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

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ABSTRACT:

Acetaminophen-induced hepatotoxicity has been attributed to covalent binding of the reactive metabolite N-acetyl-p-benzoquinone imine to cysteine groups on proteins as an acetaminophen-cysteine conjugate. We report a high-performance liquid chromatography with electrochemical detection (HPLC-ECD) assay for the conjugate with increased sensitivity compared with previous methods. Previous methods to quantitate the protein-bound conjugate have used a competitive immunoassay or radiolabeled acetaminophen. With HPLC-ECD, the protein samples are dialyzed and then digested with protease. The acetaminophen-cysteine conjugate is then quantified by HPLC-ECD using tyrosine as an internal reference. The lower limit of detection of the assay is approximately 3 pmol/mg of protein. Acetaminophen protein adducts were detected in liver and serum as early as 15 min after hepatotoxic dosing of acetaminophen to mice. Adducts were also detected in the serum of acetaminophen overdose patients. Analysis of human serum samples for the acetaminophen-cysteine conjugate revealed a positive correlation between acetaminophen-cysteine conjugate concentration and serum aspartate aminotransferase (AST) activity or time. Adducts were detected in the serum of patients even with relatively mild liver injury, as measured by AST and alanine aminotransferase. This assay may be useful in the diagnostic evaluation of patients with hepatotoxicity of an indeterminate etiology for which acetaminophen toxicity is suspect.

Acetaminophen (N-acetyl-p-aminophenol; APAP; Paracetamol) is the most commonly used drug for the treatment of pain and fever. Although safe at therapeutic doses, in overdose, acetaminophen produces severe hepatotoxicity. The mechanism of toxicity is by initial metabolism to the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome P450 (Dahlin et al., 1984). At therapeutic doses, the reactive metabolite is detoxified by glutathione, but following overdoses, glutathione is depleted and the metabolite covalently binds to proteins as 3-(cystein-yl)-acetaminophen (APAP-CYS) (Streeter et al., 1984). Covalent binding to protein is thought to be a critical step in the development of hepatotoxicity.

A number of methods have been used to quantify the amount of acetaminophen covalently bound to proteins. In initial studies showing the correlation between acetaminophen toxicity and covalent binding, Jollow and coworkers (1973) used radiolabeled acetaminophen. Subsequently, polyclonal antisera were raised that recognized the acetaminophen-cysteine adducts (Bartolone et al., 1987; Roberts et al., 1987), and the relationship between covalent binding and toxicity was studied extensively. Pumford and coworkers (1989, 1990) developed a competitive enzyme-linked immunosorbent assay to quantify acetaminophen covalent binding in the liver and serum of treated mice. In addition, Western blot assays were performed. Although these latter assays were not quantitative, they were useful in comparing the relative amounts of adduct in proteins (Bartolone et al., 1988; Pumford et al., 1990; Matthews et al., 1996). These assays yielded valuable new information concerning the mechanism of acetaminophen toxicity but have had limited clinical application since the antibodies are not readily available and the assay was relatively insensitive for the measurement of acetaminophen-cysteine in human sera of acetaminophen overdose patients (James et al., 2001).

The following article describes the development of a high-performance liquid chromatograph with electrochemical detection (HPLC-ECD) method to quantify acetaminophen-cysteine in proteins. Acetaminophen- and glutathione-derived metabolites have previously been assayed by HPLC-ECD methods (Riggin et al., 1975; Munson et al., 1978; Miner and Kissinger, 1979; Hall et al., 1986; Whitcomb and Block, 1994; Alonso et al., 1995; Bonkovsky, 1995; Rivera-Penera et al., 1997). The advantage of the HPLC-ECD assay for acetaminophen-cysteine in proteins is its ability to quantify conjugates in serum...
from acetaminophen-overdosed patients. The assay may be useful in the diagnostic evaluation of patients who develop hepatotoxicity following therapeutic misadventures with acetaminophen (Hall et al., 1986; Whitcomb and Block, 1994; Alonso et al., 1995; Bonkovsky, 1995; Rivera-Penera et al., 1997).

