J Forensic Sci, July 2007, Vol. 52, No. 4
doi: 10.1111/j.1556-4029.2007.00482.x
Available online at: www.blackwell-synergy.com

CASE REPORT

Peter P. Singer,[1] *Ph.D.; Graham R. Jones,*[2] *Ph.D.; Bernard G. Bannach,*[3] *M.D.; and Lloyd Denmark,*[4]* *M.B.B.S.*

Acute Fatal Acetaminophen Overdose Without Liver Necrosis

ABSTRACT: Two unusual cases of suicidal overdose of acetaminophen (paracetamol) without the usual extensive centrilobular necrosis of the liver are reported. Both cases were subjected to comprehensive drug screening by immunoassay, and a combination of gas chromatography with mass spectrometry, nitrogen detection, and electron capture detection. Acetaminophen was detected in both cases. No other drugs were detected in case #1, and only a small amount of olanzapine (<0.1 mg/L) was detected in case #2. No anatomical cause of death was identified in either case. If untreated, the normal outcome of a large acetaminophen overdose would be massive hepatic necrosis with delayed death and low blood and tissue acetaminophen concentrations. In contrast, particularly high postmortem acetaminophen concentrations were measured in both our cases with little hepatic tissue damage. For case #1, femoral blood acetaminophen 1280 mg/L, vitreous 878 mg/L, and liver 729 mg/kg; in case #2, cardiac blood 1220 mg/L, vitreous 779 mg/L, liver 3260 mg/kg, and gastric 11,500 mg/500 g. Acetaminophen was measured using high performance liquid chromatography with UV detection (254 nm) using 3-hydroxyacetanilide as the internal standard. The very high concentrations of acetaminophen is these cases but relatively little hepatic damage suggests an alternative, possibly cardiac, mechanism of death.

KEYWORDS: forensic science, acetaminophen, paracetamol, overdose, liver, necrosis, centrilobular, cardiac

Acetaminophen (paracetamol, *p*-hydroxyacetanilide) is an extremely widely used antiinflammatory and analgesic drug that is frequently used in suicide attempts. Overdose with acetaminophen generally produces a toxic intermediate which leads to extensive hepatic destruction, characterized by centrilobular necrosis (1). If the suicide is successful, death usually follows 3–5 days later, at which time only low concentrations of acetaminophen are present, but extensive hepatic damage may be seen on histologic examination. The following cases involve unusual sudden deaths following massive acetaminophen overdose with very high postmortem concentrations of acetaminophen, but absent or very minimal hepatic necrosis.

Case History

Case #1

This 39-year-old Caucasian male was involved in a motor vehicle accident 11 years ago that left him quadriplegic from damage at the C8 position. He had a recent history of pyelonephritis, ulcers, depression, and two suspected suicide attempts by postural asphyxia (tipping the wheelchair so he was trapped beneath it). On the weekend of his death he stated he was suffering more pain than usual and asked his helper to empty a new container of Extra Strength Tylenol (150 tablets 500 mg, equivalent to a maximum dose of 1.27 g/kg) into a cup, so that he could take them as necessary. She checked on him 2 days later and found him dead in bed, lying on his side, with full rigor. Colace, Senokot, Imovane (zopiclone), baclofen, Noroxin (norfloxacin), Clavulin (amoxacillin, clavulanate), and Novotetra (tetracycline) were also prescribed.

At autopsy he weighed 59 kg (130 lb) and was 175 cm (5 ft 9 in) in length with no evidence of recent injury. His lungs were edematous and congested, weighing 513 g (right) and 791 g (left), with some hemorrhagic mucous in the airways. The heart was unremarkable with minimal atherosclerosis and no other disease. Kidneys (186 and 194 g) were grossly normal, and on microscopic examination, showed focal interstitial nephritis but no evidence of significant destruction by any acute or chronic infection. The liver (1382 g) was somewhat soft and on microscopic examination showed only early liquefactive necrosis in the centrilobular region. An ileostomy was *in situ*. There was no anatomic cause of death.

Case #2

This 18-year-old female had a history of schizophrenia and other psychiatric problems. She was found dead in a hotel room. Several empty and partially full bottles of Extra Strength Tylenol were found in the room, in addition to a suicide note. Although the time of death is unknown, the victim was alive up to 16 h before being found dead, based on receipts for food found at the scene.

