Postmortem Analyses of Drugs in Pericardial Fluid and Bone Marrow Aspirate

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In forensic toxicology, bone marrow is often used when adequate blood samples are not available; however, pericardial fluid (PCF) has been poorly investigated. The present study comprehensively reviewed the toxicological data of blood, PCF and bone marrow aspirate (BMA) in forensic autopsy cases to investigate drug distribution. Analysis using automated gas chromatography/mass spectrometry (GC–MS) following solid/liquid phase extraction detected 36 drugs in 218 cases (8.0% among 2,724 cases examined). Drug distribution varied by drug as well as partly by case even when taken as a mixture. Most of the drugs showed overall similar distributions in right heart blood, PCF and BMA with some exceptions, however, several drugs, including phenothiazine derivatives and antidepressants, were detected at ~1.5 times (1.2–2.0) higher levels in BMA than in right heart blood, but PCF levels were mostly equivalent to blood levels. Midazolam, propofol and thiamylal (intravenous anesthetics) were detected at a substantially lower concentration in PCF than in blood or BMA. These observations suggest that PCF and BMA are useful materials to be included in the forensic toxicological routine when blood samples are not available, as well as to investigate pharmacokinetics and postmortem redistribution.

Introduction

In forensic autopsy, it is often difficult to collect adequate blood specimens; thus, an alternative material is needed for the toxicological analysis of drugs and poisons. In such cases, body fluids are easy to collect and analyze routinely. In addition, simultaneous analyses of blood and other body fluids can provide useful information to investigate antemortem distribution as well as postmortem redistribution (diffusion), degradation, incidental production and contamination, which can depend on the intake routes and chemical properties of individual drugs and poisons (1–9); this is of special significance in forensic toxicology (10, 11).

Pericardial fluid (PCF) and bone marrow aspirate (BMA) are well-preserved postmortem materials in cases without structural damage due to injury or medical intervention, and can easily be collected in larger amounts without significant contamination, compared with other body fluids including vitreous humor (12–16). PCF is an epicardial transudate, which contains plasma components that include toxicological substances, while vertebral BMA involves a substantial amount of peripheral venous blood (11). Previous studies have suggested that these materials are useful for the postmortem analysis of volatile substances including ethanol, showing good correlations with the blood level, when the influence of postmortem diffusion from stomach contents due to their locations and increased bone marrow lipid contents in elderly subjects are considered (11, 16–22); however, sufficient practical data are not available for other drugs and poisons (16, 23).

The present study comprehensively reviewed the toxicological data of forensic autopsy cases and compared blood, PCF and BMA levels of drugs to investigate the postmortem distribution.

Materials and methods

Autopsy database

All forensic autopsy cases during the past 16.7 years (January 1996–August 2012), excluding those for which adequate specimens were not available due to advanced decomposition or skeletonization, were retrospectively reviewed, and those with positive toxicological findings were collected to compare the data among right heart blood, PCF and BMA. These data analyses as well as sample collections and the analyses described below were performed within the framework of our routine medicolegal casework following the autopsy guideline (2009) and ethics guidelines (1997 and 2003) of the Japanese Society of Legal Medicine, approved by the institutional ethics committee.

Analytical procedures

Autopsy material

Besides blood, urine and stomach contents, PCF and BMA were routinely collected at autopsy and analyzed in parallel. PCF (~5–25 mL) was drawn using an aseptic syringe after opening the pericardial cavity. BMA (~2–5 mL) was collected by puncturing the lower thoracic vertebrae with a 10-mL syringe connected to a bone marrow needle (Jamshidi 11G; Baxter Healthcare Corp., McGaw, IL, USA). These specimens were subsequently stored at −20°C until analysis.

Chemicals and reagents

Sources of standard drugs and internal standards are listed in Table 1. Deionized pure water was obtained using a Milli-Q Purification System (Millipore, Bedford, MA, USA). Other common chemicals and reagents were of the highest purity that was commercially available. Bond Elut Certify columns were provided by Varian Sample (Harbor City, CA, USA).