Materials and Methods

Chemicals. L-Tyrosine, 4-acetamidophenol (acetaminophen), trichloroacetic acid, and protease type XIV from Streptomyces griseus (a 4-U/mg solid) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest grade and purchased from Fisher Scientific (Pittsburgh, PA).

Acetaminophen-Cysteine. APAP-CYS was synthesized using a modification of a previously described method (Potter and Hinson, 1986; Potter et al., 1989). Briefly, N-acetyl-p-benzoquinone imine was synthesized by oxidation of acetaminophen in chloroform in freshly prepared silver oxide (Dahlin et al., 1984). The chloroform solution was mixed with a solution of cysteine in sodium phosphate buffer (0.2 M), pH 7.05. The slurry was vigorously stirred for approximately 1 h. Subsequently, the aqueous solution was extracted with ethyl acetate and reduced to dryness under a stream of nitrogen. The APAP-CYS was purified on silica thin-layer chromatographic plates (Analtech, Wilmington, DE). The structure and purity (>99%) of the conjugate was established by 500-MHz 1H NMR spectroscopy, as previously described (Potter et al., 1986). Briefly, the NMR data showed the following proton assignments (shifts relative to tetramethylsilane, coupling pattern, number of protons): H2 (7.64, d, 1), H3 (6.86, d, 1), H4 (7.35, dd, 1), N-acetyl (2.08, s, 3), Cys-α (3.76, dd, 1), Cys-β (3.52, dd, 1), and Cys-γ (3.03, dd, 1).

Animals and Dosing. Male C57BL/6 mice (10 weeks of age) were obtained from Harlan Bioproducts for Science (Indianapolis, IN) (mean weight, 22.1 g). Animals were obtained from Harlan Bioproducts for Science (Indianapolis, IN) (mean weight, 22.1 g). Animals were obtained from Harlan Bioproducts for Science (Indianapolis, IN) (mean weight, 22.1 g). Animals were fasted the evening before the experiment. The morning of the experiment, animals were divided into five groups (n = 5 per group) and dosed with 300 mg/kg acetaminophen i.p. or an equal volume per body weight of saline. At 0 (saline group), 15, 30, 60, and 120 min, mice were anesthetized with CO2, and blood samples were removed from the retro- orbital plexus. The blood was allowed to clot at room temperature, and serum was separated by centrifugation. Subsequently, the animals were euthanized by CO2, and cervical dislocation was then performed.

The sera were surgically removed, and a portion of each liver was weighed and homogenized in a 3:1 v/w of 0.25 M sucrose, 10 mM Hepes, 1 mM EDTA buffer, pH 7.5, then stored at -80°C for analysis of GSH levels. Serum samples were analyzed for hepatic transaminase levels, as described below. The remaining serum samples were stored at -80°C before analysis.

APAP-CYS Adducts in Human Overdose Patients. A subset of banked serum samples from a previous acetaminophen overdose study in children and adolescents were analyzed for APAP-CYS adducts (James et al., 2001). In the initial study (James et al., 2001), serum samples were obtained from patients during the time of routine blood drawing for the clinical management of acetaminophen overdose. The study (James et al., 2001) was approved by the Human Research Advisory Committee of the University of Arkansas for Medical Sciences, and informed consent was obtained on all patients. Patients were treated with the antidote N-acetylcysteine by routine treatment protocols, and the treating physician determined the length of therapy. Serum samples from humans with no recent therapeutic or toxic acetaminophen exposure served as negative controls.

Assays. Serum AST and ALT levels were used as indicators of hepatotoxicity. These assays were performed in mice sera using a diagnostict kit obtained from Sigma Chemical Co. Acetaminophen concentrations in hepatic homogenates were determined using a commercial kit (Sigma Chemical Co.), as described by the manufacturer. Glutathione levels were determined in hepatic homogenates using a modification of the Elman procedure (Hinson et al., 1983).

