I.3 Pitfalls and cautions in analysis of drugs and poisons

By Fumio Moriya

Introduction

Blood and urine are the common specimens for drug analysis in both antemortem and postmortem cases. Usually, urine is used for drug screening using immunoassays at the first step; secondly, the drug detected is chromatographically quantitated with blood. The data obtained are carefully assessed with taking the values reported in references into consideration together with clinical and postmortem findings; the judgement of poisoning and its degree is made comprehensively.

The periods between samplings and analysis and the storage conditions of samples are very important for assessment of analytical results for human specimens, especially for postmortem specimens; the postmortem intervals and the degree of putrefaction should be always taken into consideration. Even in a vial (*in vitro*) after sampling and also inside the whole body postmortem, drugs may be metabolized by coexisting enzymes [1, 2]; postmortem production [3, 4] and decomposition [5] can take place by the action of bacterial growth. In the autopsy cases, the source of blood sampled should be recorded exactly; the high concentrations of drugs present in the lung, heart and liver can diffuse into the surrounding tissues, resulting in higher drug concentrations in blood there [6]. When a large amount of a drug is present in the stomach, it diffuses into the surrounding tissues and blood postmortem [7, 8]. The urinary bladder sometimes contains a large amount of urine with a high drug concentration; in such a case, diffusion of a drug from the bladder into blood of the femoral vein can take place postmortem [9]. When vomitus containing a high concentration of a drug is aspirated into the trachea or bronchus, or local anaesthetic jelly is applied to the trachea upon intubation, the concentration of the drug in heart blood may be enhanced postmortem [10, 11]. Even if analytical instruments are excellent, correct diagnosis of poisoning is impossible without considering the above phenomena. In analysis of drugs and poisons, there are many subtle points to be considered; in this chapter, pitfalls and cautions are presented for correct analysis in poisoning.

Metabolism of drugs by coexisting enzymes

Ester compounds, such as local anaesthetics, are susceptible to their metabolism by coexisting enzymes; they are easily metabolized postmortem by plasma cholinesterase in a cadaver and even *in vitro* after antemortem samplings [1, 2]. The cholinesterase activity in blood does almost not decline 3 weeks after its storage at room temperature [12]. Cocaine, one of the local anaesthetics and most popular abused drugs, is largely converted to benzoylecgonine by chemical reaction in antemortem blood at pH 7.4, and a minor part of the drug is metabolized by plasma cholinesterase to yield ecgonine methyl ester [13]. The latter is further decomposed to ecgonine by chemical hydrolysis very rapidly and thus not accumulates in blood of living sub-

jects [13]. In the case of postmortem blood, the pH value of blood rapidly declines due to anaerobic glycolysis postmortem, resulting in no chemical hydrolysis of cocaine into benzoylecgonine but in accumulation of ecgonine methyl ester by the action of the coexisting cholinesterase [13]. Therefore, the cocaine concentration in blood at the point of death was reported to be exactly estimated by summing up the concentrations of cocaine and ecgonine methyl ester [14].

To prevent ester compounds from their decomposition in blood, the addition of NaF, a cholinesterase inhibitor, at the concentration of about 1% is being recommended. Cocaine seems stable in blood for 2–3 weeks in the presence of NaF in a refrigerator [2]. However, in the case of tetracaine, the addition of neostigmine is necessary in place of NaF to suppress the *in vitro* metabolism completely. It should be mentioned that dichlorvos, an ester-type organophosphorus pesticide, is decomposed more easily in the presence of NaF [15].

Heroin is more susceptible to decomposition by plasma cholinesterase than cocaine; the half-life of the reaction in living subjects is only several minutes [13]. Therefore, it was difficult to detect heroin from blood of a cadaver, who had received intravenous injection only several minutes before [16]; but 6-monoacetylmorphine, the main metabolite of heroin, is relatively stable in blood and detectable postmortem [16].

