0090-9556/09/3708-1779–1784$20.00
DRUG METABOLISM AND DISPOSITION

DMD 37:1779–1784, 2009
Vol. 37, No. 8
26195/3492463
Printed in U.S.A.

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

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Received December 17, 2008; accepted May 6, 2009

ABSTRACT:

Acetaminophen (APAP)-induced liver toxicity occurs with formation of APAP-protein adducts. These adducts are formed by hepatic metabolism of APAP to *N*-acetyl-*p*-benzoquinone imine, which covalently binds to hepatic proteins as 3-(cystein-*S*-yl)-APAP adducts. Adducts are released into blood during hepatocyte lysis. We previously showed that adducts could be quantified by high-performance liquid chromatography 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 APAP 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 nonparametric statistical approaches. Peak concentrations of APAP-protein adducts correlated with peak aminotransferase concentrations ($r = 0.779$) in adults with APAP-related acute liver failure. Adducts did not correlate with bilirubin, creatinine, and APAP concentration at admission, international normalized ratio for prothrombin time, or reported APAP dose. After *N*-acetylcysteine therapy, adducts exhibited first-order disappearance. The mean elimination rate constant and elimination half-life were 0.42 ± 0.09 days^{-1} and 1.72 ± 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 postingestion. The persistence and specificity of APAP-protein adducts as correlates of toxicity support their use as specific biomarkers of APAP toxicity in patients with acute liver injury.

Acetaminophen [APAP; *N*-(4-hydroxyphenyl)acetamide; $C_8H_9NO_2$] overdose has recently been identified as a major cause of acute liver failure (ALF) in the United States (Larson et al., 2005). Currently, the diagnosis of APAP overdose is dependent on the history of a large dose of APAP, defined as 7.5 g of APAP in adults (Rumack et al., 1981), supported by an elevated level of APAP in peripheral blood (Smilkstein et al., 1988; Rumack, 2002). Many patients develop ALF rapidly, characterized by encephalopathy and the presence of coagulopathy [international normalized ratio (INR) ≥ 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 et al., 1984), to cysteine groups on protein as 3-(cystein-*S*-yl)-APAP adducts (Hoffmann et al., 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 et al., 1989, 1990; Roberts et al., 1991). Initial studies in the mouse model of APAP toxicity used antisera 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 et al., 1989, 1990; Roberts et al., 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 re-

This work was supported by the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grants DK06799, DK58639].

Parts of this work were previously presented as follows: James LP, Simpson PM, Letzig L, Kearns GL, and Hinson JA (2007) Examination of acetaminophen protein adducts (APAP-CYS) in children and adolescents with acetaminophen overdose. *Annual American Society for Clinical Pharmacology and Therapeutics Meeting*; 2007 Mar 22; San Diego, CA. American Society for Clinical Pharmacology and Therapeutics, Washington, DC.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.108.026195.

ABBREVIATIONS: APAP, acetaminophen; ALF, acute liver failure; INR, international normalized ratio for prothrombin time; NAC, *N*-acetylcysteine; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

leased 3-(cystein-*S*-yl)-APAP adducts are quantified by high-performance liquid chromatography with an electrochemical detector (Muldrew et al., 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, et al., 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 who received prompt treatment with *N*-acetylcysteine (NAC) and did not develop toxicity (Davern et al., 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 et al., 2006; James et al., 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 used as a diagnostic indicator have not been previously reported. Thus, as a followup to our previous studies (Davern et al., 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 with the observed concentrations of APAP adducts.

