Pharmacokinetics of Acetaminophen Protein Adducts in Adults with Acetaminophen Overdose and Acute Liver Failure

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Introduction – 494 words, Discussion – 1217 words

Abbreviations: acetaminophen (APAP); acute liver failure (ALF); alanine aminotransferase (ALT); aspartate aminotransferase (AST); elimination rate constant (k_e); half-life (t_{1/2}); high performance liquid chromatography – electrochemical detection (HPLC-EC); international normalized ratio for prothrombin time (INR); N-acetylcysteine (NAC); nonlinear mixed effects models (NONMEM); Acute Liver Failure Study Group (ALFSG)
Abstract

Acetaminophen-induced liver toxicity occurs with formation of acetaminophen–protein adducts. These adducts are formed by hepatic metabolism of acetaminophen to N-acetyl-p-benzoquinone imine which covalently binds to hepatic proteins as 3-(cystein-S-yl)acetaminophen adducts. Adducts are released into blood during hepatocyte lysis. We previously showed that adducts could be quantified by HPLC with electrochemical detection following proteolytic hydrolysis, and that the concentration of adducts in serum of overdose patients correlated with toxicity. The following study examined the pharmacokinetic profile and clinical associations of adducts in 53 adults with acute acetaminophen overdose resulting in acute liver failure. A population pharmacokinetic analysis using nonlinear mixed effects (statistical regression-type) models was conducted; individual empiric Bayesian estimates were determined for the elimination rate constant and elimination half-life. Correlations between clinical and laboratory data were examined relative to adduct concentrations using non-parametric statistical approaches. Peak concentrations of acetaminophen protein adducts correlated with peak aminotransferase concentrations (R=0.779) in adults with acetaminophen related acute liver failure. Adducts did not correlate with bilirubin, creatinine, acetaminophen concentration at admission, international normalized ratio for prothrombin time, or reported acetaminophen dose. Following N-acetylcysteine therapy, adducts exhibited first order disappearance. The mean elimination rate constant and elimination half-life were $0.42 \pm 0.09$ days$^{-1}$ and $1.72 \pm 0.34$ days, respectively, and estimates from the population model were in strong agreement with these data. Adducts were detected in some patient samples 12 days post-ingestion. The persistence and specificity of
acetaminophen protein adducts as correlates of toxicity support their utilization as specific biomarkers of acetaminophen toxicity in patients with acute liver injury.
Introduction

Acetaminophen [APAP; N-(4-hydroxyphenyl)acetamide; C₈H₉NO₂] overdose has recently been identified as a major cause of acute liver failure (ALF) in the United States. (Larson, 2005) Currently, the diagnosis of APAP overdose is dependent on the history of a large dose of APAP, defined as 7.5 grams of APAP in adults, (Rumack, 1981) supported by an elevated level of APAP in peripheral blood. (Smilkstein, 1988; Rumack, 2002) Many patients develop ALF rapidly, characterized by encephalopathy and the presence of coagulopathy (international normalized ratio [INR] of ≥1.5); in these patients, the history of ingestion and specific dosing information may be difficult to obtain. Furthermore, the interpretation of measured APAP concentrations in peripheral blood requires knowledge of the precise time of ingestion of a single large dose of APAP.

Overdoses of APAP result in the generation of APAP protein adducts, which are produced by the binding of the reactive metabolite, N-acetyl-p-benzoquinone imine (Dahlin, 1984) to cysteine groups on protein as 3-(cystein-S-yl)acetaminophen adducts. (Hoffman, 1985) Covalent binding of APAP to cysteine residues in proteins, hereafter referred to as APAP-adducts, is an excellent correlate with the severity of the APAP toxicity. (Pumford 1989; Pumford, 1990; Roberts, 1991) Initial studies in the mouse model of APAP toxicity utilized anti-sera with specificity for the 3-(cystein-S-yl) APAP epitope to elucidate dose-response and temporal relationships for APAP-adducts in mouse liver and serum. (Pumford 1989; Pumford, 1990; Roberts, 1991) In recent studies, our laboratory developed a very precise and sensitive analytical assay for the APAP-adducts. In this assay the liver or serum sample is initially proteolytically hydrolyzed and the released 3-(cystein-S-yl)acetaminophen adducts are quantified by
high performance liquid chromatography with an electrochemical detector (HPLC-EC) (Muldrew, 2002).

