated what parameters have the greatest impact on individual susceptibility to APAP liver injury and response to NAC treatment.

Methods

Simulations were performed using DILIsym™, an ordinary differential equation-based model of drug-induced liver injury implemented in the MATLAB computing platform (The MathWorks, Natick, MA). The model contains 282 differential equations and consists of the following submodels: a) a physiologically-based pharmacokinetic (PBPK) model of APAP, NAC, and the major APAP metabolites (glucuronide, sulfate and NAPQI); b) a model of GSH depletion and synthesis; c) a model of mitochondrial dysfunction based on production of reactive nitrogen and oxygen species due to NAPQI; d)
a model of the hepatocyte life cycle and the effects of ATP depletion and mitochondrial dysfunction on cell death; e) a model of pro-inflammatory, anti-inflammatory, and pro-regenerative mediator production and effects; f) a model of injury propagation; and g) a model of the dynamics of serum biomarkers such as bilirubin and ALT. Further information on the model is included in the supplementary materials.

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

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., who saw no significant binding of NAC to the reactive metabolite in rats (Lauterburg et al. 1983), and with currently accepted theories on the mechanism on NAC protection (Yuan and Kaplowitz 2009), (Jones 1998), (Liang et al. 2010). Other researchers have posited that NAC serves other functions, such as increasing IL-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 supplementary materials.

In order 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 3. 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 supplementary materials. The comparison of our population to the results reported by Davis et al is in Figure S1 in the supplementary materials.

In order to compare our population sample response to the treatment nomograms, we ran simulations on each simulated patient at four different APAP doses (7.5g, 10g, 15g, and 20g) with no NAC treatment to identify individuals with a hepatotoxic response. Clinically, APAP hepatotoxicity is generally characterized by an ALT value of greater than 1000 U/L (Rumack et al. 1981), (Green et al. 2010), (Sivilotti et al. 2005). In our model after a single overdose of APAP a peak ALT value of 1,000 U/L 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 (James et al. 2008), (Portmann et al. 1975). We then compared the predicted blood APAP concentration at 1-hour 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 their viable hepatocytes. This is consistent with estimates made from analyses of liver biopsies obtained in patients with fatal and non fatal liver injuries (Portmann et al. 1975), (Gazzard et al. 1975).

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

**Results**
NAC Treatment in Average Individual

In order to address which NAC protocol (listed in Table 1) is most effective for treatment of acute APAP overdose, we ran simulations of the DILIsym™ model for an average 70kg 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 hours from time of overdose. The nadir in viable hepatocyte fraction always occurred between 36 and 72 hours regardless of treatment protocol or latency in starting treatment. The minimal return of hepatocyte fraction at 240 hrs is consistent with the data on human hepatocyte turnover, which is much slower than observed in rodents (Portmann et al. 1975). As Figure 1 shows, we found that the 72-hour oral protocol (Protocol A in Table 1) and the 48-hour IV protocol (Protocol C in Table 1) produce remarkably similar results. However, treatment with the 21-hour standard IV protocol (Protocol B in Table 1) leads to a lower fraction of viable hepatocytes after short treatment delays when compared to Protocols A and C. For example, when administered 4 hours after overdose in our model, both Protocols A and C preserve 70% of hepatocytes, compared with 62% for Protocol B. However, this difference diminishes as the NAC treatment delay increases; if administered 34 hours after overdose, Protocol A preserves 32% of hepatocytes, while Protocol B preserves 31%. All three protocols fail to prevent death in the simulated average patient if administered more than 34 hours after overdose.

In Figure 1d), we show the results of our simulation for an IV infusion protocol similar to the 21-hour IV protocol but where the third stage infusion (6.25 mg/ hour) is extended to 67 hours for a total duration of 72 hours (Protocol D in Table 1). While this protocol is an improvement over Protocol B at short treatment delay lengths, it remains worse than the oral protocol; at 4 hours after overdose, the 72-hour IV infusion preserves 66.8% of hepatocytes vs. 70% for the oral protocol.