Sample preparation

Benzodiazepines as well as tri- and tetracyclic antidepressants were extracted using liquid/liquid-phase extraction. For...
benzodiazepines, 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. For antidepressants, 50 μL of internal standard (cocaine) and 0.5 mL of 20% sodium carbonate solution were added to 1 mL of each sample and vortexed; 6 mL of hexane was added for extraction by shaking for 20 min; the solvent was separated by centrifugation. These extracts were evaporated under a gentle stream of nitrogen at 40°C; then, the residues were solved with 100 μL of ethyl acetate, and 1 μL aliquots of the extract were injected into the GC–MS system. Extraction recovery of standards ranged from 62–67% (phenobarbital) to >95% (codeine).

Instrumental conditions

An automated GC–MS following solid/liquid-phase extraction was performed using a Shimadzu GC–MS System Model QP 5000 (Kyoto; column, DB-1, 30 m × 0.25 mm i.d., film 0.25 μm; column temperature, 60–230°C; injector temperature, 280°C; carrier gas, He at a flow rate of 40 cm/s; interface temperature, 230°C) from January 1996 to August 2009, and 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) from September 2009 to August 2012. Analytical precision was <10% for all drugs in each specimen.

Statistics

Regression equation analysis was used to study the relationship between pairs of parameters. Sporadically detected drugs (n < 3) were analyzed together by grouping according to their chemical or pharmacological properties. These analyses were carried out using Stat View (Version 5.0; SAS Institute, Inc., Cary, NC, USA).

Results

GC–MS screening detected 36 drugs in 218 cases (8.0%) among all cases reviewed (n = 2,724), including amphetamines (methamphetamine, and amphetamine as the possible metabolite), codeines (dihydrocodeine and codeine), sedatives/hypnotics...
(barbiturates, including secobarbital as the possible metabolite of thiamylal, benzodiazepines, phenothiazines and zolpidem), antidepressants (including amitriptyline, olanzapine, dosulepin and mirtazapine), analgesics (acetoaminophen), local/general anesthetics (lidocaine, midazolam, propofol and thiamylal) and other drugs (carbamazepine, chlorpheniramine, clopidogrel, methylephedrine, mexiletine, promethazine and ticlopidine) (Table II).

Among these drugs, amphetamines, barbiturates, benzodiazepines and codeines presented mostly equivalent concentrations in right heart blood, PCF and BMA, showing high site-to-site correlations (Group A in Figure 1, and Figures 2 and 3), although little data were available for pentobarbital and codeines. Relationships between blood and PCF levels were similar for a spectrum of other drugs, including acetaminophen, carbamazepine, chlorpheniramine, diphenhydramine and methylephedrine, although sufficient BMA data were not available for acetaminophen. Correlations between right heart blood ($x$) and PCF ($y_1$) or BMA ($y_2$) were: methamphetamine, $y_1 = 1.08x + 0.05$ ($n = 34$, $R = 0.96$, $P < 0.0001$) and $y_2 = 1.14x + 0.08$ ($n = 22$, $r = 0.98$, $P < 0.0001$); amphetamine, $y_1 = 0.77x + 0.03$ ($n = 30$, $R = 0.72$, $P < 0.0001$) and $y_2 = 1.12x + 0.28$ ($n = 20$, $R = 0.88$, $P < 0.0001$); acetaminophen, $y_1 = 1.16x - 0.28$ ($n = 9$, $R = 0.91$, $P < 0.0001$); codeines (dihydrocodeine and codeine), $y_1 = 0.73x + 0.23$ ($n = 5$, $R = 0.84$, $P = 0.073$); phenobarbital, $y_1 = 0.67x + 1.57$ ($n = 48$, $R = 0.80$, $P < 0.0001$) and $y_2 = 1.05x + 0.28$ ($n = 23$, $R = 0.92$, $P < 0.0001$); pentobarbital, $y_1 = 1.17x + 0.57$ ($n = 4$, $R = 1.00$, $P < 0.0001$) and $y_2 = 0.44x + 0.61$ ($n = 3$, $R = 0.876$, $P = 0.32$); benzodiazepines (diazepam, Figure 1. Relationship of drug concentrations between right heart blood ($x$) and PCF ($y_1$; a) or BMA ($y_2$; b). Group A, methamphetamine, acetaminophen, codeines (dihydrocodeine and codeine) and others, including carbamazepine, chlorpheniramine, diphenhydramine and methylephedrine: for these cases, $y_1 = 0.97x + 0.10$ ($n = 60$, $R = 0.90$, $P < 0.0001$) and $y_2 = 0.95x + 0.21$ ($n = 30$, $r = 0.91$, $P < 0.0001$). Group B, phenothiazine derivatives and antidepressants: for these cases, including mirtazapine, olanzapine, paroxetine and sertraline, $y_1 = 1.13x + 0.51$ ($n = 73$, $R = 0.95$, $P < 0.0001$) and $y_2 = 1.42x + 0.45$ ($n = 41$, $R = 1.00$, $P < 0.0001$). Group C, lidocaine. Details in the text.

