J Forensic Sci, September 2007, Vol. 52, No. 5
doi: 10.1111/j.1556-4029.2007.00526.x
Available online at: www.blackwell-synergy.com

CASE REPORT

Maria Nieddu,[1] Ph.D.; Gianpiero Boatto,[1] Ph.D.; Domenico Serra,[1]; Aldo Soro,[1] M.D.; Salvatore Lorenzoni,[2] M.D.; and Francesco Lubinu,[2] Ph.D.

HPLC–DAD Determination of Mepivacaine in Cerebrospinal Fluid from a Fatal Case

ABSTRACT: A fatal case involving mepivacaine-induced epidural anesthesia is described. The pathological findings were typical of cardiac shock from ischemic origin. Cerebrospinal fluid (CSF) was obtained several hours after death and mepivacaine was identified by gas chromatography-mass spectrometry (GC-MS). Its concentration was determined by high performance liquid chromatography with diode array detection (HPLC–DAD). Extraction from CSF was performed by deproteinization with acetonitrile. The mepivacaine concentration in the sample was 264 μg/mL. Concentrations of mepivacaine in CSF following epidural anesthesia are not reported in literature to our knowledge. This is the first reported case of death in which the mepivacaine concentration in CSF has been determined.

KEYWORDS: forensic science, toxicology, mepivacaine hydrochloride, epidural anaesthesia, cerebrospinal fluid

Mepivacaine hydrochloride (2%) is used as a local anesthetic because of its rapid absorption epidural and paracervical injection. Therapeutic concentrations in plasma are usually in the range of 2–5 μg/mL (1). It is rapidly metabolized by hydroxylation to the 3′- and 4′-hydroxy metabolites and by *N*-demethylation to 2′-6′-pipecoloxylidide (PPX).

In this case report, a fatality following mepivacaine induced epidural anesthesia is described. Mepivacaine was identified using gas chromatography-mass spectrometry (GC-MS) and its concentration determined by high performance liquid chromatography–diode array detection (HPLC–DAD) following deproteinization with acetonitrile. The distribution of mepivacaine, following epidural anesthesia in various regions of the brain has been reported (2), but there are no reports of its concentration in cerebrospinal fluid (CSF). To the best of our knowledge, this is the first reported fatality in which the CSF mepivacaine concentration has been determined.

Case History and Autopsy Findings

In March 2005, a 58-year-old male suffering from renal stones was hospitalized for lithotripsy. Routine blood and urine chemistries were reported in the normal range and there were no reported reactions to previous general anesthesia and other drugs (except pirazolo) were reported. Preanesthesia with Fentanest 1 mg I.M. was administered at 8:15 a.m. and this was followed by place-regional L3–L4 epidural anesthesia with 16 mL of mepivacaine 2%. At 9:35 a.m., while positioning the ureteroscopy, sustained bradycardia, followed by loss of consciousness, cardio-circulatory arrest, deep coma (Glasgow Coma Scale 3) and cyanosis occurred. At 10:10 a.m., the patient was taken to the intensive care unit where he was declared dead at 12:30 a.m.

[1]Dipartimento Farmaco Chimico Tossicologico, Università degli Studi di Sassari, Via Muroni 23/a, 07100 Sassari, Italy.
[2]Unità Operativa di Patologia Forense del Presidio Ospedaliero di Sassari, Via izzeddu 10, 07100 Sassari, Italy.

Received 4 June. 2006; and in revised form 14 Sept. 2006; and 10 April 2007; accepted 21 April 2007; published 7 Sept. 2007.

At 8.00 p.m., on the same day, a specimen of CSF was collected. An autopsy was performed 5 days after death. The body had been stored at −5°C. The autopsy showed widespread coronary sclerosis with calcified lesions and critical stenosis of the circumflex branch of the left coronary around 3 am after the origin from the common trunk; calcified stenosis of the interventricular artery (IVA) and some isolated area of sclerosis in well preserved myocardic tissue. Histology confirmed sclero-calcified lesions of the circumflex branch of the left coronary, which was entirely obstructed from overlapped thrombosis, and stenosis of IVA with aneurismatic desiccation that duplicated the vascular line. No other autopsy findings were observed. The cause of death was reported as cardiac shock from ischemic origin.

Toxicology

Materials

All solvents and inorganic chemicals were of analytical grade (Merck, Darmstadt, Germany). Deionized and distilled water was purified through a Milli Q system (Millipore, Bedford, MA). Mepivacaine hydrochloride 2% was provided by Angelini (Italy). Fentanest (fentanil citrate 0.1 mg/2 mL) was provided by Pharmacia Italia (Milan, Italy).

Stock solution (1.0 mg/mL) of mepivacaine was prepared in milli Q water, stored at +4°C, and diluted to appropriate concentrations before use. Drug-free CSF was used to prepare calibrators and controls and this had has been collected from a decedent whose cause was unrelated to neurological disease. The CSF, in this case, was collected shortly after dead (see above) and kept at −20°C until analysis.