Postmortem production and decomposition of compounds by putrefactive bacteria

Various kinds of compounds are postmortem produced by growing bacteria in human specimens; especially alcoholic and amine compounds should be noted in toxicological analysis. Ethanol is most commonly produced by fermentation. The *in vitro* production of ethanol in blood and urine is much less than its production inside a cadaver, and usually give no problems under storage at 4° C for a week. However, when a large amount of glucose and marked contamination by bacteria are present, non-negligible amounts of ethanol can be produced in specimens collected. To discriminate ethanol produced postmortem from the antemortem one, n-propanol can be used as an indicator, because it is produced by bacteria concomitantly [3]. The concentration of n-propanol is not lower than 5% of a postmortem ethanol concentration [3].

The most typical amine produced during putrefaction is β-phenylethylamine. Its structure is similar to those of amphetamines. The similarity of the amine sometimes gives false positive results during screening by immunoassays [17, 18].

In analysis of drugs in specimens collected from cadavers killed especially by severe injuries, followed by intensive medical treatments, a special caution is needed. In such cadavers, non-negligible amounts of ethanol and β-phenylethylamine are sometimes produced by the action of bacterial translocation [19,21].

The metabolic reactions for drugs by bacteria are essentially reductive; nitro, *N*-oxide, oxime, thiono, sulfur-containing heterocyclic and aminophenolic compounds are known to be decomposed rapidly [5]. Robertson and Drummer [22] reported that nitrobenzodiazepine drugs were metabolized to 7-amino reduced forms by enteric bacteria and that such reducing reaction could not be suppressed by adding NaF. The author et al. collected the cerebral cortex, diencephalons, cerebellum of a nitrazepam user at autopsy, and measured nitrazepam and 7-aminonitrazepam both immediately and 10 days (at 4° C) after autopsy as shown in *Table 3.1*.

Table 3.1

Postmortem changes in the level (μg/g) of nitrazepam and 7-aminonitrazepam during *in vitro* storage of specimens obtained from a nitrazepam user at autopsy

Specimen	Immediately after autopsy		10 days after autopsy*	
	Nitrazepam	7-Aminonitrazepam	Nitrazepam	7-Aminonitrazepam
Cerebral cortex	3.49	2.55	0.626	5.11
Diencephalon	6.22	2.49	4.61	3.82
Cerebellum	2.17	5.11	0.545	6.55

* Stored at 4° C.

The reductive reaction for nitrazepam proceeds upon storage of specimens at 4° C, but such reaction can be completely suppressed at –20° C [22].

Clozapine, an antipsychotic drug, is easily metabolized antemortem to an *N*-oxide form, which accumulates in blood of living subjects; the metabolite can be conversely reduced to form the precursor clozapine in a cadaver and in blood stored in a vial by the action of bacteria.

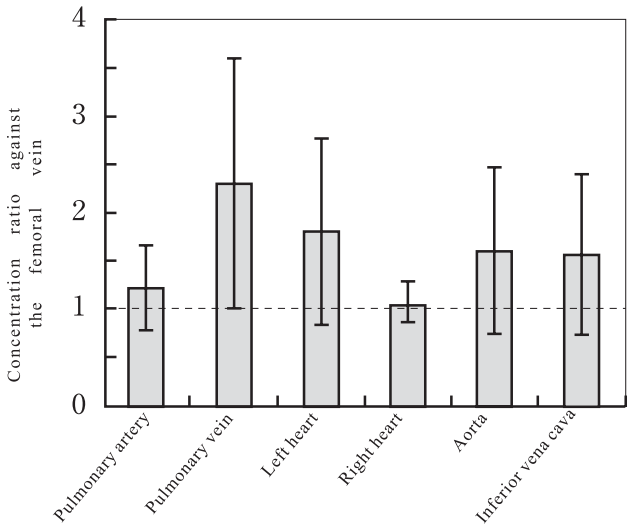
The concentration ratios of each free form to each glucuronate-conjugated form of opiates in blood are known to be helpful to estimate intervals after their administration; but the conjugated forms can be hydrolyzed to form free opiates by metabolism of bacteria, when bacteria growth is marked [23].