Materials and Methods

Study Population. Serum samples were analyzed post hoc from 53 adults with known APAP-related ALF who were enrolled in the sample/databank of the Acute Liver Failure Study Group (National Institutes of Diabetes and Digestive Diseases; William M. Lee, Principal Investigator). Clinical criteria for enrollment in the sample/databank were 1) presence of coagulopathy (INR for prothrombin time $\geq$ 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 g/day within 7 days of presentation; 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, and other etiologies). Daily serum samples are collected for 7 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. Two hundred thirty 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 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 were not standardized and were determined by the attending hepatologist. By definition, the study population did not include patients who did not have ALF; 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

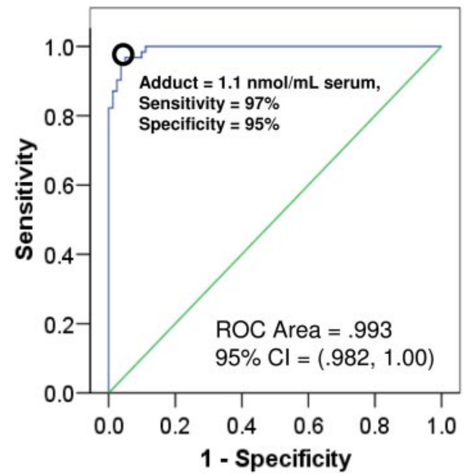

FIG. 1. Receiver operator curve analysis for APAP-protein adducts using ALT $>$1000 IU/l as a reference.

assay for APAP-cysteine derived by proteolytic cleavage of APAP adducts (Muldrew et al., 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 μM 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 interassay variations were assessed from the quality control samples. Quality control concentrations ranged from 0.031 to 17.5 μM, and three replicates were analyzed with each analysis. Intra-assay variation ranged from 3.24 to 10.0%. Interassay variation ranged from 5.29 to 10.49%. The lower limit of quantitation of the assay was determined by the lowest quality control concentration measurable with a CV of less than 15%. The lower limit of quantitation for the assay was defined as 0.03 μM (30 pmol of APAP cysteine/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 $\geq$1.1 nmol of APAP-protein adduct/ml provided a sensitivity of 96.8% and a specificity of 95% when ALT $>$1000 IU/l was used as a reference (Fig. 1).

Clinical Data. Patient data include reported dose (milligram/kilogram) 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, INR for prothrombin time, 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-h 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 (*H* test, *U* test). Statistical analysis was performed using SPSS (version 15; SPSS Inc., Chicago, IL). 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 nonlinear mixed effects model (NONMEM) program (version V, FOCE subroutine with interaction; NONMEM Project Group, University of California, San Francisco, CA). Monoexponential 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 because of the limited range and number of samples available for the analysis. Dose of the APAP ingestion and APAP concentrations were not modeled because of limited sampling, subject heterogeneity, and imprecision in self-reported APAP overdose histories.

TABLE 1

Demographic data for 53 subjects with acetaminophen-related acute liver failure

Category	Number	Percentage
Gender		
Female	35	66.0
Male	18	34.0
Race		
White	49	92.5
Asian	2	1.7
Black	2	1.7
Ethnicity		
Non-Hispanic	50	94.3
Hispanic	3	5.7
Clinical outcome		
Spontaneous survival	41	77.4
Death	9	16.9
Liver transplant	3	5.7
Ingestion type		
Acetaminophen only	46	86.8
Acetaminophen with opioid	7	13.2
N-Acetylcysteine treatment	50	94.3

Because more than 90% of the subjects received treatment with NAC, adduct formation was assumed to be complete for subjects sampled 3 or more days postingestion. 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 2 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 C_{max} (volume of distribution fixed to a value of 1). Individual empiric Bayesian estimates for the elimination rate constant (k_e) and $t_{1/2}$ were determined using the post hoc subroutine. The elimination rate and overall model goodness of fit were compared between those subjects with more than four samples and those with fewer than four samples. C_{max} (observed) 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 through 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, white, 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 in two 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 Fig. 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 (±S.D.) 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 the study samples and were compared with clinical outcomes and laboratory parameters. Because 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 with subjects who 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

TABLE 2

Summary clinical data (mean, S.D.) for 53 adults with acetaminophen-related acute liver failure

Parameter	Mean	S.D.
Age (years)	33.6	12.1
Weight (kg)	70.3	14.9
Body mass index (BMI)	25.3	5.6
Time to study enrollment from overdose (days)	3.12	1.4
Reported acetaminophen dose (mg/kg)	468	284
Acetaminophen concentration at study admission (mg/l)	69.5	80.7

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 postoverdose. The correlation for APAP adducts and AST was highest on days 3 and 4 postoverdose ($r = 0.84$; $r = 0.84$).