Measurement of APAP-adducts in clinical serum samples can accurately distinguish between known, well characterized cases of APAP-related ALF and cases of ALF of other etiologies. (Davern 2006) In previous work, we determined that high concentrations of adducts were present only in the samples of patients with well-characterized cases of APAP overdose and adducts were not detected in the samples of patients with other cases of liver failure. Moreover, no to very low concentrations of adducts were detected in patients with APAP overdose that received prompt treatment with NAC and did not develop toxicity. (Davern 2006) In further studies, high concentrations of APAP-adducts were detected in 19% of adult and 15% of pediatric samples obtained from patients with ALF of unknown etiology, thus implicating APAP as the etiology of the ALF. (Davern, 2006; James, 2006) The data indicated that APAP-adducts could accurately diagnose APAP-mediated ALF at times subsequent to the toxic event; however, the pharmacokinetics, and thus the duration of time for which adducts can be utilized as a diagnostic indicator have not been previously reported. Thus, as a follow-up to our previous studies, (Davern 2006) we examined the clinical associations and elimination characteristics of APAP-adducts in a large group of adults with APAP-related ALF. We report herein the pharmacokinetics of APAP-adducts in adults with APAP-related ALF and compare the clinical data, laboratory parameters and patient outcomes to the observed concentrations of APAP-adducts.
Methods

Study population.

Serum samples were analyzed post-hoc from 53 adults with known APAP-related ALF that were enrolled in the sample/data bank of the Acute Liver Failure Study Group (ALFSG [National Institutes of Diabetes and Digestive Diseases]; William M. Lee, Principal Investigator). Clinical criteria for enrollment in the sample/data bank were (1) presence of coagulopathy (international normalized ratio for prothrombin time [INR] ≥1.5), (2) evidence of hepatic encephalopathy, and (3) presentation within 26 weeks of illness onset without evidence of previous liver disease. Because patients were encephalopathic by definition, informed consent was obtained from their legal next of kin. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by a priori approval by participating sites’ institutional review boards. The diagnosis of APAP overdose was made by (1) history of ingestion of a large amount of APAP, defined as APAP use > 4 grams per day within 7 days of presentation and (2) detection of APAP on admission; or (3) alanine aminotransferase (ALT) levels > 1000 IU/L, with history of APAP dosing, irrespective of the APAP level. Exclusion of other causes of ALF was required (Hepatitis A, Hepatitis B, Wilson’s disease, hepatic ischemia, autoimmune hepatitis, other etiologies). Daily serum samples are collected for seven days or until time of transplantation or hospital discharge as part of the registry database. Criteria for selection of patients from the overall registry for inclusion in the present study were 1) history of suicidal ingestion based on a report of a single, “one time/acute” ingestion with admission of suicidal intent and 2) a known time of ingestion of a known amount of APAP. A total of 230 samples from 53 subjects underwent APAP-
adduct analysis. Chronic, multiple time point ingestions were not included in this analysis. Case report forms, which included detailed demographic, clinical, and laboratory data, were available for review by the investigators to determine the precise time of the APAP ingestion, history of N-acetylcysteine (NAC) use and history of concomitant ethanol ingestion. Clinical laboratory data (hepatic aminotransferase and APAP concentrations) were analyzed in the clinical laboratories of participating sites. Treatment with NAC and duration of treatment with NAC was not standardized and was determined by the attending hepatologist. By definition, the study population did not include patients that did not have ALF and thus the findings relate only to patients with ALF of APAP etiology.