Sample Preparation. Samples of mouse liver (100 mg) were homogenized in 1 ml of 10 mM sodium acetate, pH 6.5, sonicated for 10 s, and then stored at -80°C. Before analysis, liver homogenates were centrifuged at 16,000g, and the resulting supernatants were dialyzed (3500-kDa molecular mass cut off) against 4 liters of 10 mM sodium acetate buffer. Dialysis buffer was replaced at 9 and 21 h. Dialyzed samples (30 h) were stored at -80°C before digestion. Serum samples from mice were dialyzed under the same conditions as described above, with the exception that samples were diluted 1:10 before dialysis. Human serum samples were not diluted before dialysis. To confirm that dialysis removes potential, contaminating free APAP-CYS from protein bound APAP-CYS adducts, liver samples from saline-treated animals were spiked with 10 nM APAP-CYS and incubated at 25°C for 10 min. Samples were then dialyzed for 30 h, as described above, or received an additional 30-h dialysis. A portion of these dialysates was treated with and equal volume of 40% trichloroacetic acid. The resulting precipitate was washed twice with Na2HPO4, pH 7.4, then resuspended in 10 mM sodium acetate.

Protease Digestion. Protease digestion was performed according to previously published methods (Hensley et al., 1997; Walker et al., 2000) with some modifications. Briefly, a solution of APAP-CYS (8 U/ml) was dialyzed as described above to remove contaminating amino acids and stored at -80°C. Dialyzed proteins samples were mixed with an equal volume of dialyzed protease and incubated at 50°C for 18 h. After protease digestion, the samples were diluted 1:2 with 40% trichloroacetic acid to precipitate residual protein. The samples were placed on ice for 10 min and then centrifuged at 16,000g for 10 min. Supernatants were filtered through 0.2-μm polyvinylidene difluoride, Microfuge filter tubes immediately before analysis by HPLC. Samples were diluted in 20% TCA in 10 mM sodium acetate for HPLC analysis of L-tyrosine concentrations (serum, diluted 1:50; liver, diluted 1:250).

HPLC-ECD Analysis. Analysis was performed at room temperature under isocratic conditions using a model 582 solvent delivery system and two model 5600A CoulArray detectors from ESA (Chelmsford, MA). Automated injection (25 μl) was performed using a model 712, Waters Intelligent sample processor (WISP) from Waters Corp. (Milford, MA). The mobile phase was 50 mM sodium acetate and 7% methanol, pH 4.8. The flow rate was 1.0 ml/min through a reversed-phase TSK-GEL-ODS-80Tm column (4.6 mm x 25 cm; TOSOHASS, Montgomeryville, PA). Run time was 27 min. The peak detection limit was set at 100 pA, with an acceptable retention window error of 4%. Two CoulArray cell detectors (four electrodes/cell) were placed in series. In initial experiments, electrode potentials from 100 to 990 mV were divided between the eight electrodes. From the eight resulting currents, three were chosen that would detect L-tyrosine (leading, dominant, and following channels) and three that would detect APAP-CYS. A ratio analysis of the three channels along with retention time were used to identify L-tyrosine and APAP-CYS in unknown samples. Ratio 1 used the leading and dominant channels and compared the ratios of the peak heights for the standards to that of the unknown. Ratio 2 used the dominant and following channels. The closer the ratios are to 1 the greater the probability of identity. Hensley and coworkers (1997) describe this technique in detail. The optimal potentials for the analysis (channels 1–6) were 155, 280, 125, 460, 500, and 550 mV. L-tyrosine was detected on channels 4, 5, and 6, whereas APAP-CYS and APAP were detected on channels 1, 2, and 3. Once identified using ratio analysis and retention time, L-tyrosine and APAP-CYS in unknown samples were quantified using peak height of the dominant channel and standard curves for L-tyrosine and APAP-CYS. APAP was never detected in dialyzed samples. Stock solutions of APAP-CYS and L-tyrosine were prepared in 0.2 N HCl. Serial dilutions of a solution containing both APAP-CYS and L-tyrosine were prepared from 0.039 to 20 μM in 20% TCA/10 mM sodium acetate to generate standard curves. APAP-CYS values in unknown samples were expressed as moles of APAP-CYS per 100 mol of tyrosine. By normalizing the data to released L-tyrosine, values are not dependent in the degree of protease digestion.

Statistics. Ratio analysis and concentration determinations were made using CoulArray for Windows Data Processing Module, version 1.01 (ESA, Inc.). Data are expressed as mean ± S.E.M. unless noted otherwise. Statistical analysis was by analysis of variance followed by the Newman-Keuls test. Linear regression analysis was performed using Prism (GraphPad Software, Inc., Sand Diego, CA) and the Pearson correlation. Statistical significance was set at P < 0.05.