Pharmacokinetic Analysis. Because the mean (±S.D.) 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 more than four 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 four or more samples are presented in Fig. 4, A and B. Elimination half-life did not vary as a function of gender, body mass index, race, height, or age. In addition, $t_{1/2}$ did not vary as a function of reported regular ethanol use.

In subjects with sample collection initiated after day 2, APAP adduct C_{max} (observed) occurred with the first sample in 34 of 36 (94%) of subjects. In subjects with sample collections initiated before day 2, C_{max} (observed) occurred at the first collection in 71% (12 of 17) of subjects. Thus, the temporal profile of APAP adduct generation (Fig. 4B) appeared to mirror that previously reported for hepatic transferase elevation following APAP overdose, with peak expression at 2 to 3 days following APAP overdose, resulting in liver injury (Rumack et al., 1981; Rumack, 2002).

Discussion

The Acute Liver Failure Study Group 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 the patients have developed ALF by the time of the initial study sample. The mean time from ingestion to study admission was >3 days (Table 2), and the mean time from ingestion to the receipt of NAC was 3.5 days. The mean adduct $t_{1/2}$ for the study subjects was 1.72 days (±0.34 days; range, 0.94–2.55 days). In a previous, smaller study, we reported the $t_{1/2}$ of APAP adducts in four adults with APAP-related ALF ranged from 0.71 to 1.29 days (Davern et al., 2006). The slightly longer $t_{1/2}$ noted in the present study may reflect the larger subset of patients included in the present study (Davern et al., 2006).

In addition, the data showed that APAP adducts correlated with serum hepatic aminotransferases, and the highest correlation was noted for AST (Fig. 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 ad-

TABLE 3

Summary values of laboratory parameters for study population (n = 53)

	Peak ALT	Peak AST	Peak Total Bilirubin[a]	Peak Creatinine[b]	Peak INR
	IU/l	*IU/l*	*mg/dl*	*mg/dl*	
Mean	7396	7141	10.1	3.3	4.8
S.D.	4638	6028	8.0	2.9	3.5
Median	7150	7330	8.7	1.9	3.9
Minimum	609	114	1.4	0.5	1.4
Maximum	20,090	24,531	42.9	10.1	15.6

[a] To convert to μM, multiply by 17.1.
[b] To convert to μM, multiply by 88.

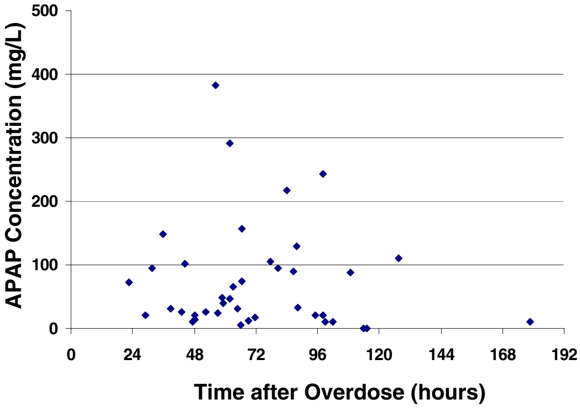

FIG. 2. Histogram plot of APAP concentrations at the time of study admission for 53 adults with APAP-related acute liver failure. The median concentration of APAP in the 1- to 99-mg/l group was 26 mg/l (range, 5.6–94.3). For two subjects, information on APAP concentrations was not available, and these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