At autopsy, she weighed 134 kg (295 lb) and measured 185 cm (6 ft 1 in) in length. The findings were unremarkable except for congested and edematous lungs (right 655 g, left 654 g) and extensive pill residue in the stomach. The heart (415 g) was grossly unremarkable with the myocardium a uniform red/brown color; the coronary arteries were free of atherosclerosis. The liver (2256 g) was grossly normal, and on histologic examination showed

[1]Deputy Chief Toxicologist, Office of the Chief Medical Examiner, Edmonton, AB, Canada.
[2]Chief Toxicologist, Office of the Chief Medical Examiner, Edmonton, AB, Canada.
[3]Assistant Chief Medical Examiner, Office of the Chief Medical Examiner, Edmonton, AB, Canada.
[4]Deputy Chief Medical Examiner, Office of the Chief Medical Examiner, Calgary, AB, Canada.
*Present address: Department of Forensic Pathology, Labplus, Auckland City Hospital, Auckland, New Zealand

Received 25 Aug. 2006; and in revised form 28 Jan. 2007; accepted 24 Feb. 2007; published 4 June 2007.

moderate macrovesicular steatosis with no evidence of centrilobular necrosis. There was no anatomic cause of death.

Methods

Both cases were subjected to routine alcohol screening by headspace gas chromatography with flame ionization detection for the presence of ethanol and other volatiles. Blood was screened for the presence of drugs by ELISA for acetaminophen, salicylate, benzodiazepines, cocaine metabolites, and opiates. Blood was also screened by gas chromatography with mass spectrometry detection (GC/MS) in combination with nitrogen–phosphorus detection (GC/NPD), as well as GC with electron capture detection. Other than acetaminophen, no other drugs were detected in case #1 and only a small amount of olanzapine was detected in blood from case #2 (<0.1 mg/L).

Acetaminophen (Sigma-Aldrich, St. Louis, MO) was quantitated by high-performance liquid chromatography (HPLC). To blood-based calibrators (5–200 mg/L), blank, controls, and specimens (0.1 mL, diluted as necessary) were added 3-hydroxyacetanilide (Sigma-Aldrich) as internal standard (10 μg) and sodium chloride (2 g). Ethyl acetate (3 mL) was used as an extraction solvent. Extracts were carefully dried, reconstituted in mobile phase (acetonitrile:phosphate buffer 0.005 M, pH 6.0; 1:9) and analyzed by HPLC using a 717 autosampler (Waters, Milford, MA), 616 pump, 996 photodiode array detector (quantitation at 254 nm), and a 15 cm LC8 Novapak column (Waters) under isocratic conditions. Data were collected and analyzed by Millennium software (ver 2.1) (Waters). Acceptance criteria for the assay required that all calibrators and controls read within ±20% of target and the calibration regression coefficient (r^2) was ≥0.995. For both cases #1 and #2, the calibration was linear (r^2 = 0.9999 and *y*-intercept of 0.0). Controls were independently prepared in-house at a target concentration of 25 mg/L. Accuracy and precision of the assay are based on the data from the 2005 quality control batch: target 25.2 mg/L, QC mean 25.4 mg/L ± 1.63 SD, CV 6.5% (n = 24).

Results and Discussion

Acetaminophen concentrations for cases #1 and #2 are shown in Table 1. Ethanol or other drugs were not detected in case #1, and only a small amount of olanzapine (<0.1 mg/L) in case #2. The screening employed was not designed to detect antibiotics or baclofen (case #1), and their presence or absence was not confirmed by any special analyses. After therapeutic doses of 1000 mg, plasma concentrations should generally be <30 mg/L (2). Postmortem blood concentrations in both cases #1 and #2 are clearly consistent with an overdose. Acetaminophen is not usually subjected to postmortem redistribution, although some degree of nonhomogeneity of blood concentrations due to incomplete distribution or postmortem diffusion from the stomach is a possibility (3,4).

TABLE 1—*Acetaminophen concentrations in postmortem specimens from case #1 and #2.*

Specimen	Case #1	Case #2
Femoral blood (mg/L)	1280	—
Cardiac blood (mg/L)	—	1220
Vitreous (mg/L)	878	779
Liver (mg/kg)	729	3260
Urine (mg/L)	1500	1780
Stomach contents (mg in total contents)	486	11,500

Acetaminophen, at normal doses, is metabolised by conjugation with glucuronic acid and sulphate. If these paths are saturated, as it readily occurs in overdose, acetaminophen is oxidized by cytochrome P450 (mainly by CYP1A2, 2E1, and 3A4 isozymes) to *N*-acetyl-*p*-benzoquinoneimine (NAPQI) (5–9). NAPQI has a particular affinity for sulphydryl groups and reacts rapidly with glutathione to produce cysteine and mercapturic acid conjugates. Once glutathione stores are depleted, the highly reactive NAPQI binds covalently to proteins (likely the sulphydryl sites in the proteins), leading to tissue damage (10–13). As the liver has the highest concentration of the P450 metabolizing enzymes, this is the site of NAPQI production and consequently leads to the hepatic cell damage.

