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Evaluation of postmortem drug concentrations in cerebrospinal fluid compared with blood and pericardial fluid

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ABSTRACT

In forensic toxicology, body fluids are important materials not only as alternatives to blood but also for investigation of postmortem drug redistributions and pharmaco-/toxicokinetic analysis; however, there are limited data on postmortem drug distributions in cerebrospinal fluid (CSF). The present study reviewed toxicological data of autopsy cases ($n = 103$), in which drugs were detected in CSF using gas chromatography/mass spectrometry (GC/MS), to investigate drug concentrations in CSF, compared with blood and pericardial fluid (PCF) concentrations. Oral/injected amphetamines ($n = 23$) showed similar CSF and blood/PCF concentrations with partly lower CSF concentrations (about $\times$0.5–1.1). CSF concentrations of the venous anesthetic midazolam ($n = 7$) were lower with poor correlations. Oral caffeine ($n = 15$), acetaminophen ($n = 7$), chlorpheniramine ($n = 6$), dihydrocodeine ($n = 6$), and phenobarbital ($n = 21$) showed equivalent to lower CSF concentrations (about $\times$0.2–1.2), compared with blood and PCF concentrations; however, CSF phenobarbital concentrations were high in a fatal intoxication case. CSF concentrations of phenothiazine derivatives ($n = 29$) were markedly lower (about $\times$0.1) than blood/PCF concentrations. The distribution of the local anesthetic lidocaine used in critical medical care ($n = 49$) markedly varied by case. These findings suggest that CSF is useful in routine forensic toxicology as an alternative to blood as well as for investigating pharmaco-/toxicokinetics and postmortem redistributions.

1. Introduction

Forensic toxicological issues in the evaluation of drug concentrations in autopsy cases include the antemortem distribution and postmortem redistribution depending on the route of intake and pharmaco- or toxicokinetics, as well as the chemical properties [1]. Previous studies showed substantial postmortem redistribution of drugs in heart and peripheral blood, which are partly characteristic of individual drugs [2,3]; thus, it is difficult to estimate blood concentrations during the process of and at the time of death from postmortem blood concentrations [4]. Against this background, simultaneous analyses of blood and other body fluids provide useful information for investigating antemortem distribution, postmortem redistribution by diffusion and degradation, as well as incidental production and contamination, which can depend on the intake routes and chemical properties of individual drugs and poisons [5–18]. With respect to this, previous studies demonstrated significant correlations of drug concentrations in pericardial fluid (PCF) and bone marrow aspirate with blood concentrations, showing differences depending on individual chemical properties and the time after intake/administration; PCF can be an alternative to blood for postmortem drug investigation, showing significant correlations between their drug concentrations when the aforementioned conditions were considered [6,7,17–19].

Cerebrospinal fluid (CSF) may also be useful as an alternative material in cases of multiple traumas where an adequate blood sample is not available. CSF is a filtrate from blood and partly

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contains transudate from the brain, with the time taken to replace itself (turnover time) of 3–6 h [19–22]. Drugs can enter the CSF through the choroid plexus from blood and also through the brain in the transudate from the cerebral interstitial fluid derived from blood across the blood-brain barrier (BBB) [23]. However, there are limited data on CSF drug delivery systems, although the influences of protein binding and volume of distribution (Vd) of drugs, as well as BBB integrity, are suggested [7,23]. Previous studies showed insufficient correlations between CSF and blood concentrations of several drugs, suggesting that CSF is not a suitable alternative to blood for quantitative analysis [5,7,20], while significant correlations were shown for various drugs, including antipyretics, such as acetaminophen and ibuprofen, the local anesthetic lidocaine, psychotropics/anticonvulsants including phenobarbital and benzodiazepines, chemotherapeutics and opioids in clinical studies and animal experiments [24–39].

From the above-mentioned observations, the present study reviewed postmortem toxicological data of autopsy cases to examine the efficacy of drug concentrations in CSF as a possible alternative to blood and a material for investigating pharmaco-/toxicokinetics and postmortem redistribution, compared with blood and PCF concentrations.

2. Materials and methods

2.1. Autopsy database

All forensic autopsy cases (n = 808) during a period of 5 years (January 2010–December 2014), excluding those where adequate specimens were not available due to advanced decomposition or skeletonization, were retrospectively reviewed, and those with positive toxicological findings in CSF were collected to compare the data ($n \geq 5$) with those of right heart blood, peripheral blood and PCF. These data analyses as well as sample collections and the analyses described below were performed within the framework of routine medicolegal casework following the autopsy guidelines (2009) and ethics guidelines (2003) of the Japanese Society of Legal Medicine, approved by the institutional ethics committee.