Results

HPLC-ECD Analysis of L-Tyrosine and APAP-CYS. Authentic L-tyrosine, APAP-CYS, and APAP were used to establish conditions for optimal separation and detection of each component. Figure 1A

HPLC-EC ASSAY FOR APAP-PROTEIN ADDUCTS
shows a representative chromatogram of a solution containing L-tyrosine, APAP-CYS, and APAP standards. Tyrosine, APAP-CYS, and APAP eluted at 6.5, 22.9, and 25.5 min, respectively. Standard curves were constructed on each day that samples were analyzed. Standard curves for L-tyrosine and APAP-CYS were linear over the concentrations tested (0.039–20 μM). For L-tyrosine the coefficient of
variation (CV) for the slope was 16.5% (n = 10 curves). CV for the 0.625 and 10 μM standards was 29 and 16% respectively. For APAP-CYS, CV for the slope was 13% (n = 12 curves). CV for the 0.625 and 10 μM standards was 13.5 and 13%, respectively. The lower limit of detection for each compound was approximately 0.8 pmol/25-μl injection at a signal-to-noise ratio of 3.

**Analysis of APAP-CYS Protein Adducts in Mice.** Liver and blood samples were collected from mice 2 h after administration of saline or APAP (300 mg/kg i.p.) (n = 5) were sacrificed at designated times. Samples of liver were analyzed for APAP-CYS and GSH (A). Samples of serum were analyzed for APAP-CYS and ALT levels (B). Data are expressed as mean ± S.E.M. *p < 0.05 compared with the saline control group (time 0).

Both retention time and peak ratio were used to define whether the unknown peaks were consistent with the standard (APAP-CYS). For liver samples, values for ratios 1 and 2 were 0.926 ± 0.008 (range, 0.817–0.978; n = 24) and 0.937 ± 0.009 (range, 0.817–0.998), respectively. Serum values for ratios 1 and 2 were 0.854 ± 0.026 (range, 0.596–0.992; n = 23) and 0.893 ± 0.011 (range, 0.825–0.983; n = 23), respectively.

Retention times for the metabolite and standard APAP-CYS were consistent throughout all runs. The average retention time for APAP-CYS was 22.71 ± 0.40 min (mean ± S.D.; n = 10) Average retention time for tyrosine was 6.45 ± 1.02 min (mean ± S.D.; n = 10).

**Table 1** Verification of APAP-CYS protein adducts

<table>
<thead>
<tr>
<th></th>
<th>APAP-CYS/100 moles L-tyrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse Serum 15 min after APAP</td>
<td></td>
</tr>
<tr>
<td>30-h Dialysis</td>
<td>0.023 ± 0.005</td>
</tr>
<tr>
<td>60-h Dialysis</td>
<td>0.019 ± 0.005</td>
</tr>
<tr>
<td>TCA precipitate of 60 h dialysis</td>
<td>0.027 ± 0.002</td>
</tr>
</tbody>
</table>

**Fig. 2.** APAP-CYS in liver and serum of APAP-treated mice.

Mice treated with APAP (300 mg/kg i.p.) (n = 5) were sacrificed at designated times. Samples of liver were analyzed for APAP-CYS and GSH (A). Samples of serum were analyzed for APAP-CYS and ALT levels (B). Data are expressed as mean ± S.E.M. *p < 0.05 compared with the saline control group (time 0).

