Confirming the Causative Role of Acetaminophen in Indeterminate Acute Liver Failure Using Acetaminophen-Cysteine Adducts

Sean M. Frey¹ · Timothy J. Wiegand² · Jody L. Green³ · Kennon J. Heard⁴ ·
Diana G. Wilkins⁵ · Rachel M. Gorodetsky^{2,6} · Richard C. Dart³

Published online: 21 April 2015
© American College of Medical Toxicology 2015

Abstract

Introduction Acetaminophen-cysteine adducts (APAP-CYS) are a serum biomarker of acetaminophen exposure, formed when the oxidative metabolite of acetaminophen binds to cysteine residues of hepatic proteins. APAP-CYS adducts become elevated in cases of acute liver failure following acetaminophen overdose and have been proposed as a diagnostic tool to identify acetaminophen-induced acute liver failure when standard testing is inconclusive.

Case Report A 26-year-old female with history of unexplained, severe hepatitis presented with a second episode of severe hepatitis including coagulopathy and transaminase levels >10,000 U/L. The patient reported ingesting “only a couple” of acetaminophen tablets several days prior to her presentation. An acetaminophen concentration of 14 mcg/mL at presentation aroused suspicion that acetaminophen might have caused the patient’s liver failure, despite her

adamant denial of overdose. APAP-CYS adduct levels measured from serum obtained 4 days after her presentation and in two consecutive serum samples are reported alongside previously reported APAP-CYS levels.

Discussion The patient’s APAP-CYS adduct levels were consistent with those seen in acute liver injury due to acetaminophen toxicity, even up to 1 week following presentation, and allowed for confirmation of acetaminophen toxicity as the cause of the her hepatitis. Overall, this case demonstrates the real-time application of APAP-CYS adducts as a biomarker to diagnose acetaminophen toxicity in patients with indeterminate acute liver failure.

Keywords Paracetamol · Gut and hepatotoxicity · Diagnostic test

This manuscript is written in US English.

✉ Rachel M. Gorodetsky
rachel.gorodetsky@gmail.com

- ¹ Department of Pediatrics, University of Rochester Medical Center, Rochester, NY, USA
- ² Department of Emergency Medicine, University of Rochester Medical Center, Rochester, NY, USA
- ³ Rocky Mountain Poison and Drug Center, Denver Health and Hospital Authority, Denver, CO, USA
- ⁴ Department of Emergency Medicine, University of Colorado School of Medicine, Denver, CO, USA
- ⁵ Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA
- ⁶ Department of Pharmacy Practice, D’Youville College School of Pharmacy, Buffalo, NY, USA

Introduction

Acetaminophen-cysteine adducts (APAP-CYS) are a biomarker of acetaminophen exposure, metabolism, and toxicity. Following therapeutic dosing of acetaminophen, a majority of the drug is cleared through hepatic glucuronidation and sulfation. The remainder is oxidized by the cytochrome P450 system to form a toxic intermediate metabolite, N-acetyl-p-benzoquinone imine (NAPQI). At therapeutic doses, NAPQI is detoxified by covalent binding to glutathione. NAPQI also binds to sulfhydryl groups on cysteine residues of hepatocellular proteins and creates APAP-CYS adducts [1, 2] (Fig. 1). These adducts are released into systemic circulation following hepatocyte injury and become measurable through the use of either a high-performance liquid chromatography electrochemical detection (HPLC-ED) assay or a liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay. While serum APAP-CYS concentrations are measurable in patients taking

Fig. 1 APAP metabolism including protein-adduct formation

therapeutic doses of acetaminophen, concentrations are significantly higher in patients following a toxic dose of acetaminophen [3]. This is because increased NAPQI production in overdose settings results in glutathione depletion and increased adduct formation.

APAP-CYS has been suggested as a diagnostic tool to identify acetaminophen-induced liver failure, but there are no examples that demonstrate how adducts can be used in a real-world setting. We present a case of recurrent acute liver failure (ALF) in a patient suspected of acetaminophen overdose, in which the quantification of serum APAP-CYS

allowed for conclusive identification of acetaminophen as the cause of her liver failure.

