Postmortem Analyses of Gaseous and Volatile Substances in Pericardial Fluid and Bone Marrow Aspirate

Mariko Tominaga1,2*, Takaki Ishikawa1,2, Tomomi Michiue1,2, Shigeki Oritani1, Izumi Koide1,2, Yuko Kuramoto1,2, Masafumi Ogawa1,3 and Hitoshi Maeda1,2

1Department of Legal Medicine, Osaka City University Medical School, 2Forensic Autopsy Section, Medico-legal Consultation and Postmortem Investigation Support Center (MLCPI-SC), Asahi-machi 1-4-3, Abeno, 545-8585 Osaka, Japan and 3Department of Clinical Pharmaceuticals, Osaka Ohtani University, Nishikiori-kita 3-11-1, 584-8540 Tondabayashi, Japan

*Author to whom correspondence should be addressed. Email: mari1216@med.osaka-cu.ac.jp

A previous study suggested the usefulness of pericardial fluid (PCF) and bone marrow aspirate (BMA) for the postmortem analysis of ethanol. The present study reviewed forensic autopsy cases (n = 2,983), which included 683 cases with the following positive toxicological findings, to reassess ethanol distribution and to investigate other gaseous and volatile substances in blood, PCF and BMA. Toxicological analyses detected ethanol (>10 mg/dL, n = 345), acetone (>0.01 mg/dL, n = 402), cyanide (n = 282), toluene (n = 47), liquefied petroleum gas (LPG, n = 1), cresol (n = 1), trichloroethylene (TCE, n = 1) and hydrogen sulfide (H₂S, n = 5) in 683 cases. Ethanol and acetone levels showed good correlations among right heart/peripheral blood, PCF and BMA with a few exceptions. Inhaled cyanide showed a higher level in PCF than in blood and BMA; however, ingested cyanide showed a lower level in PCF. Distribution of inhaled toluene was largely higher by case; however, BMA levels were substantially lower in PCF than in blood and BMA; however, ingested cresol showed similar distributions in blood and PCF. These observations suggest the usefulness of PCF and BMA as alternatives to blood for postmortem toxicological analysis. The inclusion of these materials in routine analysis may be also useful to investigate pharmacokinetics and toxicokinetics in the death process and the influence of postmortem redistribution/diffusion.

Introduction

In postmortem analyses of drugs and poisons, an alternative material may be needed when adequate blood specimens are not available. In addition, multiple site sampling of blood and other body fluids is useful to investigate postmortem redistribution and postmortem interference due to redistribution/diffusion, degradation, incidental production and contamination, depending on the intake routes and the properties of individual chemicals, which should also be considered for peripheral blood samples (1–8).

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 than vitreous humor, without contamination at autopsy (9–12). PCF is a transudate derived from the epicardium and contains plasma components involving drugs and poisons, and vertebral BMA can be an alternative to peripheral venous blood. Previous studies have suggested that these materials are useful for the postmortem analysis of ethanol, showing good correlations to blood level when considering the influence of postmortem diffusion from stomach contents due to their location and the deteriorated quality of BMA samples involving increased lipid contents in elderly subjects (13–17); however, there appears to be insufficient practical data for other chemical substances.

The present study reviewed large-scale toxicological data of serial forensic autopsy cases and compared right heart/peripheral blood, PCF and BMA levels to reassess the postmortem distribution of ethanol and to investigate the distribution of other gaseous and volatile substances.

Materials and Methods

Autopsy database

All forensic autopsy cases (n = 2,983) during the past 16 years were reviewed (1996–2012), excluding those in which adequate specimens were not available due to advanced decomposition or skeletonization, and 683 cases were collected with positive toxicological findings for gaseous and volatile substances, comprising cases of people 0–97 years of age (median, 54.5), 498 males and 185 females, approximately 6 h to 1 month postmortem (median, 20 h), and with approximately 0.5 h to 3 month survival (median, 2.5 h), to compare the data among right heart and peripheral external venous blood, PCF and BMA. In addition to blood, urine and stomach contents, PCF and BMA were routinely collected at autopsy. PCF (approximately 5–25 mL) was drawn using an aseptic syringe after opening the pericardial cavity. BMA (approximately 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). These specimens were preserved at 4°C and examined within 24 h. These sample collections and the analyses described in the following were performed within the framework of routine medicolegal casework following the autopsy guidelines (2009) and ethics guidelines (1997 and 2003) of the Japanese Society of Legal Medicine, approved by the institutional ethics committee (18).