2.2. Analytical procedures

2.2.1. Autopsy material

Beside heart blood, peripheral external iliac blood, CSF and PCF were routinely collected at autopsy and analyzed in parallel. Peripheral blood (about 5–25 mL) was drawn using and aseptic syringe from external iliac vein. PCF (about 5–25 mL) was drawn using an aseptic syringe after opening the pericardial cavity. CSF (about 2–10 mL) was collected using and aseptic syringe from the great cistern in the base of the brain at the time of opening of the skull. These specimens were subsequently stored at −20 °C until analysis.

2.2.2. Chemicals and reagents

Bond Elut Certify columns were provided by Agilent Technologies (Santa Clara, CA, USA). Deionized pure water was obtained using a Milli Q Purification System (Millipore, Bedford, MA, USA). Other chemicals and reagents, including standard drugs and internal standards, as listed below, were of the highest purity commercially available [18].

2.2.3. Sample preparation

Midazolam was extracted using liquid/liquid phase extraction: 50 μL of internal standard (prazepam) solution and 1 mL of saturated sodium bicarbonate solution were added to 1 mL of each sample and vortexed; after 0.5 g of sodium chloride was added and vortexed, 3 mL of toluene was added for extraction by shaking for 30 min; the solvent was separated by centrifugation. Other drugs and chemicals were extracted using a Gilson ASPEC XL-274 automated SPE solid/liquid phase extraction instrument (Middleton, WI, USA) as follows. To 0.5 mL of each sample, 50 μL of solution containing the following internal standards was added: allobarbital for phenobarbital; methyl p-hydroxybenzoate for acetaminophen; 3-phenylpropylamine for amphetamine and methamphetamine; and cocaine for caffeine, chlorpheniramine, chlorpromazine, dihydrocodeine, lidocaine and promethazine [18]. The pH of the sample was adjusted to approximately 7.0 by adding 6 mL of 0.1 M potassium phosphate buffer pH 6.0. The mixture was mild mixed, centrifuged at 2500 × g for 5 min, poured into HF-Bond Elut Certify columns (acetaminophen; Autoprep EDS-1) and gently aspirated. Columns had been previously conditioned with 2 mL of methanol and 2 mL of 0.1 M potassium phosphate buffer pH 6.0. After applying the samples, the columns were then successively washed with 1 mL of buffer, 1 mL of 1 M acetic acid solution and 1 mL of methanol. Finally, analytes were eluted with 3 mL of a freshly prepared mixture of dichloromethane/isopropanol (78:20) ammonium hydroxide. The eluates were collected and evaporated to dryness under a gentle stream of nitrogen at room temperature. The residues were reconstituted with 100 μL of ethyl acetate and 1 μL aliquots of the extracts were injected into the gas chromatography/mass spectrometry (GC/MS) system. Recovery of standards ranged from 60–70% (phenobarbital) to >95% (codeine) [18].

2.2.4. Instrumental conditions

Automated GC/MS following solid/liquid phase extraction was performed using Agilent Technologies GC/MS System Model 5975c MSD (column, DB-5MS, 30 m × 0.25 mm i.d., film 0.25 μm; column temperature, 100–325 °C; injector temperature, 280 °C; turbocharged carrier gas, He at a flow rate of 48 cm/s; interface temperature, 300 °C). Analytical precision ranged from 9.6% (phenobarbital) to 16.1% (midazolam) [18].

2.2.5. Statistics

Regression equation analysis was used to study the relationship between pairs of parameters. These analyses were carried out using Stat View (Version 5.0; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Drugs detected in cerebrospinal fluid

CSF was collected in 687 cases (85.0%), including those involving multiple traumas and fatal hemorrhages, in which adequate blood samples were not available (n = 4); in CSF of these four cases, acetaminophen (n = 1) or lidocaine (n = 3) was detected. In 103 cases, 25 drugs were detected in CSF, as listed in Table 1. Among these, drugs detected in more than 5 cases were included in the following statistical analysis.

3.2. Amphetamines

Oral or injected methamphetamine showed similar concentrations in CSF and the right heart or peripheral iliac venous blood, or PCF, with partly lower CSF concentrations (about ×0.5–1.1), although the ratios varied at low concentrations (Fig. 1a and Table 2). Correlations between the right heart (y) and peripheral iliac venous (x) blood concentrations were: $y = 0.497x + 0.243$ (n = 14, r = 0.624, p = 0.0172).

Amphetamine, a potential metabolite of methamphetamine, also showed similar concentrations in CSF and blood or PCF, with