**Analytical method.**

Serum samples were assayed for APAP-adducts using a modification of the previously reported HPLC with electrochemical detection assay for APAP-cysteine derived by proteolytic cleavage of APAP-adducts. (Muldrew, 2002) Assay modifications included centrifugal gel-filtration and higher efficiency proteolytic digestion, resulting in improved sensitivity and efficiency of the assay. Calibration curves were prepared over the concentration range of 0.039 to 20 uM using drug free plasma spiked with authentic APAP-cysteine. Standard curves were linear with regression coefficients >0.99. Samples having concentrations above the highest standard were diluted so their values fell within the range of the standard curve. Intra- and inter-assay variations were assessed from the quality control samples. Quality control concentrations ranged from 0.031 to 17.5 uM and three replicates were analyzed with each analysis. Intra-assay variation ranged from 3.24 - 10.0%. Inter-assay variation ranged from 5.29 - 10.49%.
The lower limit of quantitation (LLQ) of the assay was determined by the lowest quality control concentration measurable with a CV of less than 15%. The LLQ for the assay was defined as 0.03 µM (30 pmoles APAP-cysteine per ml serum). Receiver operator curve analysis was performed with an existing set of samples from patients with APAP overdose. This analysis determined that a cut point of ≥ 1.1 nmol acetaminophen protein adduct/mL provided a sensitivity of 96.8% and a specificity 95%, when ALT > 1000 IU/L was used as a reference (Fig 1).

Clinical Data.

Patient data include reported dose (mg/kg) and date of APAP ingestion, history of ethanol use, history of concomitant opioid ingestion, treatment and duration of treatment with NAC, and outcome (spontaneous survival, death or liver transplantation). Laboratory parameters included daily measurements of ALT, aspartate aminotransferase (AST), total bilirubin, international normalized ratio for prothrombin time (INR) and creatinine. Individual subject peak values for each laboratory parameter (ALT, AST, bilirubin, INR, and creatinine) were analyzed relative to peak observed concentrations of APAP-adducts (referred to as Peak APAP-adduct). Clinical endpoints and APAP-adduct values were analyzed relative to the time of reported overdose and expressed in 24 hour increments relative to the time of the overdose. The day of overdose was defined as day 0.

Statistical analysis.

Nonparametric tests were used to examine differences between subgroups (Kruskal Wallis, Mann Whitney tests). Statistical analysis was performed using SPSS
The Pearson correlation coefficient was used for comparison between clinical/laboratory parameters and adduct concentrations.

**Pharmacokinetic analysis.**

The elimination of APAP-adducts was analyzed with a population pharmacokinetic approach using the program NONMEM (version V, FOCE subroutine with interaction). Mono-exponential decay of APAP-adducts was used to describe its elimination (ADVAN2 TRANS1). A one-compartment model was used. More complex structural models were not tested due to the limited range and number of samples available for the analysis. Dose of the APAP ingestion and APAP concentrations were not modeled due to limited sampling, subject heterogeneity and imprecision in self-reported APAP overdose histories. Since greater than 90% of the subjects received treatment with NAC, adduct formation was assumed to be complete for subjects sampled 3 or more days post ingestion. A first order model that included a lag time was used to characterize APAP-adduct formation for subjects with APAP-adduct concentrations determined using samples collected within two days of ingestion to account for ongoing APAP-adduct production. The APAP-adduct “dose” or amount was estimated by fitting a scaling factor linked to the observed Cmax (Volume of distribution [Vd] fixed to a value of 1). Individual empiric Bayesian estimates for the elimination rate $k_e$ and $t_{1/2}$ were determined using the post-hoc subroutine. The elimination rate and overall model goodness-of-fit was compared between those subjects with $> 4$ samples and those with less than 4 samples. Cmax (obs) was defined as the highest observed APAP-adduct concentration for this analysis.
Results

Patient data

Summary demographic, clinical, laboratory and treatment variables for the 53 subjects are presented in Tables 1-3. Spontaneous survival occurred in 41 (77%) subjects. Nine subjects (17%) died, and three subjects (6%) required liver transplantation. The majority of the population was female, Caucasian, and non-Hispanic (Table 1).