Figure 2. Relationship of drug concentrations between right heart blood ($x$) and PCF ($y_1$; a) or BMA ($y_2$; b) for phenobarbital and pentobarbital. For all cases, $y_1 = 0.95x + 0.21$ ($n = 30$, $R = 0.91$, $P < 0.0001$) and $y_2 = 1.04x + 0.13$ ($n = 26$, $r = 0.92$, $P < 0.0001$). Details for each drug are in the text.
estazolam and bromazepam) and zolpidem, $y_1 = 1.44x + 0.05$ ($n = 15, R = 0.91, P < 0.0001$) and $y_2 = 0.76x + 0.11$ ($n = 10, R = 0.98, P < 0.0001$); secobarbital, $y_1 = 1.08x + 0.15$ ($n = 6, R = 0.80, P = 0.056$) and $y_2 = 1.30x - 0.18$ ($n = 5, R = 0.97, P < 0.01$); others, including carbamazepine, chlorpromazine, diphenhydramine and methylephedrine, $y_1 = 0.73x + 0.42$ ($n = 12, R = 0.74, P < 0.02$) and $y_2 = 0.43x + 0.59$ ($n = 6, R = 0.90, P < 0.02$). For these drugs, however, there were sporadic exceptions showing marked dissociation between PCF or BMA and right heart blood (difference of >50%): dissociation at a low concentration (<1.0 μg/mL) for methamphetamine/amphetamine ($n = 6$ in total); lower PCF phenobarbital level ($n = 1$); and higher ($n = 2$) PCF levels of chlorpromazine ($n = 1$) and zolpidem ($n = 2$); lower BMA carbamazepine level ($n = 1$); higher BMA phenobarbital level ($n = 1$); and secobarbital detected at a low concentration (0.17–1.42 μg/mL) as a possible metabolite of thiamylal, also showed almost equivalent correlations of PCF and BMA levels with right heart blood levels, with the exception of a higher level in PCF than in blood.