When the APAP dose is increased to 85g (Figure 2), the difference between oral and 21-hour IV NAC becomes more pronounced. After a 9-hour delay in treatment, Protocol A preserves 46.7% of hepatocytes, while Protocol B only preserves 35.2% of hepatocytes. After 24 hours, Protocol A preserves...
32.0% of hepatocytes while Protocol B preserves 28.8%. Neither NAC course saves the patient if administered more than 24 hours after overdose.

When we decrease the APAP dose to 30g (Figure 3), the difference between oral and 21-hour IV NAC is less apparent. After a 9-hour delay, Protocol A preserves 83.0% of hepatocytes, while Protocol B preserves 80.1%. At 24 hours after overdose, both the oral and IV courses preserve 68.5% of hepatocytes. In addition, both treatment protocols have some positive effect on the patient even after a 44-hour delay, though the effect is clearly diminished at that late stage. We see that at this dose, Protocol B administered with a 4 hour delay is slightly less effective than the same treatment offered with a 9-hour delay.

With an 85 gram overdose, the difference between the oral protocol A and the 21 hr iv protocol B becomes more pronounced (Table 2). Protocol B now fails to save the average simulated patient if administered 4 hours after the 85g overdose (less than 15% of hepatocytes preserved; Figure 2, Table 2). However, an interesting observation is that protocol B saves the patient if offered 9, 14, 19, or even 24 hours after overdose (Figure 3, Table 2). This suggests that the 21-hour IV 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 gram overdose, the 72-hour IV infusion (Protocol D) saves the patient if administered 4 hours after an 85g overdose (Figure 2d Table 2), although it is still inferior to the oral and IV bolus protocols (Table 2). When administered 4 hours after overdose, Protocol D preserves 39.3% of hepatocytes, and at 9 hours after overdose, it preserves 38.4% of hepatocytes. This is superior to Protocol B, but still well below the percentage preserved by Protocols A and C (Table 2).

We next investigated whether the entire 72-hour oral course of NAC is always required for optimal treatment. We ran simulations for a 60g APAP overdose with oral NAC treatment starting between 4 and 34 hours after overdose and varied the total number of NAC doses administered in the treatment. These results are displayed in Figure 4, which plots the minimum fraction of viable hepatocytes against the number of doses for different treatment delay lengths. When started 4 hours after overdose, the effectiveness of NAC treatment in preserving hepatocyte viability is limited to the first 10
When we begin oral NAC treatment at 14 hours after overdose, hepatocyte viability does not increase appreciably for any treatment course longer than 8 doses. When treatment is started at 24 hours after APAP overdose, the number of useful doses is even fewer; only 6 doses have a beneficial effect. In each of these cases, the final dose with a therapeutic effect is the dose taken between 44 and 48 hours after APAP overdose.

If we increase the APAP dose to 85g, we see a larger number of NAC doses having a therapeutic effect. When oral NAC is started 4h after an 85g overdose, 14 doses increase the minimum fraction of viable hepatocytes (Figure 4b). If oral NAC is started 14h after overdose, 12 doses are useful; at 24 hours after overdose, only 10 doses are useful. In each of these cases, the final dose with a therapeutic effect is taken between 60 and 64 hours after overdose.

The IV bolus protocol (Protocol C) has the same number of effective doses as the oral course for all delay lengths investigated (not shown); Since the IV bolus protocol administers the same amount of NAC as the oral protocol at each dose, this indicates that route of administration of NAC does not matter. Interestingly, with continuous iv infusion protocols (Protocols B and D), the maximum effective treatment duration is longer than the maximum effective length of the oral NAC or bolus IV courses (Figure 5). If started 4 hours after an 85g overdose, IV infusion treatment is useful up to 73 hours after overdose, and if started 14 hours after overdose, it is useful up to 71 hours after overdose. At 24 hours after overdose, the treatment is useful until 73 hours after overdose.