Case Report

A 26-year-old woman presented to the emergency department with acute onset abdominal pain, nausea, vomiting, and tremor. She was found to be in acute liver failure with severe elevations of serum alanine aminotransferase (ALT >5000 U/L), serum aspartate aminotransferase (AST >10,

000 U/L), and international normalized ratio (INR 3.3). Her initial acetaminophen concentration was 14 mg/L. On further questioning, the patient reported taking “a couple tablets” of acetaminophen several days prior to presentation. They were ingested with intent to treat chronic back pain made acutely worse by standing all day at a family member’s funeral. She denied more recent or long-term acetaminophen ingestion, including intentional overdose or the use of other acetaminophen-containing products.

Of note, the patient experienced a prior episode of severe, unexplained hepatitis approximately 1 year before this presentation. The earlier episode was thought most likely secondary to acetaminophen toxicity, although this etiology was not confirmed.

The patient had previous medical training but was still collecting disability related to a previous episode of liver failure and chronic back pain. The patient denied illicit drug use or any history of substance abuse or chemical dependency, with alcohol consumption only in social settings. Although the patient reported multiple, significant psychosocial stressors including isolation, financial, and other personal hardships, she specifically denied suicidal ideation or intent while in the hospital.

On initial exam, the patient was jaundiced with marked scleral icterus. She was hemodynamically stable, alert, and oriented with a normal neurological examination. Her abdominal examination was only notable for mild, generalized tenderness on palpation, without guarding or rebound tenderness. The patient was treated with an intravenous N-acetylcysteine (NAC) protocol (Acetadote®) at 150 mg/kg for the first hour, followed by 50 mg/kg/4 h, then 100 mg/kg over 16 h, and finally a repeat 16 h infusion bag. NAC was thus administered for 2 days until her ALT was <1000 U/L, INR was <1.5, and acetaminophen concentration was <2 mg/L. During treatment, she had paroxysms of abdominal pain requiring opioid analgesia, but no nausea or vomiting. Viral serology testing was negative.

Despite the patient’s insistence that she did not take more acetaminophen, significant concern remained that this second episode of ALF was secondary to acetaminophen toxicity. As typical therapeutic doses of acetaminophen have a half-life of 2–3 h, her serum acetaminophen concentration was inconsistent with the reported history of ingesting a few tablets; although the half-life of acetaminophen is prolonged in liver injury, a therapeutic dose would not be expected to produce an acetaminophen concentration of 14 mg/L several days later. Her multiple social stressors and insistence that she would not be able to work if she were “going into liver failure all of the time” concerned the provider team that the patient was not providing all necessary historical details. Psychiatry was consulted, but the patient refused to speak with any mental health providers.

To further assess the potential role of acetaminophen in causing this patient’s acute liver failure, serum APAP-CYS

concentrations were measured using a LC-MS/MS assay. The first measurement was obtained from a blood sample drawn 4 days after presentation to the emergency department, with two consecutive daily serum samples obtained for comparison. The initial APAP-CYS concentration on day 4 of hospitalization was 10.13 nmol/mL. By day 5, this had decreased to 6.21 nmol/mL, with a further drop to 4.90 nmol/mL by day 6 of hospitalization. These values were well above the reported 1.1 nmol/mL threshold for APAP-CYS in confirmed cases of acetaminophen-associated acute liver failure, as well as adduct concentrations seen with therapeutic dosing [3] (Fig. 2). Eventually, the patient admitted to taking an overdose of acetaminophen in order to maintain eligibility for “disability,” though dose and time of ingestion were never specified. A signed notice of agreement related to care and use of deidentified information for research and academic purposes was obtained during the admission.