Postmortem redistribution of drugs

Postmortem redistribution is more common for basic drugs, which have high affinities to the lung, heart muscle and liver and show wide distribution areas [6]. These drugs are partly liberated from tissues with high contents, penetrate vessel walls and diffuse into blood, resulting in higher concentrations of the drugs in surrounding tissues than true concentrations at the time of death. After death, the supply of oxygen and ATP, and the Na^+/K^+ pumping function of cell membranes stop; then cell membranes and organelles are damaged. In the cells, energy-requiring bindings of proteins with drugs are inhibited, and pH is lowered as a result of accumulation of lactic acid produced by anaerobic glycolysis. These conditions of cells cause basic drugs to diffuse outside the cells more easily.

Holt and Benstead [24] first demonstrated the increase of blood drug concentration postmortem as a result of redistribution; they found a higher concentration of digoxin in blood of the heart than in blood of the femoral vein in an autopsy case of a digoxin-user. Jones and Pounder [25] reported analytical results of imipramine and its metabolite desipramine in blood and various organs of a victim, who had died by ingesting imipramine and acetaminophen together with alcohol (postmortem interval: 12 h); when the concentration of imipramine (2.3 μg/mL) and desipramine (1.5 μg/mL) in peripheral blood is assumed as 1.0, the relative values were 2.3 and 2.2 in blood of the thoracic aorta, 2.1 and 1.4 in blood of the inferior vena cava, 3.5 and 3.4 in blood of the pulmonary artery, 7.0 and 7.1 in blood of the pulmonary vein, 70 and 115 in the lung, and 78 and 52 in the liver, respectively. The above data show that imipramine concentrations in blood of the pulmonary artery and vein are higher than those in blood of the inferior vena cava, although imipramine concentration in the lung was almost equal to that in the liver, suggesting that the diffusion of the drug into blood is more marked

Figure 3.1



Variation in drug concentration in blood obtained from different locations of each cadaver. Blood specimens were obtained from fresh cadavers, which had ingested various drugs, with almost no postmortem changes. Each value was expressed as a ratio of the concentration in blood of a target location to that in blood of the femoral vein for each victim and for each drug. All values obtained from blood of each location were averaged irrespective of the kinds of drugs. The bars show means ±SD (n=11–16). The drugs detected were: phenobarbital, phenytoin, ephedrine, diazepam, nordiazepam, lidocaine, methamphetamine, codeine, barbital, zotepine, amitriptyline and nortriptyline.

for the lung than for the liver. Hilberg et al. [26] reported, using rats, that the concentrations of amitriptyline and its metabolite nortriptyline in blood of the heart increased within 2 h after death, and those in blood of the inferior vena cava increased more than 5 h after death. The author et al. [27, 28] also clarified that basic drugs distributed in the lung tissue at high concentrations diffuse postmortem, through thin walls of the pulmonary vein, into blood of the vein and are further redistributed into blood of the left atrium of the heart; this is the mechanism of the higher concentrations of basic drugs in heart blood. The increase in drug levels in blood of the right heart is less than in blood of the left heart. In many of autopsy cases, drug concentrations in blood of the right heart are similar to those in peripheral blood (in the femoral vein) (*Figure 3.1)*. Therefore, blood of the right heart together with peripheral blood seems to be good specimens for determination of the correct blood drug level, when a cadaver is relatively fresh [29]. Cautions are needed against that the posture movements of a body at postmortem inspection and during its transportation can cause a flow of blood in the vessels and thus enhance such redistribution of drugs.