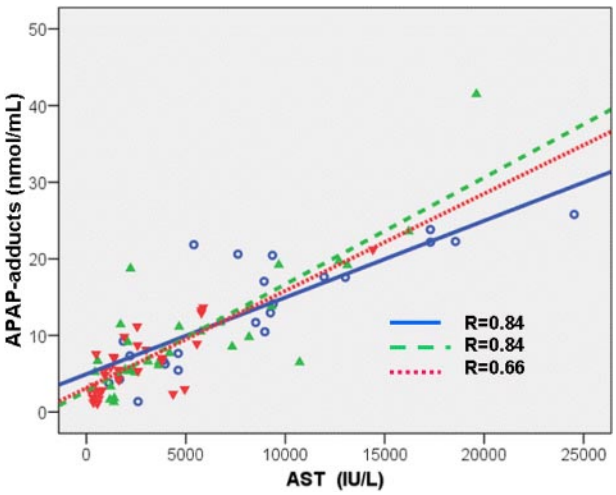

FIG. 3. Correlation of AST (IU/l) with APAP adducts in adults with APAP-related acute liver failure, plotted relative to overdose (▬, day 3; – – –, day 4; ⋯, day 5).

ducts has been established in animal models (Pumford et al., 1989, 1990; Roberts et al., 1991) and in patients (Hinson et al., 1990; Muldrew et al., 2002; James et al., 2008) and is logical as APAP-protein adducts accumulate in the hepatic cytosol (also the location of hepatic aminotransferases) and are released during toxicity. Although both AST and ALT may be found in extrahepatic tissues, the relative abundance of AST in extrahepatic tissues (e.g., heart, skeletal muscle, blood cells) is greater than that of ALT (Wroblewski, 1959; Green and Flamm, 2002). In addition, the primary cytochrome P450 enzyme responsible for the bioactivation of APAP, CYP2E1 (Gonzalez, 2007), is present in extrahepatic tissues (e.g., nasal mucosa, olfactory epithelium, lung, and kidney) (Gu et al., 2005), and the metabolic activation of APAP in these extrahepatic 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 et al., 2005) compared with 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 extrahepatic origin and may account for the better correlation of AST with APAP adducts compared with that of ALT with APAP adducts.

TABLE 4

Summary data for acetaminophen adducts in 53 adults with acetaminophen-related acute liver failure

	C_{max} (observed)	k_e	Half-life
	nmol/ml serum	*days*$^{-1}$	*days*
Mean	10.85	0.420	1.72
S.D.	9.26	0.090	0.34
Median	6.72	0.396	1.75
Minimum	0.79	0.272	0.94
Maximum	41.51	0.738	2.55

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 high-performance liquid chromatography with electrochemical detection determination of adducts and the long $t_{1/2}$ 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 (e.g., 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 h of APAP overdose (Rumack et al., 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 (Schiødt et al., 2002). The $t_{1/2}$ of APAP in patients with encephalopathy has been reported to be 3-fold longer (18.4 h) than that observed in patients without encephalopathy (6.4 h) (Schiødt et al., 2002). Whereas the $t_{1/2}$ of APAP may be prolonged in severe APAP-related liver injury, the relatively shorter $t_{1/2}$ of the parent