Hence, the usual outcome of a large untreated acetaminophen overdose is hepatic damage, with death following several days later. Acetaminophen normally has a short half-life in the body, on the order of 1–3 h. After overdose, the half-life may be increased to 6–8 h and sometimes longer (14–16). Even with a lengthened half-life, there is usually sufficient time before death to metabolize and excrete the majority of the acetaminophen such that postmortem blood concentrations are typically <50 mg/L. On microscopic examination visible centrilobular necrosis is readily observed. It is unusual to find high concentrations of acetaminophen in postmortem specimens unless the death resulted from a mixed drug overdose with other, more immediately toxic drugs, such as codeine or propoxyphene. Occasionally other tissues, such as the kidneys, may suffer damage. Nephrotoxicity appears to be due to the formation of *p*-aminophenol as well as NAPQI (17–19).

In the two cases reported here, acetaminophen was detected at very high concentrations, with only therapeutic concentrations of olanzapine found in case #2. There was only early preliminary centrilobular damage in case #1, differing from the extensive hepatic necrosis normally observed following fatal acetaminophen overdose, and no evidence of centrilobular necrosis in case #2 (although there was some steatosis).

The P450 isozymes that metabolise acetaminophen have varying activity; CYP1A2 has higher K_m and lower V_{max} (K_m = 3430 μM, V_{max} = 74 pmol/min mg) than 2E1 (K_m = 680 μM, V_{max} = 330 pmol/min mg) and 3A4 (K_m = 280 μM, V_{max} = 130 pmol/min mg) (6). The amounts of each isozyme in liver however vary quite widely in individuals; 3A4 averages about 29%, 1A2 about 13%, and 2E1 only 7%, and hence the overall contribution to acetaminophen oxidation from each isozyme varies considerably (20). These isozymes may be induced or inhibited, usually each by different agents. In the past, the occurrence of toxicity in one individual and not another with the same blood acetaminophen concentrations was a puzzle. It can be seen that the occurrence of toxicity depends on two conjugation paths, glutathione stores and three variable oxidative isozymes; therefore the unpredictability of toxicity is understandable.

CYP2E1 can be induced by phenobarbital and alcohol, and alcoholics are particularly susceptible to acetaminophen toxicity (21). However, neither decedent had a history of alcohol abuse. It is therefore unlikely that their 2E1 levels would have been elevated.

The heart appears to be a vulnerable secondary site for acute acetaminophen toxicity. There are several examples in the literature but none involves acetaminophen concentrations as high as those described here. In both our cases the heart appeared grossly normal and was not saved for microscopy, but acute cardiotoxicity remains the most likely cause of sudden death. This may involve inhibition of endothelium-derived relaxing factor (22) or myocardial necrosis (23–25).

Acetaminophen is a virtually neutral molecule and it is rare for it to lead to metabolic acidosis as is found with salicylate, but has

been reported (26). Electrolyte imbalance due to kidney damage is also a possible contributor to death. The kidney in our case #1 showed no necrosis on microscopic examination, but injury cannot be ruled out as histopathology may not always reveal cellular damage; for case #2 there was no evidence of damage on gross examination. Acetaminophen frequently leads to arrhythmias, but whether this is due solely to hepatic failure or other factors described is not known (27). Certainly any electrolyte imbalance will increase the cardiotoxicity of acetaminophen.

The cause of death in case #1 was determined to be "acetaminophen toxicity" and the manner was suicide and for case #2 the cause of death was "acute acetaminophen toxicity" and the manner, suicide.

Conclusion

Acetaminophen after overdose is primarily regarded as a hepatotoxin. However, the cases presented here support the supposition that following a very large overdose acetaminophen may cause death by an alternative, possibly cardiac, mechanism.