We also investigated the length of time the reactive metabolite NAPQI is 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 is administered between 4 and 34 hours after an 85g overdose, the predicted NAPQI concentration in the liver falls to zero at 60h (Figure S2, supplemental materials) and the GSH is replenished between 28 hours (for the 4h delay) and 44 hours (for the 34 hour delay) prior to rising above baseline levels. For the 60g overdose, NAPQI is eliminated from the liver at 48 hours after overdose for all lengths of NAC delay, and the GSH is replenished between 22.5 hours (for the 4 hour delay) and 40 hours (for the 34 hour delay). The NAPQI residence time in the liver varies with the infusion length when
IV NAC is administered; however, for the longest NAC infusions, NAPQI is cleared from the liver at 72 hours after overdose (Figure S3, supplemental materials).

Population Effects

Reaction to APAP overdose and NAC treatment is likely to be highly dependent 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 hours after APAP ingestion); at the FDA’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 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 (Reid and Hazell 2003).

We administered four doses of APAP (7.5g, 10g, 15g, and 20g) to our SimPops™ virtual population of 957 individuals. At 7.5g, 1.04% of the population developed hepatotoxicity (i.e. a viable hepatocyte fraction of ≤82%), and at 20g, 56.6% of the population developed hepatotoxicity. We plotted the APAP concentration of each patient at one-hour intervals from 4 hours to 24 hours 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, while 3.19% of those falling below the 100 line
developed hepatotoxicity. However, there is a difference in risk between patients presenting at 4 hours and patients presenting at 24 hours. At 4 hours after overdose, 17.16% of individuals falling below the 150 line developed hepatotoxicity, while 5.21% of individuals falling below the 150 line at 24 hours after overdose developed hepatotoxicity. A similar disparity was present for the 100 line; 6.28% of individuals below the 100 line at 4 hours after overdose developed hepatotoxicity, while only 2.33% of individuals below the 100 line at 24 hours developed hepatotoxicity. The risk of hepatotoxicity below the 150 line for our simulated patients declines to 7.77% at 12 hours after overdose.

In order 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 (17g, peak ALT = 1000 U/L) to the nomogram lines (Figure 6). The time course of APAP concentration has a flatter trajectory than the nomogram treatment lines until about 12 hours after overdose; in fact, the average model human concentration-time curve crosses over the 200 line at about 8 hours after overdose. This demonstrates that the slope of the current nomogram lines does not reflect the pharmacokinetics of APAP in the blood, and that 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 30g and 60g acute overdoses of APAP with a) no NAC treatment, b) oral NAC treatment using Protocol A, c) IV infusion NAC treatment using Protocol D, or d) IV bolus NAC treatment using Protocol C extended to 72 hours. The effects of these treatment courses on the simulated population are described in Table S1. As with the average patient, Protocol A and Protocol C perform better than Protocol D when administered to the simulated population soon after overdose. After a 30g overdose and a 4-hour treatment delay, 11.1% of the population
developed hepatotoxicity after Protocol A, compared to 11.5% after Protocol C and 22.9% after Protocol D. However, when administered with a 14-hour delay, the difference among the treatment protocols shrinks 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 60g overdose, the oral and IV bolus courses outperform the IV infusion course at delay lengths of 4 and 14 hours. After a 14-hour 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 60g overdose and a 14-hour treatment delay and as expected from our simulations in the average individual, this protocol was the least efficacious as 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 hours after either a 30g or a 60g overdose.

