Fulminant liver failure in a patient on carbamazepine and levetiracetam treatment

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INTRODUCTION AND CASE REPORT

A 22-year-old female with a history of developmental delay and seizures was presented to the hospital following secondary generalized seizures of 35 min duration and a one day history of fever to 38.9 °C at home. She had been successfully treated with carbamazepine for years and levetiracetam for several weeks, and was acutely given midazolam. On arrival at the intensive care unit she developed fulminant hepatic failure and subsequently died of multiple organ failure. A circulatory shock as well as intoxication was taken into consideration during the clinical course (ca. 21 hours).
Autopsy failed to reveal a macroscopically discernible cause of death. Significant findings on microscopic examination were an acute tubular necrosis in the kidneys, pre-existing marked accumulation of neutral lipid within the hepatocytes (Fig. 1a) and hyper acute liver damage with evidence of almost complete hepatocyte necrosis (Fig. 1b). Suspecting a toxic cause of death, a liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay was expanded to determine levetiracetam along with carbamazepine in post mortem blood and tissues.

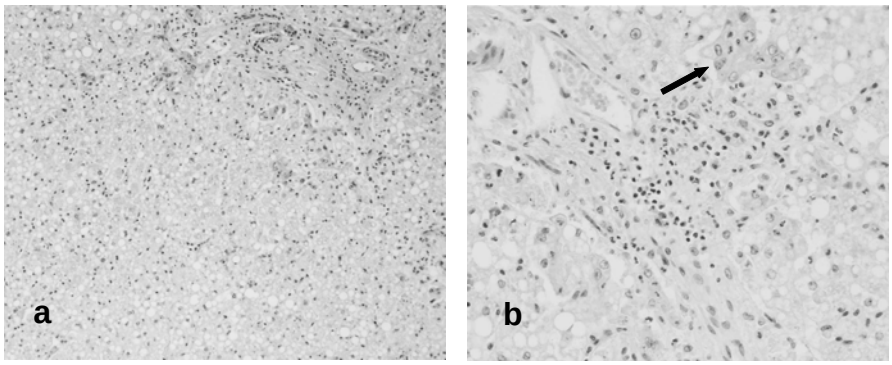

Fig. 1: Microscopic examination of the liver. (a) Almost complete hepatocyte necrosis and accumulation of neutral lipid, (b) some cellular preservation near the portal triads (arrow)

MATERIALS AND METHODS

Toxicology specimens collected at autopsy: Femoral venous blood, gastric contents, liver, lungs, muscle, and kidneys. The following standards were used: levetiracetam (UCB, Brussels, Belgium), carbamazepine (Promochem, Wesel, Germany), and lamotrigine as internal standard (GlaxoSmithKline, Munich, Germany). Chemicals and solvents were of analytical grade, and methanol and acetonitrile were of LC grade.

Sample preparation (carbamazepine, levetiracetam): To 250 µL of blood or tissue homogenate (1 g tissue/4 mL acetate buffer pH 4.6), calibrator or blank value was added 500 µL of acetate buffer pH 4.6 and 500 ng of the internal standard. Samples were extracted using 1.0 mL ethyl acetate. The residue was resuspended in 50 µL of mobile phase and 5 µL-aliquots were injected into the LC-MS/MS system.
LC-MS/MS: Analysis of carbamazepine and levetiracetam was performed using a quaternary HPLC pump equipped with an autosampler (series 200 Perkin Elmer, Überlingen, Germany). Samples were eluted from a Zorbax Eclipse XDB-C8 column (2.1 mm • 150 mm, 5µm particle size, Agilent, Waldbronn, Germany) using 4 mM ammonium acetate buffer (pH 3.2)/methanol/acetonitrile 44:11.2:44.8 by volume as the mobile phase at a flow rate of 0.25 mL/min. The chromatographic system was coupled to an API 365 tandem mass spectrometer via a TurboIon interface (Applied Biosystems, Toronto, Canada). Following optimization of ion source and M/MS parameters in positive ion mode, data were acquired in the multiple reaction monitoring mode (Masschrom 1.1.1, Applied Biosystems, Toronto, Canada).
The concentration of midazolam in blood was determined by gas chromatography/electron capture detection following liquid/liquid extraction. The toxicology investigation also included a blood volatiles screen, a screen for basic, neutral and acidic drugs by gas chromatography/mass spectrometry and a screen for basic drugs by LC/UV in blood and gastric contents, resp..