APAP concentrations, measured by the clinical laboratories of participating sites, were measurable in 90.6% of the study population (three patients had reported concentrations of 0 mg/L and no information was available on APAP concentrations concentrations in 2 subjects). Concentrations of the parent drug, APAP, at the time of study enrollment, plotted as a function of time lapsed since the APAP overdose, are shown in Figure 2. Of patients with detectable APAP, 72.2% had concentrations of APAP that were < 100 mg/L APAP and 49% had concentrations of APAP that were < 50 mg/L APAP.

Eighty-six percent of study subjects ingested overdoses that were exclusive to APAP (Table 1) and the remaining 13% ingested opioids in addition to APAP. Ninety-four percent (n=50) of the patients received NAC and the mean (+ SD) time to start of NAC treatment was day 3.5 (+ 1.7; day of overdose defined as day 0). The mean duration of NAC treatment was 4.3 (+ 2.9 days).

APAP-adducts were detected in all study samples and were compared to clinical outcomes and laboratory parameters. Since multiple measures were available from each patient, the peak APAP-adduct was used for this analysis. No differences were found in
peak APAP-adduct in subjects who received a transplant (p=0.34) or died (p=0.89), compared to subjects that survived. Of the clinical laboratory variables, peak APAP-adduct had the strongest correlation with peak AST (R=0.779). The correlation for peak APAP-adduct and ALT was (0.726). No significant correlations for peak APAP-adduct and other clinical laboratory values (peak creatinine R=0.17, peak bilirubin R=0.03; and peak INR R=0.17) were found. No correlation was observed between reported APAP dose and peak APAP-adduct (R=0.03).

In further analysis, the relationship between APAP-adducts and AST was examined as a function of time lapsed since overdose. Figure 3 shows the correlation of APAP-adducts and AST on days 3, 4 and 5 post-overdose. The correlation for APAP-adducts and AST was highest on days 3 and 4 post-overdose (R=0.84; R=0.84).

Pharmacokinetic analysis.

Since the mean (± SD) time of sample collection for the first study sample was 3.12 ± (1.4) days after the APAP overdose (Table 1), the pharmacokinetic analysis was limited to the elimination phase of APAP-adducts. Summary data for the Bayesian estimates for the patients with > 4 samples are presented in Table 4. The population model generated very similar elimination half-lives (1.69 days) to the Bayesian estimates. Individual and summary concentration time profiles for 20 subjects with ≥ 4 samples are presented in Figures 4A and 4B. Elimination half-life did not vary as a function of gender, BMI, race, height or age. In addition, t1/2 did not vary as a function of reported regular ethanol use.

In subjects with sample collection initiated after day 2, APAP-adducts Cmax (obs) occurred with the first sample in 34/36 (94%) of subjects. In subjects with sample
collections initiated before day 2, Cmax (obs) occurred at the first collection in 71% (12/17) of subjects. Thus, the temporal profile of APAP-adduct generation (Figure 4B) appeared to mirror that previously reported for hepatic transferase elevation following APAP overdose, with peak expression at two to three days following APAP overdose resulting in liver injury. (Rumack, 1981; Rumack 2002)
Discussion

The ALFSG registry afforded an ideal opportunity to examine the pharmacokinetic profile of APAP-adducts in a large number of well-characterized severe, acute APAP overdoses (Table 2). In this registry, all patients have developed ALF by the time of the initial study sample. The mean time from ingestion to study admission was > 2.9 days (Table 1) and the mean time from ingestions to the receipt of NAC was 3.5 days. The mean adduct t1/2 for the study subjects was 1.72 days (+ 0.34 days; range 0.94 to 2.55 days). In a previous, smaller study, we reported the t1/2 of APAP-adducts in four adults with APAP-related ALF to range from 0.71 to 1.29 days. (Davern, 2006) The slightly longer t1/2 noted in the present study may reflect the larger subset of patients included in the present study. (Davern, 2006)