Postmortem diffusion of drugs from the stomach and urinary bladder

Ethanol is best studied for its postmortem diffusion from the stomach. Pounder and Smith [7] reported that the body fluids most influenced by the diffusion of the stomach ethanol were pericardial fluid, followed by blood of the left pulmonary vein, aorta, left heart, pulmonary artery, superior vena cava, inferior vena cava, right heart and right pulmonary vein; the blood in the femoral vein was almost not affected. The postmortem diffusion of ethanol from the stomach is dependent upon the residual amounts of ethanol in the stomach, physique and postmortem intervals. In actual cases, such diffusion is a problem, when more than 100 g contents containing more than several percent of ethanol are present in the stomach and the postmortem interval is longer than one day.

Not many basic studies have not been reported on the postmortem diffusion of general drugs from the stomach. A drug can diffuse from the stomach postmortem into the surrounding tissues and body fluids in the presence of a large amount (more than several ten mg) of the drug in the stomach with a long postmortem interval. However, the blood of the femoral and subclavian veins is almost not affected about 2 days after death [8].

Although the postmortem diffusion of a drug from the urinary bladder is rare, it can take place in the presence of a large amount of urine containing a high content of a drug. The author et al. [9] experienced an autopsy case of a drug abuser, in which diphenhydramine and dihydrocodeine diffused from the urinary bladder, resulting in the remarkable increase in their concentrations in the femoral vein; although the postmortem interval was 9 days, the putrefaction was not so marked because of the winter season. The amount of urine in this case was as large as 600 mL, and diphenhydramine and dihydrocodeine concentrations in it were 22.6 and 37.6 μg/mL, respectively; their concentrations in the femoral vein were 1.89 and 3.27 μg/mL, which were much higher than those (0.204–0.883 and 0.173–1.01 μg/mL) obtained from other parts of circulation, respectively. Although it is unequivocally accepted by forensic chemists that blood of the femoral vein is most suitable for postmortem analysis of drugs, it seems dangerous to use only femoral vein blood for drug analysis because of our above experience.

Postmortem diffusion of drugs from the trachea into heart blood

In the autopsy cases, in which vomitus containing a large amount of a drug is aspirated into the trachea, postmortem diffusion of a drug into the surrounding tissues of the trachea, especially into heart blood, should be taken into consideration [10]. In forensic science practice, ethanol is the case for such diffusion from the trachea [10]. In the ethanol-aspirated case, the story becomes complicated, because both diffusions from the trachea and from the stomach take place concomitantly. There are not many reports dealing with comparison of the diffusion from the trachea with that from the stomach. The postmortem diffusion velocity of toluene from the trachea was reported to be faster than that from the stomach, after thinner solvent had been injected into both trachea and stomach of a human cadaver [30]. According to the experiments, in which ethanol, paracetamol and dextropropoxyphene were introduced into the trachea, the drugs diffused into blood of the pulmonary vein and artery most rapidly, followed by blood of the heart, superior vena cava and aorta [31].

Table 3.2

Lidocaine concentrations in various body fluids and organs obtained from 4 victims who did not regain heart beats after resuscitation treatments*

Specimen	Lidocaine concentration (μg/mL or μg/g)			
	Case 1	Case 2	Case 3	Case 4
Pulmonary artery blood	–	–	–	2.04
Pulmonary vein blood	–	–	–	2.29
Left heart blood	0.349	1.02	–	1.55
Right heart blood	0.102	0.209	–	0.699
Aorta blood	–	–	0.642	–
Superior vena cava blood	–	–	0.746	–
Inferior vena cava blood	0.195	0.163	0.133	0.491
Iliac vein blood	–	0.074	0.057	0.152
Femoral vein blood	–	0.015	ND	ND
Cerebrospinal fluid	ND	–	–	0.191
Vitreous humor	–	–	–	0.007
Pericardial fluid	0.193	0.097	0.171	0.489
Bile	–	–	–	ND
Urine	–	–	–	ND
Cerebrum	ND	ND	ND	0.044
Left lung	–	10.9	1.37	9.33
Right lung	–	2.65	1.41	2.60
Heart muscle	–	–	–	0.186
Liver	ND	ND	ND	0.183
Right kidney	–	ND	ND	0.020
Right femoral muscle	ND	ND	ND	ND

* Xylocaine™ jelly was used at intubation. ND: not detected.
Case 1: 3.5 month female, resuscitation 5 min, postmortem interval about 20 h.
Case 2: 44 year male, resuscitation 5 min, postmortem interval about 20 h.
Case 3: 38 year male, resuscitation 60 min, postmortem interval about 20 h.
Case 4: 60 year female, resuscitation 20 min, postmortem interval about 12 h.