**Biomarker Analysis**

Woo et al. suggested the use of serum APAP concentration as a biomarker to guide termination of NAC treatment (Woo et al. 2000). Others have suggested that treatment may be safely stopped once serum ALT or AST have reached their peak and are decreasing, or are 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 (60g and 30g) and treatment latency (4, 14, 24, 34, and 44 hours). 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 to 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 over treat 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 to the time required for blood APAP concentration to drop below 5 μg/mL is in Figure 7a; statistics for the biomarker analysis can be found in Table S2 (supplemental materials). Only 0.16% of patients would be undertreated by stopping NAC treatment when blood APAP is below 5 μg/mL (and none are undertreated more than 4 hours, meaning a missed oral or IV bolus dose). The maximum overtreatment is 48 hours, and the median overtreatment is 14 hours. At a 24 hour delay length, however, 4.60% of patients are undertreated (0.21% more than 4 hours), and at a 44 hour delay length, 73.9% of patients are undertreated (55.38% more than 4 hours). Since APAP is often undetectable in the bloodstream so long after overdose, it is unsurprising that this biomarker is a weak one at long delay times.

Serum ALT performed better than blood APAP concentration at the 30 gram APAP dose. Using peak ALT (Figure 7b) would undertreat the fewest patients (maximum of 1.88% at 4 hours after overdose), but median overtreatment time using ALT was 15 to 20 hours, which is the worst of the four biomarkers. Using peak AST would also undertreat 1.88% of patients at 4 hours after overdose, but only has a median overtreatment time of 16.6 hours at 4 hours after overdose and 7.15 hours at 44 hours after overdose (Figure 7c, Table S2). Interestingly, using blood bile acid concentration (Figure 7d) undertreats 7.84% of patients after a 4 hour delay (though only 0.10% for more than 4 hours), but the median overtreatment time is between 2 and 3 hours for all treatment delays, making it the best of the biomarkers by that measure. We also tracked peak international normalized ratio (INR), which is a commonly used clinical biomarker for the cessation of NAC treatment (Figure 7e). We found that using INR only undertreats 0.10% of the population; however, the median overtreatment with peak INR is between 56 and 59 hours, 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 60g overdose, however, the results are different. Stopping treatment at serum APAP concentration of 5 μg/mL (Figure 8a) now undertreats only 7.21% of the population after a 44-hour treatment delay, and using peak ALT (Figure 8b) undertreats no one at any delay length. Conversely, using peak AST (Figure 8c) undertreats between 6% and 19% of the population, while using peak blood bile acids (Figure 8d) undertreats between 5% and 10% of the population (Table S2). Like ALT, using peak INR (Figure 8e) does not undertreat anyone; however, the mean and median overtreatment with INR is about twice the mean and median overtreatment for peak ALT (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 (not shown). Even for a massive overdose, the ideal treatment time does not extend far beyond the current clinical recommendations; after a 100g overdose, the longest ideal treatment time in our simulated population was 81 hours for NAC administered after a 4-hour delay and 63 hours for NAC administered after a 24-hour delay. According to our model, the current 72-hour clinically recommended oral course would undertreat 1.57% of the population when administered 4 hours after a 100g 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 $V_{\text{max}}$ (CYP2E1 activity) $\geq$ RNS-ROS generation $V_{\text{max}}$ $> \text{ATP decrement } V_{\text{max}}$ $> \text{injury propagation trigger point}$. Other less important factors were: body mass $> \text{GSH baseline level} > \text{GSH transport } V_{\text{max}}$. Variation in sulfation $V_{\text{max}}$ and APAP uptake rate had no effect on hepatotoxicity susceptibility.
With oral NAC dosing (Protocol A), the effect of the variables were similar, but glucuronidation $V_{\text{max}}$, baseline GSH concentration, and GSH transport $V_{\text{max}}$ 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 is higher after a 34-hour NAC treatment delay ($R = 0.3500$, 3rd) than after a 4-hour delay ($R = 0.1243$, 5th). It is also interesting to note that the GSH precursor transport $V_{\text{max}}$ correlation coefficient was essentially not correlated with hepatocyte fraction after a 4-hour treatment delay ($p = 0.1261$), but was the 5th most important variable after a 34-hour 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 are similar after a 60g overdose.