In addition, the data showed that APAP-adducts correlated with serum hepatic aminotransferases and the highest correlation was noted for AST (Figure 4). Both AST and ALT are abundant hepatic cytosolic enzymes that are released with hepatic injury. The correlation between serum aminotransferases and serum APAP-protein adducts has been established in animal models (Pumford, 1989; Pumford 1990, Roberts 1991) and in patients (Hinson, 1990; Muldrew 2002; James 2008) and is logical as APAP-protein adducts accumulate in the hepatic cytosol (also the location of hepatic aminotransferases) and are released during toxicity. While both AST and ALT may be found in extrahepatic tissues, the relative abundance of AST in extrahepatic tissues (eg., heart, skeletal muscle, blood cells) is greater than that of ALT. (Green, 2002; Wroblewski, 1959) In addition, the primary cytochrome P450s enzyme responsible for the bioactivation of APAP, CYP2E1, (Gonzalez, 2007) is present in extra-hepatic tissues (e.g., nasal mucosa, olfactory
epithelium, lung and kidney)(Gu, 2005) and the metabolic activation of APAP in these extra-hepatic tissues can vary among tissues and is dependent on the tissue distribution of CYP2E1. For example, the nasal mucosa has relatively high levels of microsomal P450 enzymes, and is highly active in the metabolic activation of APAP,(Gu, 2005) as compared to the activity levels in the kidney and lung. Thus, it is likely that a small proportion of APAP-adducts in peripheral blood may be of extra-hepatic origin and may account for the better correlation of AST with APAP-adducts, compared to that of ALT with APAP-adducts.

A primary finding of this study was the long elimination half-life of APAP-adducts in human serum following APAP overdose (Fig 4). The significance of this observation is that it suggests that measurement of APAP-adducts may offer a considerable advantage to traditional methods (i.e. determination of APAP concentrations and hepatic transferase levels) used for the diagnosis of APAP overdose in patients with liver failure. The sensitivity provided by the HPLC-EC determination of adducts and the long t1/2 of APAP-adducts in human serum is in contrast to the relatively narrow window of time for which the parent compound, APAP, can be detected in peripheral blood. The Rumack nomogram, based on the measurement of APAP concentrations in peripheral blood relative to the reported time of overdose, is used in the clinical setting (eg., Emergency Departments) to assess the risk of developing toxicity following acute APAP overdose. It is the cornerstone of evaluation and management for patients with single, time-point ingestions who present within 24 hours of APAP overdose.(Rumack, 1981; Rumack 2002) However, beyond the acute stages of APAP toxicity, or in patients with unclear histories regarding the time of the overdose, or ingestions at multiple time points,
the utility of the Rumack nomogram is limited. As an alternative approach, prolongation of the $t_{1/2}$ of APAP has been evaluated as a potential surrogate marker for the severity of hepatotoxicity following APAP overdose. (Schiodt, 2002) The $t_{1/2}$ of APAP in patients with encephalopathy has been reported to be three fold longer (18.4 hours), than that observed in patients without encephalopathy (6.4 hours). (Schiodt, 2002) While the $t_{1/2}$ of APAP may be prolonged in severe APAP related liver injury, the relatively shorter $t_{1/2}$ of the parent compound limits its diagnostic usefulness for patients that present to medical centers after the onset of clinical symptoms in the late stages of toxicity. In the present study, the mean APAP concentration for the study population at study admission was 69 mg/L (Table 1) and almost half of the patients with detectable APAP concentrations had concentrations < 50 mg/L (Fig 2). No guidance for the interpretation of APAP concentrations in this setting exists and measurement of APAP by commercial assays may be biased towards elevated concentrations due to interference from bilirubin. (Bertholf, 2003; Polson, 2008) Therefore, measurement of APAP-adducts, a highly specific (Davern, 2006) and persistent biomarker of APAP toxicity, has considerable advantages over existing non-specific diagnostic methods and represents a potential new clinical parameter that can be utilized in patients that present with established acute liver injury or liver failure of unknown etiology. Understanding the pharmacokinetics of APAP-adducts in the setting of ALF is critical to the future utilization of the biomarker in this clinical setting.

