Letter

LC-MS analyses of *N*-acetyl-*p*-benzoquinone imine-adducts of glutathione, cysteine, *N*-acetylcysteine, and albumin in a plasma sample: A case study from a patient with a rare acetaminophen-induced acute swelling rash

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ABSTRACT — Acetaminophen (Paracetamol, APAP) has been widely used for many decades as an analgesic and antipyretic agent but APAP overdose often causes acute adverse reactions, particularly liver damage. The metabolically oxidized form of APAP, *N*-acetyl-*p*-benzoquinone imine (NAPQI), is chemically reactive and binds covalently to proteins. Therefore, NAPQI is believed to be the key metabolite that causes hepatotoxicity, especially under conditions of glutathione depletion. Other APAP-induced adverse reactions, such as skin damage, are rare and remain poorly studied. Here, we report a case study of a male patient who presented with an acute swelling skin rash (without hepatotoxicity) caused by therapeutic doses of APAP. Plasma samples were collected at 17 hr after dosing (during the manifestation of symptoms) and at one month (after recovery) and were subjected to LC-MS analysis of NAPQI-adducts. A significant concentration of NAPQI-cysteine adduct (33 pmol/mL) was found together with low concentrations of NAPQI-*N*-acetylcysteine adduct (2.0 pmol/mL) and NAPQI-glutathione adduct (0.13 pmol/mL). However, the NAPQI-albumin adduct was below the detection limit (below 0.001% modification on albumin) despite a previous report of high concentrations of NAPQI-albumin adduct following acute liver injury. Therefore, the observed APAP-induced skin damage may have had a different cause from APAP-induced liver injury.

Key words: Acetaminophen, *N*-acetyl-*p*-benzoquinone imine, Adduct, Skin damage, LC-MS

INTRODUCTION

Acetaminophen (*N*-acetyl-*p*-aminophenol, Paracetamol, APAP, Fig. 1A) has been widely used for many decades as an analgesic and antipyretic agent (Aminoshariae and Khan, 2015). APAP is normally safe and effective at therapeutic doses and thus is readily available as an OTC drug. However, serious idiosyncratic toxic reactions, such as acute liver failure, have been reported (James *et al.*, 2003). *N*-Acetyl-*p*-benzoquinone imine (NAPQI, Fig. 1B) is suspected to be the causative toxic metabolite, is chemically reactive, and binds covalently to proteins (Dahlin *et al.*, 1984). This adverse reaction

occurs following an overdose due to inadequate sulfation (Fig. 1C)/glucuronidation (Fig. 1D) of APAP and glutathione (GSH, Fig. 1E) and subsequent conjugation with NAPQI (McGill and Jaeschke, 2013; James *et al.*, 2003). Treatments for acute APAP-induced toxicity include injecting cysteine (Cys, Fig. 1F) derivatives, mainly *N*-acetyl-Cys (AcCys, Fig. 1G), because AcCys increases GSH levels and/or scavenges reactive NAPQI (Heard, 2008) and reduces NAPQI to the original unreactive APAP drug (Shayani-Jam and Nematollahi, 2010). Therefore, APAP metabolites (Vliegenthart *et al.*, 2017) and the NAPQI-human serum albumin (HSA, Fig. 1H) adduct (Geib *et al.*, 2018) are analyzed as biomarkers of acute liver injury.

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Fig. 1. Metabolic pathway of acetaminophen (APAP) and adduct formation with biological compounds. (A) APAP, (B) *N*-acetyl-*p*-benzoquinone imine (NAPQI), (C) APAP-sulfate, (D) APAP-glucuronide, (E) glutathione (GSH), (F) cysteine (Cys), (G) *N*-acetylcysteine (AcCys), (H) human serum albumin (HSA), (I) NAPQI-GSH, (J) NAPQI-Cys, (K) NAPQI-AcCys, and (L) NAPQI-HSA (analyzed as the chemotryptic peptide, NAPQI-LQQC³⁴PF).

ry/failure. However, the causes of APAP-induced hepatotoxicity are complex due to possible contributions from mitochondrial oxidative and nitrosative stress, downstream signaling, specific mediators, autophagy, and innate immunity (Ramachandran and Jaeschke, 2018). APAP is also known to induce idiosyncratic skin reactions. This is very rare but can result in severe and fatal skin damage, such as Stevens-Johnson syndrome (Trujillo *et al.*, 2010) and toxic epidermal necrolysis (Halevi *et al.*, 2000), resulting in a warning of this risk in 2013 from The U.S. Food and Drug Administration (FDA) (Kuehn, 2013;

FDA, 2013). However, few mechanistic studies, including studies of biomarkers, have been conducted because of the rarity of this reaction.

Here, we report a case study of a Japanese man in his early forties who presented with a rare APAP-induced acute swelling skin rash (without hepatotoxicity) which was treated with Cys. The NAPQI-adducts of GSH (Fig. 1I), Cys (Fig. 1J), AcCys (Fig. 1K), and HSA (Fig. 1L) in plasma samples obtained during the manifestation of symptoms and following recovery were analyzed by liquid chromatography/electrospray ionization-select-

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Fig. 2. LC/ESI-SRM/MS analyses of NAPQI adducts. (A, E) NAPQI-GSH, (B, F) NAPQI-Cys, (C, G) NAPQI-AcCys, and (D, H) NAPQI-HSA (analyzed as the chemotryptic peptide, NAPQI-LQQC³⁴PF). A–D, samples collected during the presence of symptoms; E–G, samples collected after recovery. Upper, adducts from patient; lower, corresponding IS (NAPQI-[¹³C₂, ¹⁵N₁]-GSH, NAPQI-[¹³C₃, ¹⁵N₁]-Cys, NAPQI-[²H₃]-AcCys, and intact LQQC³⁴PF).

ed reaction monitoring/mass spectrometry (LC/ESI-SRM/MS).