Analytical procedures

Chemicals and reagents

Deionized pure water was obtained using a Milli-Q Purification System (Millipore, Bedford, MA). Other common chemicals and reagents were of the highest purity commercially available.
Sample preparation

For automated headspace gas chromatography–mass spectrometry (HS-GC–MS), each sample (1 g) was added to an internal standard solution (0.5 mg/mL of t-butanol, 1 mL) and incubated at 60 °C for 30 min.

For the analysis of hydrogen sulfide (H\textsubscript{2}S), sulfide was detected as bis(pentfluorobenzyl)sulfide (C\textsubscript{8}F\textsubscript{6}Cl\textsubscript{2}SCH\textsubscript{2}C\textsubscript{8}F\textsubscript{6}) as follows: 0.2 mL (or g) of the sample was added to the mixture of 0.5 mL of 20 mM pentfluorobenzyl bromide (PFBBr) solution in ethyl acetate, 100 μL of internal standard (IS) solution [200 μM 3,5-triethylbenzene (TBB) in ethyl acetate] and 0.8 mL of 5 mM tetradecyldimethylbenzylammonium chloride (TDMA) solution in oxygen-free water saturated with sodium tetraborate. The preparation was vortexed for 1 min, and 0.1 g potassium dihydrogenphosphate was added to the mixture as a buffer to prevent excessive alkylation by tissue protein. The preparation was vortexed again for 10 s and centrifuged at 2,500 rpm for 15 min. An aliquot of the organic phase was injected onto the GC–MS (19, 20).

Instrumental conditions

HS-GC–MS was performed using a Shimadzu GC–MS system Model QP 5000 (Kyoto, Japan) with a DB-624 column, 60 m \( \times \) 0.32 mm i.d.; film, 1.8 μm (column temperature, 60–150 °C; injector temperature, 150 °C; carrier gas, He at a flow rate of 34 cm/s and with a split ratio of 1:30; interface temperature, 230 °C) or an Agilent Technologies GC–MS System, Model 5975c MSD (DB-5MS column, 30 m \( \times \) 0.25 mm i.d.; film, 0.25 μm; column temperature, 100–325 °C; injector temperature, 280 °C; carrier gas, He at a flow rate of 48 cm/s; interface temperature, 300 °C).

Results

Toxicological analysis detected eight gaseous and volatile substances, including ethanol (>10 mg/dL, \( n = 345 \)), acetone (>0.01 mg/dL, \( n = 402 \)), cyanide (\( n = 282 \)), toluene (\( n = 47 \)), liquefied petroleum gas (LPG; \( n = 1 \)), cresol (\( n = 1 \)), trichloroethylene (TCE, \( n = 1 \)), and H\textsubscript{2}S (\( n = 5 \)) in blood, PCF and/or BMA in 683 cases. For ethanol, acetone, cyanide and toluene, right heart and peripheral blood levels were mostly equivalent, showing good correlations (\( R = 0.90–0.98 \), \( p < 0.0001 \)) when a few exceptional cases of ethanol and toluene were excluded, as described in the following. Other substances showed similar findings when adequate peripheral blood samples were available.

For ethanol, a dissociation (a difference greater than 20%) was observed between right heart and peripheral blood levels with blood ethanol >100 mg/dL in two cases of blunt head injury (stomach content levels, 823 and 8,872 mg/dL); right heart blood levels (220 and 561 mg/dL) were markedly higher than respective peripheral blood levels (114 and 243 mg/dL). The PCF level (\( y_1 \)) was slightly higher than right heart/peripheral blood levels (\( x \)) in most cases, showing a good correlation (\( y_1 = 1.15x - 0.03 \), \( R = 0.97, p < 0.0001 \); \( y_1 = 1.08x + 0.03 \), \( R = 0.98, p < 0.0001 \)) when a case of marked dissociation was excluded; the PCF level (2,009 mg/dL) was markedly higher in the previously mentioned case with high stomach content levels (8,872 mg/dL) (Figure 1A). The BMA level (\( y_2 \)) was mostly equivalent to the right heart blood level (\( y_2 = 0.90x + 0.04 \), \( R = 0.95, p < 0.0001 \)), including three cases of moderate dissociation (a difference of 20–50%); an elderly subject (82 years old) and the case of a decomposed drowning victim (approximately 3–4 days postmortem) had lower BMA levels, whereas a case of multiple trauma with critical medical care had a higher BMA level (Figure 1B). BMA levels were slightly lower than the peripheral blood level (\( y = 0.85x + 0.04 \), \( R = 0.98, p < 0.0001 \), except for a case of markedly high BMA level (593 mg/dL) in the previously mentioned case with high stomach ethanol content (8,872 mg/dL).