Discussion

There is controversy about whether oral or IV NAC provides the best protection against APAP toxicity and when completing the full protocol is unnecessary. We addressed these questions 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-hour oral course (Protocol A) is superior to the 21-hour IV course (Protocol B) when treatment is started within 24 hours of overdose. There is little difference between Protocol A and the iv bolus protocol C in the outcome of the simulated patient for all APAP doses and treatment latencies. Since these two protocols administer NAC at similar rates, the difference in effectiveness between standard oral and iv NAC courses is not method of delivery but rather the duration and structure of the treatment. As Figure 9 shows, there is a significant difference in the total amount of NAC administered by the standard oral and iv infusion protocols beyond 8 hours after starting treatment, and this may become more important the earlier NAC is administered.
Interestingly, the opposite conclusion concerning the merits of oral vs iv NAC was reached based on a retrospective analysis of 4048 patients (Yarema et al. 2009). These investigators found that, for patients treated <12 hours post-ingestion, the incidence of hepatotoxicity was lower in those receiving iv treatment. However, the oral and iv-treated cohorts were different; the former was US and the latter was Canadian, and the median time from ingestion to NAC initiation differed significantly. 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 (Figures 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 hours after overdose; however, if NAC is administered >24 hours 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 due to its shorter half-life in serum. However, terminating NAC treatment after peak ALT is observed will lead to ≥20 hours of unnecessary treatment for some patients. Since 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 hours generally provides no additional benefit, terminating NAC treatment at 72 hours regardless of ALT measures would help minimize overtreatment issues.

Peak serum bile acid concentration also appeared 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 APAP overdose patients (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 since serum assays of bile acids are slow and not generally available in the clinic.

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

The DILIsym™ model predicted that initiation of NAC therapy is not effective in the average human when administered >34 hours after an overdose (however, some members of a sample population benefit from treatment starting as late as 44 hours 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 to 72 hours after overdose. The explanation for these apparently discordant observations is the critical role of the injury propagation response. Limaye et al note that propagation effects continue in liver injury after the toxic compound has cleared the liver and likely are a contributor to lethality (Limaye et al. 2003). Mehendale and Limaye implicate calpain as a contributor to this propagation effect (Mehendale and Limaye 2005); 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 60g and 85g, the propagation effect is triggered at 48h; 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 hours after overdose in the average patient in order to provide enough therapeutic effect to avoid the
injury propagation that leads to death; however if propagation does not occur, as with non-lethal 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 30g overdose, the correlation coefficient of the variable controlling the location of the trigger point increased almost threefold when NAC was administered at 34 hours after overdose compared to a 4-hour 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 hours, hepatocyte injury is already well underway and the die is cast on whether the propagation response will occur.

We also found that variation in Vmax 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 present early (4-8 hours after overdose) and who appear on the nomogram below the 100 line (and would likely not receive NAC therapy) have a higher risk of hepatotoxicity than patients who present 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 early-presenting APAP overdose patients who
fall below but near either the 100 or 150 nomogram line 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 hour iv protocol in preventing hepatotoxicity after acetaminophen overdose. The modeling also indicates that due to slow absorption of large APAP overdoses, current treatment nomograms may underestimate 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.

Acknowledgements

We wish to acknowledge the DILI-sim Initiative partners for many informative conversations.

Authorship Contributions

Participated in research design: JW, BH, YY, HC, MA, SS, PW
Conducted experiments: AH
Performed data analysis: JW
Wrote or contributed to the writing of the manuscript: JW, BH, MA, SS, PW
References


Footnotes

This research was supported by the DILI-sim Initiative.