MATERIALS AND METHODS

Clinical experiment

Design

The aim of this investigative biomarker study was to estimate drug-induced idiosyncratic toxicity. The experimental setting was approved by the ethics committees of the Graduate School of Pharmaceutical Sciences, Tohoku University (15-01). All clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki.

Patient

A Japanese man in his early forties who drinks alcohol daily, is a non-smoker, and had a surgical operation (unknown, blind test).

History of drug administration

Before the surgical operation, Bactroban® nasal ointment (2%, 3 g) and Cefamezin®a (i.v.d., 1 g); during operation, Bridion® (i.v., 200 mg/2 mL), 1% Propofol® (i.v., 200 mg/20 mL), Lidocaine (i.v., 1%, 10 mL), Vecuronium (i.v., 10 mg/vial), Fentanyl (i.v., 0.1 mg/2 mL, 4 mL), Sevofrane® (inhalation, 28 mL), and Acelio® (i.v., 1000 mg APAP/100 mL).

Symptoms and treatment

Abdominal distension, swelling red rash (17 min. after Acelio® injection). Treatment, Acelio® terminated, Minofit® (i.v., 20 mL, 20 mg L-Cys), Polalamine® (i.v., 5 mg/mL), Loxoprofen (tablet, 60 mg), and Cefamezin®a (i.v.d., 1 g).

Physiological data from blood (taken 17 hr after the manifestation of symptoms)

Bilirubin, 0.6 mg/dL; AST, 20 U/L; ALT, 22 U/L; ALP, 162 U/L; LDH, 124 U/L; γ -GTP, 38 U/L; CK, 222

U/L; albumin, 3.7 g/dL; creatinine, 0.73 mg/dL; BUN, 11.9 mg/dL; eGFR, 93.6; Na, 140 mEq/L; K, 3.5 mEq/L; Cl, 104 mEq/L; Ca, 8.9 mg/dL; P, 1.5 mg/dL; Mg, 2.3 mg/dL; sugar, 192 mg/dL; WBC count, $6.9 \times 10^3/\mu\text{L}$; RBC count, $511 \times 10^4/\mu\text{L}$; hemoglobin, 14.6 g/dL; hematocrit, 41.9%; MCV, 82 fL; MCH, 28.6 pg; MCHC, 34.8%; thrombocyte, $19.1 \times 10^4/\mu\text{L}$; neutrophil, 79%; lymphocyte, 17%; monocyte, 4%; eosinophil, 0%; basophil, 0%; RET, 13 %; CRP, 0.50 mg/dL. A control plasma sample was taken from the same patient after recovery from the symptoms (1 month later).

Materials, reagents, and analytical procedures

Detailed information is provided in Supplemental Data.

RESULTS AND DISCUSSION

The blood sample obtained 17 hr after the start of the APAP-induced acute swelling red rash and following cysteine treatment showed a significant level of NAPQI-Cys (Fig. 2B; 33 pmol/mL) and an order of magnitude lower level of NAPQI-AcCys (Fig. 2C; 2.0 pmol/mL). However, the sources of these two adducts could not be assigned to whether endogenous thiols (GSH or Cys) or exogenous thiol (Cys in Minofit®). In addition, the NAPQI-GSH adduct was unexpectedly low (Fig. 2A; 0.13 pmol/mL), perhaps due to GSH depletion caused by daily alcohol intake and/or because NAPQI-GSH had already been metabolized to NAPQI-Cys or -AcCys adducts. Furthermore, the NAPQI-HSA adduct was unexpectedly below the detection limit (Fig. 2D; relative modification ratio < 0.001%). Several articles have reported adduct formation in patients with acute liver failure and severe hepatotoxicity (Damsten *et al.*, 2007; Geib *et al.*, 2018) as well as in patients without hepatotoxicity (O'Malley *et al.*, 2015). We previously found that Cys³⁴ in HSA is the most likely binding site for the NAPQI *in vitro* (Osaki *et al.*, 2014). A blood sample taken after the patient had recovered (1 month later) showed dramatic reductions in NAPQI-GSH (Fig. 2E; undetectable), NAPQI-Cys (Fig. 2F; undetectable), and NAPQI-AcCys (Fig. 2G; < 4 fmol/mL). The patient drank alcohol daily but did not show APAP-induced hepatotoxicity (AST < 30 U/L, ALT < 30 U/L, γ -GTP < 50 U/L), despite many reports of APAP-treatment for alcoholic patients and hepatotoxicity (Dart *et al.*, 2000). Therefore, the observed APAP-induced skin damage may have had a different cause. Furthermore, the patient was under oxidative stress, judging from high methionine oxidation levels in his skin keratin (data not shown; to be published

elsewhere), suggesting that Cys³⁴ in HSA was taking non-nucleophilic forms of disulfides with endogenous thiols such as Cys (Nagumo *et al.*, 2014) and/or oxidized forms such as sulfuric acid (Colombo *et al.*, 2012).

In conclusion, this study reported the concentrations of APAP-metabolites and NAPQI-adducts in plasma samples from a patient exhibiting rare APAP-induced skin damage. Although only one patient was studied, to our knowledge this is the only study to analyze plasma NAPQI adducts from a patient presenting with an APAP-induced acute swelling rash treated with Cys. Our findings may help clarify the toxicological mechanisms underlying APAP-induced skin damage.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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