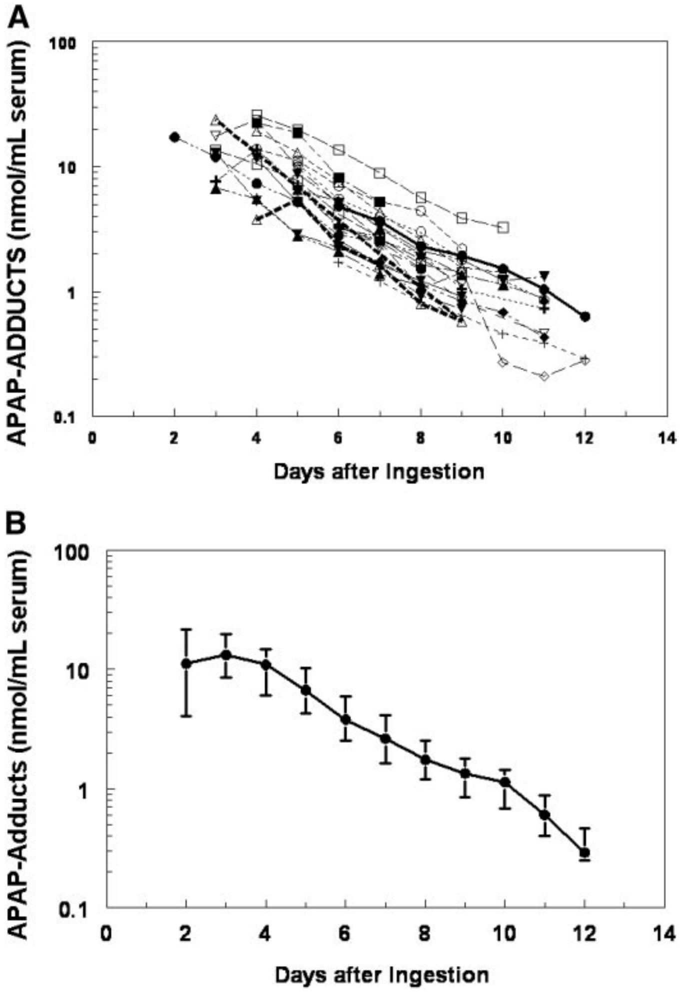

FIG. 4. A, individual line plots for 18 subjects with four samples available for APAP adduct analysis. One subject in this subset received concomitant opioids. B, summary data for APAP adducts presented as median and interquartile range.

compound limits its diagnostic usefulness for patients who 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 toward elevated concentrations as a result of interference from bilirubin (Bertholf et al., 2003; Polson et al., 2008). Therefore, measurement of APAP adducts, a highly specific (Davern et al., 2006) and persistent biomarker of APAP toxicity, has considerable advantages over existing nonspecific diagnostic methods and represents a potential new clinical parameter that can be used in patients who 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 use 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 et al., 2008). In the study of children and adolescents, the mean (±S.D.) 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 C_{max}, a surrogate marker for the degree of toxicity (James et al., 2008). An additional important finding was that significantly higher concentrations of adducts were detected in patients who had delays in treatment with NAC. The similarity in adduct elimination between these two studies, despite substantial differences in disease severity between the populations, suggests that determination of adduct concentrations may have potentially broad clinical relevance across the clinical spectrum of APAP toxicity, ranging from patients who receive early treatment with NAC to those who 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 supratherapeutic exposures to APAP and may be complicated by use of combination APAP/narcotic preparations (Larson et al., 2005). The severity of liver injury (ALT elevation), incidence of encephalopathy, and rate of transplant listings are very similar among patients with deliberate suicidal gestures and patients with unintentional overdoses (Larson et al., 2005). Nonetheless, further analysis of APAP adducts in patients who are victims of unintentional or inadvertent APAP overdose is warranted to examine the potential influence of concomitant opioid exposure and other comorbidities on the elimination of APAP adducts in this population.

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

References

Bertholf RL, Johannsen LM, Bazooband A, and Mansouri V (2003) False-positive acetaminophen results in a hyperbilirubinemic patient. *Clin Chem* **49:**695–698.

Dahlin DC, Miwa GT, Lu AY, and Nelson SD (1984) *N*-Acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. *Proc Natl Acad Sci U S A* **81:**1327–1331.

Davern TJ 2nd, James LP, Hinson JA, Polson J, Larson AM, Fontana RJ, Lalani E, Munoz S, Shakil AO, and Lee WM (2006) Measurement of serum acetaminophen-protein adducts in patients with acute liver failure. *Gastroenterology* **130:**687–694.

Davern TJ (2006) Indeterminate acute liver failure: a riddle wrapped in a mystery inside an enigma. *Hepatology* **44:**765–768.

Gonzalez FJ (2007) The 2006 Bernard B. Brodie Award Lecture Cyp2e1. *Drug Metab Dispos* **35:**1–8.

Green RM and Flamm S (2002) AGA technical review on the evaluation of liver chemistry tests. *Gastroenterology* **123:**1367–1384.