Methods

APAP-CYS Protein Adduct Assay

Blood samples obtained on days 4, 5, and 6 of hospitalization were used to quantitate serum APAP-CYS adduct concentrations. Serum samples were frozen and stored at -80°C until analysis, which was conducted at the University of Utah within the Center for Human Toxicology laboratory using a LC-MS/MS method [4]. Samples were analyzed using a modification of this previously described method [5]. Briefly, a 500- μL aliquot of each serum sample was dialyzed overnight to remove any free APAP-CYS. The samples were then filtered two times through separate Bio-Spin 6 columns following the manufacturer’s instructions; after which, a 50- μL aliquot of the filtrate was added to 50 μL of protease type XIV enzyme in water (8 U/mL) and incubated at 37°C for 24 h to release APAP-CYS from the bound protein. After digestion, the internal standard norbuprenorphine-d3 was added to all assay tubes followed by acetonitrile to precipitate the enzyme. Enzyme was then pelleted using equal volumes of methanol:water. After centrifugation, the supernatant was evaporated to dryness and then reconstituted using 200 μL of 0.1 % formic acid in water. The supernatant was then passed through an Ultrafree-MC centrifugal filter device and transferred into autosampler vials for LC-MS/MS analysis.

Discussion

Acetaminophen toxicity is the most common cause of acute liver failure in the USA. Prior studies estimate that up to 50 % of all cases of ALF in adults are secondary to acetaminophen toxicity [6]. Current acetaminophen treatment protocols

Fig. 2 Comparing our patient's [APAP-CYS] with values observed in therapeutic dosing

require direct measurement of a patient's serum acetaminophen concentration, which is interpreted using the Rumack-Matthew nomogram. This nomogram, however, was only validated in acute, single ingestion, immediate release acetaminophen overdoses with known time of ingestion [7]. Most cases of ALF due to acetaminophen are not from single acute ingestions but rather from repeated supratherapeutic use of acetaminophen-containing products including OTC preparations and APAP/opioid combination products.

Use of the nomogram is further problematic as it has only been validated up to 15 h post ingestion. Patients with acetaminophen-related ALF often present for medical care at a later time and in later stages of hepatotoxicity than patients with ALF from other causes [2, 8]. Late entry to care (>15 h) after acetaminophen overdose in patients without ALF on presentation is also associated with an increased risk of later developing liver failure [9]. This delay means that serum acetaminophen concentrations may no longer be detectable at the time of presentation, and patients may be unable to provide an ingestion history due to altered mental status or unwilling to disclose a history of overdose [6].

Finally, falsely elevated acetaminophen concentrations can be found in patients with other ALF-related lab abnormalities. For example, very high serum bilirubin concentrations can cause false positives for acetaminophen with certain assay methods [8]. Together, these limitations suggest that there may be many patients with indeterminate-cause ALF in whom a true association with acetaminophen is not identified by standard diagnostic methods. Overall, up to 14 % of ALF in adult patients is of indeterminate cause [6]. There is a clear need for an improved ability to evaluate for acetaminophen toxicity in these patients.

APAP-CYS adducts are a potentially useful biomarker in such an indeterminate clinical setting for a number of reasons.

The initial rises in serum adduct concentrations parallel the increases seen with aminotransferases, with a significant correlation identified between peak serum aminotransferase and adduct concentrations [10]. Adducts also have a long elimination half-life, increasing the diagnostic window for identifying acetaminophen exposure. Compared with a typical acetaminophen half-life in patients with ALF ranging from 5.4 to 18.4 h, the mean elimination half-life of APAP-CYS adducts is 41.3 ± 8.3 h [2, 8]. In one study, APAP-CYS adducts were detectable in serum for up to 12 days after acetaminophen ingestion [10]; in another, adducts were detected for up to 7 days after enrollment into the study [8]. This delayed clearance is critical in considering the utility of APAP-CYS adducts as a diagnostic test. If a patient presents for care in later stages of hepatotoxicity, the prolonged presence of elevated adduct concentrations may be essential in helping clinicians to diagnose acetaminophen-associated ALF [7].

Several studies have examined APAP-CYS concentrations in instances of indeterminate ALF and suggested that up to 18–19 % of adult cases may be associated with acetaminophen toxicity on the basis of adduct concentrations [6, 8]. Moreover, adult patients with indeterminate ALF and elevated APAP-CYS concentrations shared the clinical and biochemical profile of patients with acetaminophen-associated ALF: hyperacute liver injury, high aminotransferase concentrations, low total bilirubin, a lower rate of transplantation, and a higher rate of spontaneous survival [6].