**Fig. 3.** APAP-CYS in human serum samples.

A, linear regression analysis of APAP-CYS in sera versus serum AST levels in APAP overdose patients (Pearson r, 0.831; p < 0.05). Two points overlap at APAP-CYS levels of zero. Dotted lines indicate the 95% confidence interval. B, linear regression analysis of the time to treatment with N-acetylcysteine versus APAP-CYS in sera (Pearson r, 0.607; P < 0.05). Dotted lines indicate the 95% confidence interval. Symbols indicate estimated dose of APAP: ≤225 mg/kg (squares), 226 ≤ 350 mg/kg (diamonds), >350 mg/kg (circles).
Analysis of APAP-CYS Protein Adducts in APAP-Treated Mice. A time course for the formation of APAP-CYS protein adducts and GSH depletion in liver and serum APAP-CYS protein adducts and ALT levels were determined in mice treated with APAP (300 mg/kg). Figure 2A shows APAP-CYS protein adducts and GSH levels in the liver. As GSH levels fell, APAP-CYS increased. APAP-CYS protein adducts were detected 15 min after administration of APAP and increased approximately 20-fold from 0.035 ± 0.005 to 0.800 ± 0.150 mol of APAP-CYS/100 mol of tyrosine by 120 min. During this time period, GSH levels declined approximately 80%. Figure 2B shows APAP-CYS protein adducts and ALT activity in the serum of the same mice. As expected, serum ALT activity did not change over the 2-h time course. However, APAP-CYS protein adducts were detected 15 min after administration of APAP and were increased approximately 20-fold from 0.022 ± 0.005 to 0.418 ± 0.046 mol of APAP-CYS/100 mol of tyrosine by 120 min. These amounts, approximately 0.05 mmol/mg of protein and 1 nmol/mg of protein [based on the estimated abundance of tyrosine in protein of 3.2% (Doolittle, 1989)] are similar to values previously reported using the competitive enzyme-linked immunosorbent assay (Pumford et al., 1989).

Two types of experiments were performed to validate that the assay measured APAP-CYS protein adducts and not free APAP-CYS. In the first, authentic APAP-CYS was added to serum from mice that had not received acetaminophen. The serum was then dialyzed 30 h, treated with protease, and assayed by HPLC-EC for APAP-CYS adducts. APAP-CYS was not detectable in these samples, indicating that dialysis efficiently removed free APAP-CYS (data not shown). In the second experiment, serum obtained from the mice 15 min after administration of APAP was subject to extended dialysis. Table 1 shows that detected APAP-CYS did not change even after 60 h of dialysis. Furthermore, all detectable APAP-CYS was accounted for in the TCA precipitate. These data show protease digestion of dialyzed or TCA-precipitated serum measures APAP-CYS protein adducts.

APAP-CYS Adducts in Human Overdose Patients. Mean values of APAP-CYS adducts, AST, and ALT for overdose patients by hepatotoxicity severity group are listed in Table 1. No adducts were detected in control subjects (humans with no recent therapeutic or toxic APAP exposure). From linear regression analysis, positive linear relationships were found for APAP-CYS adduct concentration and AST levels (Fig. 3A; Pearson $r$, 0.831; $P < 0.05$). Thus, the large majority of patients fell within the 95% confidence interval. Only one patient was dramatically out of this range (Fig. 3A). It is unclear whether this patient had other pathology that may have lead to an increased AST level. Also, there was a significant correlation between APAP-CYS adduct concentration and the time from the acetaminophen ingestion to therapy (Fig. 3B; Pearson $r$, 0.607; $P < 0.05$).

APAP-CYS adducts were detected in patients even with relatively mild hepatotoxicity, as measured by AST and ALT levels (Table 2).

**Table 2 Human acetaminophen APAP overdose patients**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>AST</th>
<th>ALT</th>
<th>APAP-CYS/100 mol Tyrosine</th>
<th>Estimated APAP Dose</th>
<th>Time from Ingestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>21</td>
<td>0.009</td>
<td>230</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>45</td>
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<td>147</td>
<td>5</td>
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<tr>
<td>3</td>
<td>13</td>
<td>15</td>
<td>None detected</td>
<td>214</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>35</td>
<td>0.003</td>
<td>1186</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>46</td>
<td>0.006</td>
<td>200</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>106</td>
<td>130</td>
<td>0.015</td>
<td>429</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>502</td>
<td>638</td>
<td>0.020</td>
<td>263</td>
<td>26</td>
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<tr>
<td>8</td>
<td>1314</td>
<td>2111</td>
<td>0.016</td>
<td>806</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>2376</td>
<td>1874</td>
<td>0.080</td>
<td>184</td>
<td>25.5</td>
</tr>
<tr>
<td>10</td>
<td>3317</td>
<td>6269</td>
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<td>379</td>
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</tr>
<tr>
<td>11</td>
<td>4021</td>
<td>5442</td>
<td>0.024</td>
<td>333</td>
<td>29</td>
</tr>
</tbody>
</table>