Reprint requests should be directed to Dr. Paul B. Watkins, The Hamner-UNC Institute for Drug Safety Sciences, 6 Davis Drive, Research Triangle Park, NC 27709
Legends for Figures

Figure 1. Fraction of hepatocytes viable versus time for a simulated 60g acute overdose of acetaminophen (APAP) followed by NAC treatment given by the a) 72-hour oral; b) standard 21-hour IV; c) 48-hour IV bolus; d) 72-hour IV infusion protocols. The treatment was initiated between 4 and 44 hours after overdose as noted. A surviving hepatocyte fraction less than 15% was considered lethal (see methods).

Figure 2. Fraction of hepatocytes viable versus time for a simulated 85g acute overdose of acetaminophen (APAP) followed by NAC treatment given by the a) 72-hour oral; b) standard 21-hour IV; c) 48-hour IV bolus; d) 72-hour IV infusion protocols. The treatment was initiated between 4 and 44 hours after overdose.

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

Figure 4. Minimum fraction of hepatocytes viable after oral NAC treatment with a varying number of doses. Simulated APAP doses given were a) 60g and b) 85g. NAC treatment was delayed by 4, 14, and 24 hours.

Figure 5. Minimum fraction of hepatocytes viable after intravenous NAC treatment (Protocol B/D) with a varying length of final (6.25 mg/kg) infusion. Simulated APAP doses given were a) 60g and b) 85g. NAC treatment was delayed by 4, 14, and 24 hours.
Figure 6. A diagram of the APAP overdose risk assessment nomogram lines in current clinical use and the risk of hepatotoxicity below each reported by Ali et al. (Ali et al. 2008) and predicted by DILIsym™. The concentration-time curve for a hepatotoxic (peak ALT = 1000 U/L) dose of APAP to the average human is shown in blue.

Figure 7. Plot of the time required for NAPQI to reach 1% of its peak value against the time required for a) blood APAP concentration to reach 5 μg/mL; b) ALT to reach its peak value; c) AST to reach its peak value; d) blood bile acid concentration to reach its peak value; e) INR to reach its peak value after a 30g APAP overdose. 72-hour oral NAC treatment was modeled with delays of 4, 14, 24, 34, and 44 hours. The blue line denotes where the biomarker matches the disappearance of NAPQI perfectly; below and to the right of the blue line is overtreatment, while above and to the left of the blue line is undertreatment.

Figure 8. Plot of the time required for NAPQI to reach 1% of its peak value against the time required for a) blood APAP concentration to reach 5 μg/mL; b) ALT to reach its peak value; c) AST to reach its peak value; d) blood bile acid concentration to reach its peak value; e) INR to reach its peak value after a 60g APAP overdose. 72-hour oral NAC treatment was modeled with delays of 4, 14, 24, 34, and 44 hours. The blue line denotes where the biomarker matches the disappearance of NAPQI perfectly; below and to the right of the blue line is overtreatment, while above and to the left of the blue line is undertreatment.

Figure 9. A diagram of the total NAC dosed over time (in mg/kg) for the IV infusion protocol ( ) and the oral/IV bolus protocol ( ).
Table 1. Description of NAC treatment protocols simulated in this study. Note that clinically, treatment is frequently extended beyond the suggested treatment duration length.

<table>
<thead>
<tr>
<th>Protocol identifier</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 hours (Yarema et al. 2009)</td>
<td>72 hours</td>
</tr>
<tr>
<td>B</td>
<td>IV infusion</td>
<td>Loading infusion of 150 mg/kg over 1 hour, followed by 50 mg/kg over 4 hours, followed by 100 mg/kg over 16 hours (Stravitz et al. 2007)</td>
<td>21 hours</td>
</tr>
<tr>
<td>C</td>
<td>IV bolus</td>
<td>Loading dose of 140 mg/kg over 1 hour followed by 70 mg/kg doses over 1 hour administered every 4 hours (Smilkstein et al. 1991)</td>
<td>48 hours; extended to 72 hours for some simulations</td>
</tr>
<tr>
<td>D</td>
<td>IV infusion</td>
<td>Loading infusion of 150 mg/kg over 1 hour, followed by 50 mg/kg over 4 hours, followed by 418.75 mg/kg over 67 hours</td>
<td>72 hours</td>
</tr>
</tbody>
</table>
Table 2: Nadir hepatocyte fraction as a function of simulated APAP dose and delay in onset of treatment for each of the four NAC treatment protocols. D indicates that the simulated patient died despite treatment (i.e. reached a fraction of viable hepatocytes of $\leq 0.15$).