In Japan, Xylocaine™ jelly is usually used at endotracheal intubation in emergency medicine; we frequently experience the detection of lidocaine from blood due to such intubation in cadavers, which had received the cardiopulmonary resuscitation [32]. Although many victims without regaining heart beat were included in such resuscitation cases, relatively high concentrations of lidocaine could be detected from their heart blood [11]. The distribution of lidocaine, which had been used at endotracheal intubation, in body fluids and organs of 4 victims, who did not regain the heart beat, is shown in *Table 3.2*. The postmortem intervals were as short as 12~20 h, but rapid postmortem diffusion of the drug from the trachea into heart blood (especially left heart blood) was observed; there was no influence on the femoral vein blood. The lidocaine level was remarkably increased in the left heart blood, probably because lidocaine in the trachea diffused through the thin walls of the pulmonary vein into blood and then moved to the left atrium of the heart. Lidocaine in the trachea seems to diffuse into blood of the pulmonary artery. However, the diffusion velocity is slow because of thick walls of the

artery; the blood of the pulmonary artery hardly flows backward to the right ventricle of the heart. These seem to be reasons why the concentration of lidocaine is higher in the left heart blood than in the right heart blood. The postmortem diffusion of lidocaine from the trachea was also confirmed by experiments with rabbits [11]. Analytical chemists should be always aware of such a phenomenon for victims who had received emergency medical treatments.

Countermeasures

As stated above, when the handling of specimens is careless, it may cause serious variations of drug concentrations depending on the kinds of drugs upon their analysis. The temporary storage of specimens can be made at 4° C in a refrigerator; but they should be kept at –20° C or preferably at –80° C until analysis, when the intervals between samplings and analysis are more than one week. When ester and nitro compounds are analyzed, the addition of a suitable preservative (usually NaF and/or NaN_3) should be considered.

In autopsy cases, blood specimens should be collected from the atrium/ventricle of both sides, and also from the femoral vein; the analytical data from different locations should be assessed. For the victims, who had received medical treatments, the analysts should be aware of the details of the treatments and clinical process.

References

1) Kalow W (1952) Hydrolysis of local anesthetics by human serum cholinesterase. J Pharmacol Exp Ther 104: 122–134
2) Baselt RC (1983) Stability of cocaine in biological fluids. J Chromatogr 268:502–505
3) Nanikawa R (1977) Legal Medicine. Nippon-iji-shinpo-sha, Tokyo, pp 239–260 (in Japanese)
4) Oliver JS, Smith H, Williams DJ (1977) The detection, identification and measurement of indole, tryptamine and 2-phenethylamine in putrefying human tissue. Forensic Sci 9:195–203
5) Stevens HM (1984) The stability of some drugs and poisons in putrefying human liver tissues. J Forensic Sci Soc 24:577–589
6) Anderson WH, Prouty RW (1989) Postmortem redistribution of drugs. In: Baselt RC (ed) Advances in Analytical Toxicology, Vol 2. Year Book Medical Publishers, Chicago, pp 70–102
7) Pounder DJ, Smith DRW (1995) Postmortem diffusion of alcohol from the stomach. Am J Forensic Med Pathol 16:89–96
8) Pounder DJ, Fuke C, Cox DE et al. (1996) Postmortem diffusion of drugs from gastric residue: an experimental study. Am J Forensic Med Pathol 17:1–7
9) Moriya F, Hashimoto Y (2001) Postmortem diffusion of drugs from the bladder into femoral venous blood. Forensic Sci Int 123:248–253
10) Marraccini JV, Carroll T, Grant S et al. (1990) Differences between multisite postmortem ethanol concentrations as related to agonal events. J Forensic Sci 35:1360–1366
11) Moriya F, Hashimoto Y (1997) Postmortem diffusion of tracheal lidocaine into heart blood following intubation for cardiopulmonary resuscitation. J Forensic Sci 42:296–299
12) Coe JI (1993) Postmortem chemistry update: emphasis on forensic application. Am J Forensic Med Pathol 14:91–117
13) Karch SB (1996) The Pathology of Drug Abuse, 2nd edn. CRC Press, Boca Raton
14) Isenschmid DS, Levine BS, Caplan YH (1992) The role of ecgonine methyl ester in the interpretation of cocaine concentrations in postmortem blood. J Anal Toxicol 16:319–324
15) Moriya F, Hashimoto Y, Kuo T-L (1999) Pitfalls when determining tissue distributions of organophosphorus chemicals: sodium fluoride accelerates chemical degradation. J Anal Toxicol 23:210–215