Instrumentation

GC-MS identification was performed on a HP 5989-A mass spectrometer upgraded with an HP 5890 series II gas chromatograph coupled with an HP 5988 GC/MS direct interface. The GC column was HP 1 (25 m × 0.2 mm ID, film thickness 0.11 μm).

The temperature of the column was held at 100°C for 3 min, and raised to 300°C (10°C/min). Injector, detector and ion source temperatures were set at 200, 250 and 200°C, respectively. The carrier gas was ultra high purity helium and the electron impact was 70 eV.

Chromatographic separation were performed using a 1090 L liquid chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a diode array detector HP 1040A. All separations were accomplished on a Hypersil ODS 200 × 4.6 mm, 5 μm ID. The selected wavelengths were 230 nm. The mobile phase used in the separation consisted of (A) water: 0.14% ethylamine and (B) methanol. For the elution program, the following proportions of solvent B were used: 0–3 min, 25% B; 3–10 min, 25–60% B; 10–14 min, 60–100% B. The flow-rate was 1 mL/min with an injection volume of 20 μL.

Extraction Procedure from CSF

One milliliter of CSF was deproteinized with 1 mL of acetonitrile; the suspension vortexed, mixed and centrifuged at 1000 g for 10 min. The organic phase was evaporated under a nitrogen gas stream at 40°C. The residue was dissolved in 1 mL of milli Q water and injected into the HPLC system.

Method Validation

The method was validated according to international guidelines (3,4). Linearity was obtained with an average regression coefficient (r^2) of >0.99. Calibration curves were prepared in drug-free CSF by spiking with mepivacaine. Concentrations ranging from 1 to 500 μg/mL were prepared and analyzed using the above procedure. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as $y\text{LOD} = b + 3\text{SD}_b$ and $y\text{LOQ} = b + 10\text{SD}_b$, where b, intercept and SD_b, the standard deviation of intercept (5).

Precision was evaluated by analyzing a sample containing 100 μg/mL of mepivacaine on the same day in five replicate (intraday precision) and over five consecutive days in triplicate (interday precision) and by calculating the relative standard deviation (RSD) of the experimentally determined concentrations.. Finally, precision of the HPLC instrument was evaluated by calculating the RSD of migration times of a standard solution (100 μg/mL) of mepivacaine over 10 sample injections (with washing every third injection).

Accuracy was expressed in terms of recovery %. Recovery values were studied by spiking drug-free CSF samples at three fortification levels (50–100–400 μg/mL) and analyzing six replicates.

Results and Discussion

In the present paper, the extract obtained from CSF was analyzed by HPLC–DAD to determine mepivacaine concentrations in a fatality following epidural anesthesia. Data for precision and accuracy were within required limits (3,4). The intraday and interday RSD % were 0.20–2.20%, respectively, and the RSD of migration times was 0.35%. The LOD and the LOQ were 14.5 and 48.9 μg/mL, respectively. Recoveries obtained from spiked CSF were better than 65%. The recoveries were calculated by comparing the peak areas obtained from the extract of the spiked CSF sample with those obtained by direct injection of standard solution. The relatively simple extraction from CSF resulted in chromatograms free of interfering peaks.

The CSF mepivacaine concentration was determined to be 264 μg/mL. Concentrations of mepivacaine in CSF following epidural anesthesia have not been reported in literature. This is the first reported fatality in which a CSF mepivacaine concentration in CSF was determined. Cause of death was reported as cardiac shock of ischemic origin.

The CSF mepivacaine concentration was approximately 250 μg/mL equating to approximately 30 mg in a CSF volume of 120 mL. This compares to an injected amount of 320 mg and therefore it is improbable that mepivacaine contributed to the patient's death.

References

1. Moffat AC, Osselton DM, Widdop B, editors. Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material. London: Pharmaceutical Press, 2004.
2. Narumi J, Kudo K, Imamura T, Rimura K, Ikeda N. Distribution of drugs in various tissues in a brain dead man. Forensic Sci Int 1991;90:103–9.
3. Peters FT, Maureer HH. Bioanalytical method validation and its implications for forensic and clinical toxicology. A review. Accred Qual Assur 2002;7:441.
4. Taveniers I, de Loose M, Van Bockstaele E. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trend Analyt Chem 2004;23:533.
5. United States Pharmacopoeia 24 NF 19. Validation of compendial methods. Philadelphia, PA: National Publishing, 2000.

Additional information and reprint requests:
Gianpiero Boatto, Ph.D.
Associate Professor
Dipartimento Farmaco Chimico Tossicologico
Università degli Studi di Sassari
Via Muroni 23/a
07100 Sassari
Italy
E-mail: gboatto@uniss.it