Chlorpromazine, levomepromazine and promethazine as well as antidepressants showed ~1.2–2.0 times higher levels in BMA than in right heart blood, and their PCF levels were mostly equivalent to blood levels (Group B in Figure 1). Correlations between right heart blood ($x$) and PCF ($y_1$) or BMA ($y_2$) were: chlorpromazine, $y_1 = 1.23x - 0.01$ ($n = 15, R = 0.95, P < 0.0001$) and $y_2 = 2.12x + 0.08$ ($n = 9, R = 0.49, P = 0.18$); levomepromazine, $y_1 = 0.65x + 0.15$ ($n = 6, R = 0.79, P = 0.060$) and $y_2 = 1.35x + 0.042$ ($n = 4, R = 0.86, P = 0.14$); promethazine, $y_1 = 0.87x + 0.07$ ($n = 31, R = 0.92, P < 0.0001$) and $y_2 = 2.12x - 0.21$ ($n = 15, R = 0.72, P < 0.01$); for these three phenothiazine derivatives, $y_1 = 1.13x + 0.59$ ($n = 58, R = 0.95, P < 0.0001$) and $y_2 = 1.42x + 0.65$ ($n = 30, R = 1.00, P < 0.0001$); tricyclic antidepressants (amitriptyline, doxepin, clomipramine and imipramine), $y_1 = 0.81x + 0.02$ ($n = 18, R = 0.99, P < 0.0001$) and $y_2 = 1.20x + 0.05$ ($n = 5, R = 0.97, P < 0.01$). However, there were several dissociations between PCF or BMA and right heart blood (difference of >50%), including higher PCF levels of chlorpromazine ($n = 2$), levomepromazine ($n = 1$) and olanzapine ($n = 1$), as well as lower BMA levels of chlorpromazine ($n = 1$) and levomepromazine ($n = 2$) at a low concentration.

Lidocaine, used as a local anesthetic in critical medical care, showed similar levels in right heart blood, PCF and BMA (Group C in Figure 1). Correlations between right heart blood ($x$) and PCF ($y_1$) or BMA ($y_2$) were $y_1 = 0.96x + 0.05$ ($n = 55, R = 0.88, P < 0.001$) and $y_2 = 1.90x - 0.45$ ($n = 42, R = 0.80, P < 0.0001$). However, right heart blood levels were markedly higher than PCF and BMA levels in some cases of a low level (<1.5 μg/mL), and three cases had markedly higher BMA level than blood level (difference of >50%). Midazolam, propofol and thiamylal, used as an intravenous anesthetic in critical medical care, showed a substantially lower concentration in PCF than in blood or BMA in most of the cases, while BMA levels largely varied by case; the ratio of the BMA-to-blood level ranged from 0.28 to 45.99, including markedly higher BMA levels of midazolam ($n = 1$) and propofol ($n = 2$), and markedly higher ($n = 1$) and lower ($n = 1$) BMA thiamylal levels (Figure 4). Correlations between right heart blood ($x$) and PCF ($y_1$) or BMA ($y_2$) were: midazolam, $y_1 = 0.77x + 0.00$ ($n = 16, R = 0.86, P < 0.0001$) and $y_2 = 0.65x + 0.21$ ($n = 10, R = 0.66, P = 0.37$); propofol, $y_1 = 0.34x - 0.07$ ($n = 9, R = 0.92, P < 0.001$) and $y_2 = 2.25x + 1.19$ ($n = 5, R = 0.82, P = 0.092$); thiamylal, $y_1 = 0.48x - 0.31$ ($n = 8, R = 0.91, P < 0.01$) and $y_2 = 1.48x - 3.98$ ($n = 6, R = 0.87, P = 0.24$).

Discussion

Previous studies suggested correlations between bone marrow and blood drug concentrations, involving different pharmacokinetic processes, and the influences of the sampling site, age of...
the subject, water/lipid contents and postmortem interference were discussed (2, 24); however, the practical data of bone marrow analysis are insufficient, and PCF has been poorly investigated (25–27). With regard to these, the present study demonstrated characteristic distributions of a spectrum of drugs in right heart blood, PCF and BMA of forensic autopsy cases, as follows.

Postmortem concentrations of benzodiazepines, including alprazolam, bromazepam, diazepam and estazolam, were overall similar in right heart blood, PCF and BMA with good site-to-site correlations, although previous studies showed accumulation in the bone marrow (28, 29); this suggests that aspirated bone marrow samples, containing a substantial amount of peripheral blood, have a property as an alternative to blood in toxicology, different from bone marrow tissues (11, 20). Most other drugs, including acetaminophen, barbiturates, carbamazepine, chlorpheniramine, codeine/dihydrocodeine, diphenhydramine, methamphetamine and amphetamine as the possible metabolite, methylephedrine, and zolpidem, also showed similar concentrations at each site with good correlations, irrespective of the hydro-/lipophilic chemical structure. Drugs in circulating blood are gradually diffused into PCF as a transudate from the epicardium (30–33), depending on the concentration gradient; PCF drug levels are lower and higher than adjacent right heart blood levels in early and delayed phases after intake, respectively, and the different half-life of drugs can modify the distribution.