<table>
<thead>
<tr>
<th>Dose (g)</th>
<th>4h</th>
<th>14h</th>
<th>24h</th>
<th>34h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>30</td>
<td>0.855</td>
<td>0.801</td>
<td>0.854</td>
<td>0.809</td>
</tr>
<tr>
<td>60</td>
<td>0.702</td>
<td>0.626</td>
<td>0.699</td>
<td>0.668</td>
</tr>
<tr>
<td>85</td>
<td>0.534</td>
<td>D</td>
<td>0.527</td>
<td>0.393</td>
</tr>
</tbody>
</table>
Table 3. List of parameters varied within the sample population and their ranges and standard deviations.*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Std. dev. up</th>
<th>Std. dev. down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>82</td>
<td>130</td>
<td>50</td>
<td>19.2</td>
<td>12.8</td>
</tr>
<tr>
<td>Baseline GSH (mol/mL)</td>
<td>5.50E-06</td>
<td>8.00E-06</td>
<td>3.00E-06</td>
<td>1.00E-06</td>
<td>1.00E-06</td>
</tr>
<tr>
<td>1APAP uptake rate from gut (1/hour)</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Glucuronidation maximum rate (V_{max}) (mol/mL/hour)</td>
<td>2.82E-04</td>
<td>3.88E-04</td>
<td>1.76E-04</td>
<td>5.29E-05</td>
<td>5.29E-05</td>
</tr>
<tr>
<td>Sulfation maximum rate (V_{max}) (mol/mL/hour)</td>
<td>2.62E-05</td>
<td>3.44E-05</td>
<td>1.80E-05</td>
<td>3.2775E-06</td>
<td>3.2775E-06</td>
</tr>
<tr>
<td>NAPQI maximum rate (V_{max}) (mol/hour)</td>
<td>9.50E-04</td>
<td>1.71E-03</td>
<td>1.88E-04</td>
<td>6.10E-04</td>
<td>6.10E-04</td>
</tr>
<tr>
<td>2RM creation of RNS-ROS V_{max} (1/hour)</td>
<td>2.21</td>
<td>2.91</td>
<td>1.51</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>3RNS-ROS effect on ATP V_{max} (1/hour)</td>
<td>13</td>
<td>21</td>
<td>8</td>
<td>3.2</td>
<td>2</td>
</tr>
<tr>
<td>4Necrotic flux effect on propagation K_{m} (bn cells/hour)</td>
<td>1.28</td>
<td>1.584</td>
<td>0.25</td>
<td>0.1216</td>
<td>0.412</td>
</tr>
<tr>
<td>5GSH precursor transport V_{max} (mol/mL/hour)</td>
<td>7.00E-07</td>
<td>1.12E-06</td>
<td>2.80E-07</td>
<td>1.68E-07</td>
<td>1.68E-07</td>
</tr>
</tbody>
</table>

* Sex, a binary variable, is not included in the table. The influence of sex on the model is limited to the differences in lean body mass between a male and female of the same overall body mass.

1. This parameter approximates the absorption of drug into the blood from the gut as a first-order rate equation.

2. This parameter is the V_{max} in the equation that approximates the creation of reactive oxygen and nitrogen species from reactive metabolite using Michaelis-Menten kinetics.

3. This parameter is the V_{max} in the equation that approximates the loss of ATP due to reactive oxygen and nitrogen species as a Hill function.