Acetone concentrations in PCF (\( y_1 \)) and BMA (\( y_2 \)) were mostly equivalent to right heart blood levels (\( x \)) for all cases (\( n = 402 \)), although PCF levels were often evidently lower (\( n = 60 \)), especially in cases of <5 mg/dL: \( y_1 = 1.01x - 0.53, R = 0.98, p < 0.0001 \) and \( y_2 = 1.10x - 0.85, R = 0.98, p < 0.0001 \) (Figures 2A and 2B). In cases of acetone <5 mg/dL, a dissociation (a difference greater than 20%) between blood and BMA levels was also detected (\( n = 43 \)), including lower BMA levels (\( n = 30 \)), frequently in elderly subjects of >60 years of age (\( n = 17 \)), and higher BMA levels (\( n = 13 \)), primarily in prolonged death (\( n = 10 \)). The PCF level was substantially higher than blood levels (\( n = 9 \)), primarily in prolonged death (\( n = 6 \)).

Cyanide concentrations in acute fire fatalities (\( n = 98 \)) showed an excellent correlation between BMA (\( y_2 \)) and right heart blood (\( x \)), with two exceptional cases of substantially higher BMA levels, whereas PCF cyanide levels (\( y_1 \)) were usually substantially lower than blood levels with one exceptional case, showing no significant correlation: \( y_1 = 0.02x + 0.15, R = 0.18, p > 0.05 \); \( y_2 = 0.96x + 0.04, R = 0.88, p < 0.0001 \) (Figures 3A and 3B). Ingested cyanide in suicide victims (\( n = 2 \)) showed higher levels in PCF (53.88 and 13.91 μg/mL) than in blood (20.29 and 10.50 μg/mL) independently of stomach contents; however, the relationship to BMA levels varied (10.59 and 17.02 μg/mL).

The distribution of inhaled toluene (\( n = 47 \)) varied by case: right heart blood. PCF and BMA levels moderately correlated at lower concentrations (<1.5 μg/mL) in fire fatalities involving petroleum (\( n = 34 \)) (Figures 4A and 4B); however, abusers with higher toluene concentrations (\( n = 7 \)) showed approximately 1.2–6.8 times (median, 2.28) higher levels in PCF than in blood (20.29 and 10.50 μg/mL), independently of stomach contents; however, the relationship to BMA levels varied (10.59 and 17.02 μg/mL).

The distribution of inhaled toluene (\( n = 47 \)) varied by case: right heart blood. PCF and BMA levels moderately correlated at lower concentrations (<1.5 μg/mL) in fire fatalities involving petroleum (\( n = 34 \)) (Figures 4A and 4B); however, abusers with higher toluene concentrations (\( n = 7 \)) showed approximately 1.2–6.8 times (median, 2.28) higher levels in PCF than in blood (20.29 and 10.50 μg/mL), independently of stomach contents; however, the relationship to BMA levels varied (10.59 and 17.02 μg/mL).

Inhaled LPG (\( n = 1 \)) and TCE (\( n = 1 \)) were also higher in BMA (14.38 and 0.51 μg/mL, respectively) than in right heart blood (4.09 and 0.05 μg/mL, respectively). Ingested cresol
(n = 1) showed similar distribution in right heart blood and PCF (212.03 and 215.46 mg/mL, respectively; BMA data not available). H$_2$S in suicidal inhalation (n = 5) was detected at a substantially lower concentration in PCF (0.38–12.10 mg/mL; median, 0.79 mg/mL) than in right heart blood (0.09–84.91 mg/mL; median, 2.71 mg/mL) and BMA (1.00–61.55 mg/mL; median, 3.50 mg/mL). The relationship between blood and BMA levels differed by case.

Discussion

Previous studies showed good correlations of ethanol levels in PCF and BMA to blood, in which the PCF level was slightly higher than heart and peripheral blood levels and the BMA level was almost equivalent to heart blood level and slightly lower than peripheral blood level, however, an influence of alcoholic stomach contents or vomit on PCF ethanol level was detected (20, 21). The present study confirmed correlations among ethanol levels in blood, PCF and BMA, involving the approximate equivalency of BMA and blood levels and a slightly higher PCF level when the influence of postmortem diffusion on heart blood, PCF or BMA levels was excluded due to extremely high stomach ethanol contents. Dissociation was otherwise sporadically detected between BMA and right heart blood or PCF levels (evidently lower BMA level) in elderly subjects, possibly depending on the quality of BMA samples (20), and with decomposition, suggesting a less significant influence of putrefaction on BMA than on heart blood or PCF. When these postmortem influences or artifacts are excluded, BMA level is comparable to blood level. Differences between PCF and blood levels can primarily indicate the phase after alcohol ingestion, reflecting a delay in the change of PCF level compared to that of blood level, with the partial contribution of water contents in the blood (20).