**Discussion**

Studies in the mouse have revealed that APAP-CYS adducts are excellent biomarkers of acetaminophen toxicity. Initial data using radiolabeled acetaminophen suggested that adducts are formed only under conditions of toxicity (Jollow et al., 1973, 1974). Subsequently, these findings were confirmed using immunological assays (Roberts et al., 1987; Pumford et al., 1989; Birge et al., 1990; Pumford et al., 1990). Similarly, data in adult overdose patients also indicated that APAP-CYS adducts detected immunologically correlated with development of significant hepatotoxicity (ALT activity > 4000 IU/l) (Birge et al., 1990; Hinson et al., 1990; James et al., 2001).

In the present study, we have developed an assay for APAP-CYS protein adducts that uses HPLC and electrochemical detection. This assay is highly specific, extremely sensitive, and does not require specific antibodies that are generally unavailable. Thus, the assay may have general utility. Protein samples are enzymatically hydrolyzed and APAP-CYS released from protein is separated by HPLC and detected by an electrochemical method. Another advantage is that the assay uses tyrosine as an internal standard, eliminating the need to determine the absolute amount of protein hydrolyzed. This assay also can detect and quantify APAP, as others have previously reported (Rigg et al., 1975; Munson et al., 1978; Miner and Kissinger, 1979). However, dialysis or protein precipitation before proteolysis is effective in removing any contamination of free compounds.

Using this assay, we demonstrated that APAP-CYS adducts are generated in the liver and serum of mice before detectable increases in serum transaminase levels occur. With a competitive immunoassay, APAP-CYS adducts were detected in serum only after significant increases in serum ALT levels (Pumford et al., 1989, 1990). An overdose of APAP depletes liver GSH because GSH is an effective scavenger of NAPQI. We observed a 60% decrease in liver GSH content at the time APAP-CYS adducts began to appear. This is consistent with the concept that NAPQI will react with cysteine when GSH is depleted.

Also, we have used this assay to analyze samples obtained from APAP-overdosed patients. Western blot analysis only detected APAP-CYS adducts in serum when ALT activity exceeded 6000 IU/l (James et al., 2001). Analyzing these same serum using the HPLC-EC method, APAP-CYS adducts were detectable in serum of patients who overdosed with APAP even though the patients may not have had large increases in serum AST or ALT levels. Thus, the HPLC-EC assay seems to be more sensitive than immunological assays. There was a significant correlation between serum AST levels and serum,
APAP-CYS protein adducts. Furthermore, there was a significant correlation between serum APAP-CYS protein adducts and time to treatment. Additional studies are needed to assess the effect of N-acetylcysteine therapy on APAP-CYS adduct formation over time.

The development of this assay provides a tool for the systematic study of APAP toxicity in humans following toxic exposures or therapeutic misadventures with APAP. Recent literature has highlighted the occurrence of therapeutic misadventures with APAP (Seeff et al., 1986; Eriksson et al., 1992; Bonkovsky et al., 1994; Whitcomb and Block, 1994; Rivera-Penera et al., 1997; Heubi et al., 1998). These articles describe the development of hepatotoxicity in patients using APAP with therapeutic intent. Although the development of toxicity in the setting of therapeutic intent is poorly understood (Prestcott, 2000), several frequently cited risk factors include dose miscalculation or lack of age appropriate dose formulation (Rivera-Penera et al., 1997; Heubi et al., 1998), alcoholism (Eriksson et al., 1992; Johnston and Pelletier, 1997), malnutrition with associated depletion of glutathione (Bonkovsky et al., 1994), and concurrent therapy with drugs known to cause CYP2E1 induction (Wilson et al., 1978; Murphy et al., 1990). The development of this assay may be helpful in delineating the relative contribution of APAP covalent binding to the development of toxicity in these clinical settings and establishing whether serum levels of APAP-CYS early after APAP ingestion can be used as a predictor of the severity of hepatotoxicity. In addition, this assay may be useful in the diagnostic evaluation of patients with acute hepatic failure of indeterminate etiology for which APAP toxicity is suspect.

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References