RESULTS

1. LC-MS/MS assay

The single liquid workup step was sufficient to isolate levetiracetam along with carbamazepine from the specimens without interfering endogenous material. Retention times were 1.70, 2.04 and 5.85 min for levetiracetam, carbamazepine and lamotrigine, resp.. All compounds produced an intensive, protonated molecular ion [$M+H^+$]. The most prominent parent to product transition used for quantification was m/z 171 → 126 for levetiracetam, and m/z 237 → 194 for carbamazepine. For lamotrigine, monitoring of the surviving parent ion was necessary to achieve the required sensitivity. No cross talk interference between MS/MS channels occurred, and ion suppression/-enhancement was absent. The assay was precise across a linear dynamic range of 0.24-10.0 µg levetiracetam/mL blood or g tissue ($r > 0.99$). Some validation data are summarized in Table 1.

Table 1: Validation data of levetiracetam in post mortem blood including relative recovery (%, mean ± SEM, n=5), between- and within-run precision (%, coefficient of variation) and lower limits of detection and quantitation (LLOQ, LLOQ, resp.; µg/mL) according to DIN 32645 (1)

Concentration (µg/mL)	Relative recovery	Between-run precision	Within-run precision
0.25	82.7 ± 5.9	5.4	5.2
1.0	88.2 ± 4.7	4.7	6.0
10.0	85.7 ± 4.5	0.9	1.0
LLOD	0.06 µg levetiracetam/mL post mortem blood		
LLOQ	0.24 µg levetiracetam/mL post mortem blood		

2. Drug findings in autopsy specimens

Carbamazepine and levetiracetam were present in all specimens submitted for toxicology investigation (Table 2). Carbamazepine concentration decreased in the following order: kidneys > liver > muscle, lungs. Drug concentration in blood could not be considered to result from overdose, for toxic levels were reportedly > 12 µg/mL. The half-life is 10-20 h following long-term use (2).
Levetiracetam was uniformly distributed among tissues. The blood concentration was already below the tentative target range of 10–37 µg/mL. The drug has a half-life of 6-8 h (2).
Midazolam which had been administered after the last epileptic seizure was detectable in blood at a concentration of 0.22 µg midazolam/mL blood. Screens also revealed presence of lidocaine and adrenalin, which had been administered at the intensive care unit.

Table 2: Concentrations of carbamazepine and levetiracetam in blood [µg/mL] and tissues [µg/g]

Specimen	Carbamazepine	Levetiracetam
Femoral blood	2.0	2.8
Liver	9.3	3.5
Lungs	7.3	3.6
Muscle	7.2	3.6
Kidneys	9.8	3.8

CONCLUSIONS

GC/MS and HPLC/UV methods following deproteinization, liquid/ liquid or solid phase extraction have been described for the determination of levetiracetam (3-5). The present assay is very quick, inexpensive and precise and can successfully be applied to samples that are derived from murky sources.
The uniform distribution of levetiracetam among tissues is consistent with a use of the drug lasting several weeks. The analytical results suggest a low affinity of levetiracetam towards tissues.
Carbamazepine findings also suggest long-term administration of the anticonvulsant drug, which is in accordance with the medical history. Haematological disturbances which are regarded as primary safety concerns were absent.
Carbamazepine is mainly metabolized in the liver by CYP3A4 and CYP2A8, and to a lesser extent by CYP1A2 and CYP2C19. Levetiracetam is not metabolized by the hepatic CYP450 system (2), and thus interaction with carbamazepine is unlikely.
After excessive drug concentrations or severe interactions could be ruled out, the question arises whether the previously well-tolerated medication could give rise to fulminant liver failure in the present case:
A likely mechanism of injury to the hepatocyte is oxidative membrane damage by either an increased production of free radicals or a decreased free radical scavenger capacity (6). Carbamazepine metabolites are conjugated with glutathione, which is one of the major protective mechanisms utilized by the liver. Further, induction of mixed oxidase activity during long term administration of carbamazepine may increase production of reactive oxygen species, leaving the hepatic cell more vulnerable to oxidative injury (7). In the present case, a large amount of free radicals might have been acutely produced during prolonged seizure activity and hyperthermia. Thus, fulminant hepatic failure is suggested to be due to glutathione depletion of hepatic stores and an excessive generation of reactive oxygen species.

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