Concentrations of phenothiazine derivatives, including chlorpromazine, levomepromazine and promethazine, as well as antidepressants also showed equivalency between PCF and right heart blood; however, their BMA levels were ~1.5 times (1.2–2.0) higher than in right heart blood, as was seen for inhaled toluene (11), although a small number of case were available for levomepromazine. These findings may partly be attributed to larger volumes of distribution (V_d) of these drugs (6–50 L/kg), involving tissue components including bone marrow (23). For several drugs, however, marked dissociations among blood, PCF and BMA were detected by case; individual drug concentrations in each specimen were different even when taken as a mixture (e.g. Vegetamin, containing chlorpromazine, promethazine and phenobarbital). These incidental discrepancies may have been caused by pharmacokinetic factors before death, involving delayed distribution and redistribution in PCF as well as in tissues including bone marrow; lower and higher drug concentrations in PCF and/or BMA than in blood may indicate early and delayed phases after intake, respectively. Rapid degradation (short half-life) of the drug may increase the difference. Such factors may also affect postmortem distribution in central and peripheral blood (34).

Lidocaine, which was used in critical medical care, was detected in blood, BMA and PCF, showing correlations; however, the concentration was often higher in right heart blood than in PCF and BMA, suggesting death before distribution throughout the whole body. Dissociation between blood and BMA levels may also be caused by terminal circulatory disturbance involving shock during intensive medical care.

Different from the above-mentioned drugs, intravenous anesthetics, including midazolam, propofol and thiamylal, were detected at a lower concentration in PCF than in blood and BMA, as was seen for inhaled cyanide (11). This dissociation can occur in early pharmacokinetic processes. Varied distribution in blood and BMA may indicate terminal circulatory disturbance involving shock during intensive medical care as mentioned above; however, large V_d of propofol (2–12 L/kg), involving tissue components including bone marrow (23), may contribute to a higher level in BMA than in right heart blood, as for the phenothiazine derivatives described above. Secobarbital as a possible metabolite of thiamylal was detected at similar levels in right heart blood, PCF and BMA, despite well-known postmortem redistribution (35), suggesting rapid biotransformation of thiamylal (short half-life) and distribution of the metabolite throughout the whole body as well as postmortem stability.

Major limitations in the present study of comprehensive autopsy data analysis were the small number of cases for several drugs (details shown in Table I) and the partial lack of PCF and/or BMA data, as well as therapeutic levels in most of the cases,

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**Figure 4.** Relationship of drug concentrations between right heart blood (x) and PCF (a) or BMA (b) for midazolam, propofol and thiamylal. Details for each drug are in the text.
with a few cases of toxic/lethal levels for some drugs (e.g. antidepressants) due to the retrospective review, further investigation is needed, to supplement and confirm these data. Although right heart blood may be less influenced by redistribution (10), further comparison with peripheral blood is needed, especially for drugs with large \( V_d \), including phenothiazine derivatives and antidepressants. For such drugs, however, postmortem redistribution should also be considered for peripheral blood (36, 37); thus, multiple site comparison including PCF and BMA is useful for toxicological analysis in routine forensic analysis.

In conclusion, the present comprehensive study of autopsy data demonstrated significant correlations of drug concentrations in right heart blood, PCF and BMA, and partial differences among the drugs, suggesting that CSF and BMA are useful materials to be included in routine forensic toxicological analysis when blood samples are not available, as well as to investigate pharmaco-/toxicokinetics and postmortem redistribution.

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