The findings of the present study are in agreement with data recently reported for children and adolescents with APAP overdose (James, 2008). In the study of children and adolescents, the mean ($\pm$SD) elimination rate constant and half-life for adducts were
0.486 ± 0.084 days\(^{-1}\) and 1.47 ± 0.30 days, respectively, similar to the data of the present study. The majority (83%) of study subjects were > 12 years age and the population in general represented a broader range of liver toxicity following APAP overdose, than the present study. Only 15% of patients had ALT values > 1000 IU/L; no deaths occurred and two patients required liver transplantation. Elimination half-life did not vary as a function of Cmax, a surrogate marker for the degree of toxicity (James, 2008). An additional important finding was that significantly higher concentrations of adducts were detected in patients that had delays in treatment with NAC. The similarity in adduct elimination between these two studies, despite substantial differences in disease severity between the populations, suggest that determination of adduct concentrations may have potentially broad clinical relevance across the clinical spectrum of APAP toxicity, ranging from patients that receive early treatment with NAC to those that develop severe liver failure.

Several limitations of the present study should be noted. The pharmacokinetic data reported herein do not necessarily reflect the disposition of APAP-adducts in patients with chronic APAP overdose, which typically involves multiple daily supra-therapeutic exposures to APAP and may be complicated by use of combination APAP/narcotic preparations.(Larson, 2005) The severity of liver injury (ALT elevation), incidence of encephalopathy, and rate of transplant listings is very similar among patients with deliberate suicidal gestures and patients with unintentional overdoses.(Larson, 2005) None-the-less, further analysis of APAP-adducts in patients that are victims of unintentional or inadvertent APAP overdose is warranted to examine the potential...
influence of concomitant opioid exposure, and other co-morbidities, on the elimination of APAP-adducts in this population.

Measurement of APAP-adducts and characterization of its pharmacokinetics will have application for the diagnosis of ALF of unknown etiology, which is thought to represent approximately 20% of all cases of ALF in the US. (Davern, 2006) In addition, measurement of this biomarker will be important in the diagnosis of patients that present in the later stages of APAP toxicity, particularly those that present more than 1 day (> 24 hours) after overdose.
REFERENCES


Footnotes

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Figure Legends

Figure 1. Receiver operator curve analysis for acetaminophen protein adducts, using ALT > 1000 IU/L as a reference.

Figure 2. Histogram plot of acetaminophen (APAP) concentrations at the time of study admission for 53 adults with APAP-related acute liver failure. The median concentration of APAP in the 1-99 mg/L group was 26 mg/L (range: 5.6 - 94.3). For two subjects, information on APAP concentrations was not available and these subjects are included in the 0 group.

Figure 3. Correlation of aspartate aminotransferase (AST) (IU/L) with acetaminophen (APAP)-adducts in adults with APAP-related acute liver failure, plotted relative to overdose (----, Day 3; ---, Day 4; ·····, Day 5).

Figure 4A. Individual line plots for 18 subjects with 4 samples available for acetaminophen (APAP)-adduct analysis. One subject in this subset received concomitant opioids.