16) Goldberger BA, Cone EJ, Grant TM et al. (1994) Disposition of heroin and its metabolites in heroin-related deaths. J Anal Toxicol 18:22–28
17) Kintz P, Tracqui A, Mangin P et al. (1988) Specificity of the Abott TDx assay for amphetamine in post-mortem urine samples. Clin Chem 34:2374–2375
18) Moriya F, Hashimoto Y (1997) Evaluation of Triage™ screening for drugs of abuse in postmortem blood and urine samples. Jpn J Legal Med 51:214–219
19) Carrico CJ, Meakins JL, Marshall JC et al. (1986) Multiple-organ-failure syndrome. Arch Surg 121:196–208
20) Border JR, Hassett J, LaDuca J et al. (1987) Gut origin septic states in blunt multiple trauma (ISS=40) in the ICU. Ann Surg 206:427–446
21) Moriya F, Hashimoto Y (1996) Endogenous ethanol production in trauma victims associated with medical treatment. Jpn J Legal Med 50:263–267
22) Robertson MD, Drummer OH (1995) Postmortem drug metabolism by bacteria. J Forensic Sci 40:382–386
23) Moriya F, Hashimoto Y (1997) Distribution of free and conjugated morphine in body fluids and tissues in a fatal heroin overdose: is conjugated morphine stable in postmortem specimens? J Forensic Sci 42:734–738
24) Holt WD, Benstead JG (1975) Postmortem assay of digoxin by radioimmunoassay. J Clin Pathol 28:483–486
25) Jones GR, Pounder DJ (1987) Site dependence of drug concentrations in postmortem blood-a case study. J Anal Toxicol 11:186–190
26) Hilberg T, Bugge A, Beylich K-M et al. (1993) An animal model of postmortem amitriptyline redistribution. J Forensic Sci 38:81–90
27) Moriya F, Hashimoto Y (1999) Redistribution of basic drugs into cardiac blood from surrounding tissues during early-stages postmortem. J Forensic Sci 44:10–16
28) Moriya F, Hashimoto Y (2000) Redistribution of methamphetamine in the early postmortem period. J Anal Toxicol 24:153–154
29) Moriya F, Hashimoto Y (2000) Criteria for judging whether postmortem blood drug concentrations can be used for toxicologic evaluation. Legal Med 2:143–151
30) Fuke C, Berry CL, Pounder DJ (1996) Postmortem diffusion of ingested and aspirated paint thinner. Forensic Sci Int 78:199–207
31) Pounder DJ, Yonemitsu K (1991) Postmortem absorption of drugs and ethanol from aspirated vomitus: an experimental model. Forensic Sci Int 51:189–195
32) Moriya F, Hashimoto Y (1998) Absorption of intubation-related lidocaine from the trachea during prolonged cardiopulmonary resuscitation. J Forensic Sci 43:718–722