The distribution of acetone in blood, PCF and BMA was similar to ethanol, described previously, showing overall correlations and equivalency. Some cases of dissociation with a higher blood level than PCF level, which were usually observed in cases of low acetone (<5 mg/dL), may be attributed to the progressive increase of acetone in blood due to metabolic deterioration during the survival period after a fatal insult (22); however, similar dissociation between blood and BMA levels may primarily depend on the quality of BMA samples, as observed in ethanol distribution (20). Higher acetone levels in PCF and/or BMA than in blood, primarily observed in prolonged deaths, may be the consequence of terminal metabolic disorder and circulatory failure; however, further investigation of these discrepancies is needed.
Inhaled cyanide in a fire fatality and H₂S in a suicidal inhalation were detected at substantially lower concentrations in PCF than in blood and BMA. An explanation for these findings is the high affinity of cyanide and H₂S for hemoglobin (23–25) and the short survival time before distribution into pericardial effusion; this blood/BMA-to-PCF dissociation can occur in pharmacokinetic processes before death rather than being attributable to redistribution after death. In contrast, a higher PCF level in suicidal ingestion cases is indicative of a longer survival time, although the partial contribution of postmortem diffusion from stomach contents should also be considered. Some cases of higher cyanide or H₂S in BMA than in blood suggest the less significant postmortem decrease of these gaseous substances in bone marrow than in blood (26).

The distribution of inhaled toluene in fire fatalities showed equivalency at each site, whereas BMA level was approximately

Figure 3. Relationship of cyanide concentrations between right heart blood \(x\) and pericardial fluid \(y_1\) or bone marrow aspirate \(y_2\) in acute fire fatalities: \(y_1 = 0.02x + 0.15, n = 53, R = 0.18, p > 0.05\) (A); \(y_2 = 0.96x + 0.04, n = 91, R = 0.88, p < 0.0001\) (B).

Figure 4. Relationship of toluene concentrations between right heart blood \(x\) and pericardial fluid \(y_1\) and bone marrow aspirate \(y_2\) in acute fire fatalities \(<1.5 \mu g/mL\) and in abusers: \(y_1 = 0.10x + 0.03, n = 22, R = 0.78, p < 0.0001\) (A); \(y_1 = 0.30x + 0.03, n = 32, R = 0.86, p < 0.0001\) (B); \(y_1 = 0.62x + 0.27, n = 4, R = 0.99, p < 0.05\) (C); \(y_1 = 1.88x + 0.71, n = 6, R = 0.99, p < 0.0001\) (D).
twice as high as the right heart blood level in abusers; however, sporadically detected dissociations between heart and peripheral blood levels in multiple trauma and fire victims suggest the instability of volatile substances in blood because of body destruction due to trauma or fire in addition to postmortem interference and artifacts. Inhaled LPG and trichloroethylene were also detected at higher levels in BMA than in blood and PCF; however, ingested cresol \((n = 1)\) showed similar distributions in right heart blood and PCF. These findings suggest that these substances are readily distributed in PCF and bone marrow and are retained longer in the bone marrow than in blood. A highly lipophilic property and a larger volume of distribution \((V_d)\) involving tissue components including bone marrow, e.g., for toluene \((V_d = 57–66 \, L/kg)\) \((27)\), may partly contribute to the higher BMA level at the time of death; the dissociation between blood and BMA levels may occur in pharmacokinetic processes before death, partly modified by redistribution after death. PCF levels may primarily depend on the survival time after intake, as described previously, with the additional influence of postmortem diffusion from the stomach contents in cases of ingestion. Thus, PCF and BMA are useful fluid materials available for routine analysis to investigate antemortem pharmacokinetics, toxicokinetics and postmortem redistribution. PCF reflects changes in plasma components with a delay, whereas BMA, containing a substantial amount of venous blood, appears to have properties between peripheral blood and tissues. In the present study, however, the hypothetical distributions and site-to-site correlations of \(H_2S\), LPG, cresol and TCE are tentative owing to the limited number of cases because of their rare incidences; thus, further case studies are needed. Further investigation is also needed regarding the relationship of gaseous and volatile substances in PCF/BMA and other body fluids.

In conclusion, the present study demonstrated significant correlations among concentrations of gaseous and volatile substances in right heart/peripheral blood, PCF and BMA with partial differences, suggesting that PCF and BMA are useful alternative materials for toxicological analysis; however, the quality of BMA samples should be carefully assessed, especially in elderly subjects. The inclusion of these materials in routine analysis may also be helpful to investigate pharmacokinetics and toxicokinetics in the death process and the influence of postmortem redistribution/diffusion.

References