Figure 4B. Summary data for acetaminophen (APAP)-adducts, presented as median and interquartile range.
**Table 1. Demographic Data for 53 Subjects with Acetaminophen-Related Acute Liver Failure**

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Female</td>
<td>35</td>
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</tr>
<tr>
<td>Male</td>
<td>18</td>
<td>34.0</td>
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<td><strong>Race</strong></td>
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<tr>
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<td>1.7</td>
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<td><strong>Ethnicity</strong></td>
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<tr>
<td>Hispanic</td>
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<td>5.7</td>
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<tr>
<td><strong>Clinical Outcome</strong></td>
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<tr>
<td>Spontaneous Survival</td>
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<tr>
<td>Death</td>
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</tr>
<tr>
<td>Liver Transplant</td>
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<td>5.7</td>
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<tr>
<td><strong>Ingestion Type</strong></td>
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<tr>
<td>Acetaminophen with Opioid</td>
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<td><strong>N-acetylcysteine Treatment</strong></td>
<td>50</td>
<td>94.3</td>
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Table 2. **Summary Clinical Data (Mean, Standard Deviation [SD]) for 53 Adults with Acetaminophen-Related Acute Liver Failure.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
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<tbody>
<tr>
<td>Age (years)</td>
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</tr>
<tr>
<td>Weight (kg)</td>
<td>70.3</td>
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<tr>
<td>Body Mass Index (BMI)</td>
<td>25.3</td>
<td>5.6</td>
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<tr>
<td>Time to Study Enrollment from Overdose (days)</td>
<td>3.12</td>
<td>1.4</td>
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<tr>
<td>Reported Acetaminophen Dose (mg/kg)</td>
<td>468</td>
<td>284</td>
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<tr>
<td>Acetaminophen Concentration @ Study Admission (mg/L)</td>
<td>69.5</td>
<td>80.7</td>
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Table 3. Summary Values of Laboratory Parameters for Study Population (n=53).

<table>
<thead>
<tr>
<th></th>
<th>Peak ALT\textsuperscript{a} (IU/L)</th>
<th>Peak AST\textsuperscript{b} (IU/L)</th>
<th>Peak Total Bilirubin (mg/dL)\textsuperscript{c}</th>
<th>Peak Creatinine (mg/dL)\textsuperscript{d}</th>
<th>Peak INR\textsuperscript{e}</th>
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<tr>
<td>Mean</td>
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<tr>
<td>Standard Deviation</td>
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<tr>
<td>Median</td>
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<td>7,330</td>
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<td>1.9</td>
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<tr>
<td>Minimum</td>
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<td>114</td>
<td>1.4</td>
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<td>Maximum</td>
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<td>42.9</td>
<td>10.1</td>
<td>15.6</td>
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</table>

\textsuperscript{a}ALT, alanine aminotransferase; \textsuperscript{b}AST, aspartate aminotransferase; \textsuperscript{c}to convert to umol/L multiply by 17.1; \textsuperscript{d}to convert to umol/L multiply by 88.4; \textsuperscript{e}INR, international normalized ratio for prothrombin time
Table 4. Summary Data for Acetaminophen-Adducts in 53 Adults with Acetaminophen-Related Acute Liver Failure

<table>
<thead>
<tr>
<th></th>
<th>Cmax (obs -nmol/mL serum)</th>
<th>$k_e$ (days$^{-1}$)</th>
<th>Half-life (days)</th>
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</thead>
<tbody>
<tr>
<td>Mean</td>
<td>10.85</td>
<td>0.420</td>
<td>1.72</td>
</tr>
<tr>
<td>SD</td>
<td>9.26</td>
<td>0.090</td>
<td>0.34</td>
</tr>
<tr>
<td>Median</td>
<td>6.72</td>
<td>0.396</td>
<td>1.75</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.79</td>
<td>0.272</td>
<td>0.94</td>
</tr>
<tr>
<td>Maximum</td>
<td>41.51</td>
<td>0.738</td>
<td>2.55</td>
</tr>
</tbody>
</table>
Adduct = 1.1 nmol/mL serum, Sensitivity = 97%, Specificity = 95%

ROC Area = 0.993
95% CI = (0.982, 1.00)

Figure 1
Figure 2
Figure 3
Figure 4A
Figure 4B

APAP-Adducts (nmol/mL serum)

Days after Ingestion

0 2 4 6 8 10 12 14

0.1 1 10 100