4. This parameter is the K_{m} in the equation that approximates the effect of necrotic flux on injury propagation as a Hill function.

5. This parameter is the V_{max} in the equation describing GSH precursor uptake into hepatocytes using Michaelis-Menten kinetics.
Table 4. Correlation coefficients (R) between each varied parameter and the fraction of hepatocytes viable. *** denotes a statistically significant correlation (p < 0.001).

<table>
<thead>
<tr>
<th>APAP dose (g)</th>
<th>Treatment</th>
<th>APAP uptake</th>
<th>Glucuronidation</th>
<th>Sulfation</th>
<th>NAPQI</th>
<th>RNS-ROS</th>
<th>ATP</th>
<th>GSH</th>
<th>Glutathione Transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>4</td>
<td>0.2603***</td>
<td>0.0329</td>
<td>-0.0046</td>
<td>0.0040***</td>
<td>0.0117</td>
<td>-0.5425***</td>
<td>-0.4252***</td>
<td>-0.2780***</td>
</tr>
<tr>
<td>30</td>
<td>34</td>
<td>0.1234***</td>
<td>0.1098***</td>
<td>-0.0060</td>
<td>-0.0227</td>
<td>0.0331</td>
<td>-0.4462***</td>
<td>-0.4926***</td>
<td>-0.3099***</td>
</tr>
<tr>
<td>60</td>
<td>4</td>
<td>0.1618***</td>
<td>0.0555</td>
<td>-0.0159</td>
<td>-0.0266</td>
<td>0.0281</td>
<td>-0.5249***</td>
<td>-0.4328***</td>
<td>-0.2789***</td>
</tr>
<tr>
<td>60</td>
<td>34</td>
<td>-0.1000</td>
<td>0.0800</td>
<td>-0.0333</td>
<td>-0.0282</td>
<td>0.0004</td>
<td>-0.4095***</td>
<td>-0.5803***</td>
<td>-0.3026***</td>
</tr>
<tr>
<td>15</td>
<td>no NAC</td>
<td>0.2269***</td>
<td>0.1167***</td>
<td>-0.0173</td>
<td>0.0239***</td>
<td>0.0538</td>
<td>-0.5323***</td>
<td>-0.4385***</td>
<td>-0.2928***</td>
</tr>
<tr>
<td>20</td>
<td>no NAC</td>
<td>0.2070***</td>
<td>0.1316***</td>
<td>-0.0224</td>
<td>0.0009***</td>
<td>0.0591</td>
<td>-0.5087***</td>
<td>-0.4480***</td>
<td>-0.3147***</td>
</tr>
<tr>
<td>30</td>
<td>no NAC</td>
<td>0.1904***</td>
<td>0.1138***</td>
<td>-0.0143</td>
<td>-0.0097***</td>
<td>0.0428</td>
<td>-0.4710***</td>
<td>-0.4743***</td>
<td>-0.3122***</td>
</tr>
<tr>
<td>60</td>
<td>no NAC</td>
<td>-0.0152***</td>
<td>0.0722</td>
<td>-0.0385</td>
<td>-0.0414</td>
<td>0.0059</td>
<td>-0.4284***</td>
<td>-0.5223***</td>
<td>-0.2842***</td>
</tr>
</tbody>
</table>
Figure 2

(a) to (d) show the fraction of hepatocytes viable over time (h) with different time points labeled. The graphs illustrate the decay in viability over time for each condition.
Figure 4

Minimum fraction of hepatocytes viable

(a) 4h delay
(b) 14h delay
(c) 24h delay

Number of oral doses
Figure 5

(a) Minimum fraction of hepatocytes viable vs. length of IV infusion protocol (h) for different delays:
- 4h delay
- 14h delay
- 24h delay

(b) Minimum fraction of hepatocytes viable vs. length of IV infusion protocol (h) for different delays:
- 4h delay
- 14h delay
- 24h delay
Figure 7