References

1. Roberts LJ II, Morrow JD. Analgesic-antipyretic and antiinflammatory agents and drugs. In: Hardman JG, Limbird LE, Gilman AG, editors. The pharmacological basis of therapeutics. New York: McGraw-Hill, 2001;703.
2. Prescott LF, Sansur M, Levin W, Conney AH. The comparative metabolism of phenacetin and N-acetyl-p-aminophenol in man, with particular reference to effects on the kidney. Clin Pharm Ther 1968;9:605–14.
3. Jones GR, Pounder DJ. Site dependence of drug concentrations in postmortem blood — a case study. J Anal Tox 1987;11:186–90.
4. Pounder DJ, Fuke C, Cox DE, Smith D, Kuroda N. Postmortem diffusion of drugs from gastric residue: an experimental study. Am J Forensic Med Pathol 1996;17(1):1–7.
5. Corcoran GB, Mitchell JR, Vaishnav YN, Horning EC. Evidence that acetaminophen and N-hydroxyacetaminophen form a common arylating intermediate, N-acetyl-p-benzoquinoneimine. Mol Pharmacol 1980;18(3):536–42.
6. Dahlin DC, Nelson SD. Synthesis, decomposition kinetics, and preliminary toxicological studies of pure N-acetyl-p-benzoquinone imine, a proposed toxic metabolite of acetaminophen. J Med Chem 1982;25(8):885–6.
7. Raucy JL, Lasker JM, Lieber CS, Black M. Acetaminophen activation by human liver cytochromes. P450IIE1 and P450IA2. Arch Biochem Biophys 1989;271(2):270–83.
8. Thummel KE, Lee CA, Kunze KL, Nelson SD, Slattery JT. Oxidation of acetaminophen to N-acetyl-p-aminobenzoquinone imine by human CYP3A4. Biochem Pharmacol 1993;45(8):1563–9.
9. Patten CJ, Thomas PE, Guy RL, Lee M, Gonzalez FJ, Guengerich FP, et al. Cytochrome P450 enzymes involved in acetaminophen activation by rat and human liver microsomes and their kinetics. Chem Res Toxicol 1993;6(4):511–8.
10. Streeter AJ, Dahlin DC, Nelson SD, Baillie TA. The covalent binding of acetaminophen to protein. Evidence for cysteine residues as major sites of arylation in vitro. Chem Biol Interact 1984;48(3):349–66.
11. Hoffmann KJ, Streeter AJ, Axworthy DB, Baillie TA. Identification of the major covalent adduct formed in vitro and in vivo between acetaminophen and mouse liver proteins. Mol Pharmacol 1985;27(5):566–73.
12. Hoffmann KJ, Axworthy DB, Baillie TA. Mechanistic studies on the metabolic activation of acetaminophen in vivo. Chem Res Toxicol 1990;3(3):204–11.
13. Dietze EC, Schafer A, Omichinski JG, Nelson SD. Inactivation of glyceraldehyde-3-phosphate dehydrogenase by a reactive metabolite of acetaminophen and mass spectral characterization of an arylated active site peptide. Chem Res Toxicol 1997;10(10):1097–103.
14. Forrest JA, Adriaenssens P, Finlayson ND, Prescott LF. Paracetamol metabolism in chronic liver disease. Eur J Clin Pharmacol 1979; 5(6):427–31.
15. Forrest JA, Clements JA, Prescott LF. Clinical pharmacokinetics of paracetamol. Clin Pharmacokinet 1982;7(2):93–107.
16. Baselt RC. *Disposition of toxic drugs and chemicals in man*. Foster City, CA: Biomedical Publications, 2004.
17. Newton JF, Bailie MB, Hook JB. Acetaminophen nephrotoxicity in the rat. Renal metabolic activation in vitro. Toxicol Appl Pharmacol 1983;70(3):433–44.
18. Nicholls AW, Caddick S, Wilson ID, Farrant RD, Lindon JC, Nicholson JK. High resolution NMR spectroscopic studies on the metabolism and futile deacetylation of 4-hydroxyacetanilide (paracetamol) in the rat. Biochem Pharmacol 1995;49(8):1155–64.
19. Mugford CA, Tarloff JB. The contribution of oxidation and deacetylation to acetaminophen nephrotoxicity in female Sprague-Dawley rats. Toxicol Lett 1997;93(1):15–22.
20. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 1994;270(1):414–23.
21. Schiodt FV, Rochling FA, Casey DL, Lee WM. Acetaminophen toxicity in an urban county hospital. N Engl J Med 1997;337(16):1112–7.
22. Armour A, Slater SD. Paracetamol cardiotoxicity. Postgrad Med J 1993;69(807):52–4.
23. Sanerkin NG. Acute myocardial necrosis in paracetamol poisoning. Br Med J 1971;3(772):478.
24. Will EJ, Tomkins AM. Acute myocardial necrosis in paracetamol poisoning. Br Med J 1971;4(784):430–1.
25. Wakeel RA, Davies HT, Williams JD. Toxic myocarditis in paracetamol poisoning. Br Med J (Clin Res Ed) 1987;295(6606):1097.
26. Flanagan RJ, Mant TG. Coma and metabolic acidosis early in severe acute paracetamol poisoning. Hum Toxicol 1986;5(3):179–82.
27. Weston MJ, Talbot IC, Horoworth PJ, Mant AK, Capildeo R, Williams R. Frequency of arrhythmias and other cardiac abnormalities in fulminant hepatic failure. Br Heart J 1976;38(11):1179–88.

Additional information and reprint requests:
Peter P. Singer, Ph.D.
Office of the Chief Medical Examiner
7007 - 116 Street NW
Edmonton, AB
Canada, T6H 5R8
E-mail: peter.singer@gov.ab.ca