Gu J, Cui H, Behr M, Zhang L, Zhang QY, Yang W, Hinson JA, and Ding X (2005) In vivo mechanisms of tissue-selective drug toxicity: effects of liver-specific knockout of the NADPH-cytochrome P450 reductase gene on acetaminophen toxicity in kidney, lung, and nasal mucosa. *Mol Pharmacol* **67:**623–630.

Hinson JA, Roberts DW, Benson RW, Dalhoff K, Loft S, and Poulsen HE (1990) Mechanism of paracetamol toxicity. *Lancet* **335:**732.

Hoffmann KJ, Streeter AJ, Axworthy DB, and Baillie TA (1985) Structural characterization of the major covalent adduct formed in vitro between acetaminophen and bovine serum albumin. *Chem Biol Interact* **53:**155–172.

James LP, Alonso EM, Hynan LS, Hinson JA, Davern TJ, Lee WM, and Squires RH (2006) Detection of acetaminophen protein adducts in children with acute liver failure of indeterminate cause. *Pediatrics* **118:**e676–e681.

James LP, Capparelli EV, Simpson PM, Letzig L, Roberts D, Hinson JA, Kearns GL, Blumer JL, Sullivan JE, and the Network of Pediatric Pharmacology Research Units (2008) Acetaminophen-associated hepatic injury: evaluation of acetaminophen protein adducts in children and adolescents with acetaminophen overdose. *Clin Pharm Ther* **84:**684–690.

Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO, et al. (2005) Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. *Hepatology* **42:**1364–1372.

Muldrew KL, James LP, Coop L, McCullough SS, Hendrickson HP, Hinson JA, and Mayeux PR (2002) Determination of acetaminophen-protein adducts in mouse liver and serum and human serum after hepatotoxic doses of acetaminophen using high-performance liquid chromatography with electrochemical detection. *Drug Metab Dispos* **30:**446–451.

Polson J, Wians FH Jr, Orsulak P, Fuller D, Murray NG, Koff JM, Khan AI, Balko JA, Hynan

LS, and Lee WM (2008) False positive acetaminophen concentrations in patients with liver injury. *Clin Chim Acta* **391:**24–30.
Pumford NR, Hinson JA, Potter DW, Rowland KL, Benson RW, and Roberts DW (1989) Immunochemical quantitation of 3-(cystein-S-yl)acetaminophen adducts in serum and liver proteins of acetaminophen-treated mice. *J Pharmacol Exp Ther* **248:**190–196.
Pumford NR, Roberts DW, Benson RW, and Hinson JA (1990) Immunochemical quantitation of 3-(cystein-S-yl)acetaminophen protein adducts in subcellular liver fractions following a hepatotoxic dose of acetaminophen. *Biochem Pharmacol* **40:**573–579.
Roberts DW, Bucci TJ, Benson RW, Warbritton AR, McRae TA, Pumford NR, and Hinson JA (1991) Immunohistochemical localization and quantification of the 3-(cystein-S-yl)-acetaminophen protein adduct in acetaminophen hepatotoxicity. *Am J Pathol* **138:**359–371.
Rumack BH (2002) Acetaminophen hepatotoxicity: the first 35 years. *J Toxicol Clin Toxicol* **40:**3–20.
Rumack BH, Peterson RC, Koch GG, and Amara IA (1981) Acetaminophen overdose. 662 cases with evaluation of oral acetylcysteine treatment. *Arch Intern Med* **141:**380–385.
Schiødt FV, Ott P, Christensen E, and Bondesen S (2002) The value of plasma acetaminophen half-life in antidote-treated acetaminophen overdosage. *Clin Pharmacol Ther* **71:**221–225.
Smilkstein MJ, Knapp GL, Kulig KW, and Rumack BH (1988) Efficacy of oral *N*-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985). *N Engl J Med* **319:**1557–1562.
Wroblewski F (1959) The clinical significance of transaminase activities of serum. *Am J Med* **27:**911–923.

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