It is important to note that APAP-CYS adducts form and are detectable in the setting of therapeutic dosing [3], even in the absence of liver injury [1]. While they are detectable, however, “normal” adduct concentrations are significantly lower than those measured in patients with acetaminophen-associated ALF [3]. In a study of patients with ALF, 95 % of patients with clinically defined acetaminophen overdose had

APAP-CYS concentrations exceeding 1.0 nmol/mL, with a median APAP-CYS concentration of 11.1 nmol/mL [6]. In contrast, therapeutic dosing typically yields adduct concentrations less than 0.5 nmol/mL [3] (Fig. 2). Differences in the rate of adduct formation between immediate- and extended-release preparations have been observed in patients administered supra-therapeutic (but sub-toxic) doses, though peak adduct concentrations for both preparations remained well below those measured in acetaminophen-associated ALF [2]. Overall, the predictable rise and delayed half-life of adducts, in concert with previous work suggesting an adduct threshold associated with toxic acetaminophen overdose, strongly suggests that APAP-CYS assays can be a valuable tool in the identification of liver failure from occult acetaminophen poisoning.

Limitations

This is a single case report involving APAP-CYS adduct measurement and so may have limited generalizability to other patients and clinical contexts. As these assays are currently confined to research centers, they are not readily accessible to most practitioners. Finally, the assays took 3 days to run as overdose samples. While this timeline was reasonable given our patient's length of hospitalization, it may limit perceptions of usability among practitioners.

Conclusion

The patient in this case presented with a second episode of acute liver failure from indeterminate cause. Although intentional acetaminophen toxicity was suspected, initial data did not allow for conclusive determination of this etiology. Evaluating serum APAP-CYS concentrations revealed values consistent with toxic ingestion, even up to a week after presentation to care. Using this emerging biomarker allowed for the confirmation of acetaminophen toxicity as the cause of the patient's acute liver failure. This case illustrates the feasibility and real-time application of measuring acetaminophen

adducts in hospitalized patients with indeterminate acute liver failure.

Conflict of Interest Drs. Frey, Wiegand, and Gorodetsky have no conflicts of interest to disclose.

Drs. Green, Heard, and Dart received research funding from McNeil Consumer Healthcare via contracts with their institution. They did not receive personal compensation related to performance of any grant.

Dr. Wilkins is the primary investigator on an analytical service contract for acetaminophen-cysteine adducts analysis for McNeil Consumer Healthcare.

References

1. Bond GR. Acetaminophen protein adducts: a review. *Clin Toxicol (Phila)*. 2009;47(1):2–7.
2. James LP et al. Acetaminophen protein adduct formation following low-dose acetaminophen exposure: comparison of immediate-release vs extended-release formulations. *Eur J Clin Pharmacol*. 2013;69(4):851–7.
3. Heard KJ et al. Acetaminophen-cysteine adducts during therapeutic dosing and following overdose. *BMC Gastroenterol*. 2011;11:20.
4. McGill MR et al. HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. *Hepatology*. 2011;53(3):974–82.
5. Cook SF et al. Quantification of a biomarker of acetaminophen protein adducts in human serum by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry: clinical and animal model applications. *J Chromatogr B Anal Technol Biomed Life Sci*. 2015;985:131–41.
6. Khandelwal N et al. Unrecognized acetaminophen toxicity as a cause of indeterminate acute liver failure. *Hepatology*. 2011;53(2):567–76.
7. James LP et al. Pharmacokinetics of acetaminophen-protein adducts in adults with acetaminophen overdose and acute liver failure. *Drug Metab Dispos*. 2009;37(8):1779–84.
8. Davern TJ et al. Measurement of serum acetaminophen-protein adducts in patients with acute liver failure. *Gastroenterology*. 2006;130(3):687–94.
9. Waring WS. Criteria for acetylcysteine treatment and clinical outcomes after paracetamol poisoning. *Expert Rev Clin Pharmacol*. 2012;5(3):311–8.
10. James LP et al. Acetaminophen-associated hepatic injury: evaluation of acetaminophen protein adducts in children and adolescents with acetaminophen overdose. *Clin Pharmacol Ther*. 2008;84(6):684–90.