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Postmortem Drug Redistribution—Human Cases Related to Results in Experimental Animals

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ABSTRACT: Femoral blood is widely accepted as the most reliable postmortem specimen for drug analysis in forensic toxicology. There is considerable evidence that the drug concentrations in peripheral blood samples are closer to the antemortem level than the concentration in cardiac blood. In the present study drug concentrations measured in postmortem femoral and/or heart blood samples from eight cases were compared with the concentration found in serum samples from the same subject collected antemortem or perimortem. The drugs involved were amitriptyline, nortriptyline, imipramine, verapamil and chloroquine. Two additional cases with very early postmortem blood samples, as well as femoral blood samples from later autopsy, involved amphetamine and tetrahydrocannabinol. The results from the human cases were compared with results from rat experiments on similar drugs. The samples were analyzed by high performance liquid or gas chromatography. The cases with tricyclic antidepressants had a median postmortern femoral blood to antemortem serum drug concentration ratio of 3.3, the 95% reference range being from 1.1 to 6.0 (pooled data). Large variations of the ratios were seen. The extremes noted were a postmortem femoral blood to antemortem serum drug concentration ratio of 0.9 in a case with nortriptyline and 49 in the case with chloroquine. The low ratio in the former case could be due to attempted resuscitation, while the high ratio in the latter case is probably due to the extremely high apparent volume of distribution and a high blood to plasma concentration ratio for chloroquine. Accordingly, it is dubious whether the drug concentration found in femoral blood at autopsy can be accepted as being representative for the antemortem level. The results obtained from the human cases in the present study were generally in reasonable agreement with previous rat experiments, confirming that the animal studies when interpreted carefully, are indicative of the changes observed in man as well as a previous study in pigs. Studies on drug concentrations in pigs are not necessarily more representative for the findings in humans than experiments with a smaller animal like the rat. The postmortem concentration changes observed for tetrahydrocannabinol in man were found to be unpredictable, while in the accompanying experimental rat study there was a significant postmortem decrease in the tetrahydrocannabinol blood concentration measured in blood from the inferior vena cava. In special cases where the diagnosis of overdose is to be used as judicial evidence, a single sample of blood may prove insufficient. In such cases, analyses of several samples of blood and tissue will increase the possibility of reaching a correct conclusion, but reference values on drug concentrations in tissues are often missing.

KEYWORDS: forensic science, forensic toxicology, postmortem redistribution, pharmacokinetics, amitriptyline, nortriptyline, imipramine, chloroquine, amphetamine, tetrahydrocannabinol, rat, pig

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The concentration of drugs in postmortem blood specimens often exhibits sample collection site dependence, particularly for drugs with a high apparent volume of distribution (1,2). Blood from the heart and central vessels have been reported to show higher concentrations than blood from peripheral sites such as femoral blood, and consequently femoral blood is recommended as the preferred sample for drug analysis and interpretation (1–4). However, in some cases this relationship is reversed, indicating the unpredictable nature of postmortem drug redistribution (5,6). It has been questioned whether the drug concentration found in a femoral sample can be regarded as reasonably representative for the antemortem situation in all cases. To answer this question one has to compare the drug concentration in the blood sample obtained at autopsy with a specimen sampled preferably immediately prior to death or very soon after. Previously, Prouty and Anderson have reported a series of cases where blood from autopsy was compared with earlier samples of blood taken by cardiac puncture (1,7), but only a limited number of cases has been reported where antemortem samples have been available for comparison (3,7). In the present study we have collected a series of ten human cases in which either an ante- or perimortem (collected within 1 h postmortem) serum or blood sample was available for comparison with the sample(s) obtained at autopsy performed 1 to 7 (range) days postmortem. The aims of this study were to investigate to which extent femoral and heart blood samples can be considered reasonably representative for the antemortem situation and to compare the present results from the human cases with results from previous and present experimental animal models (6,8).

Materials and Methods

Human Sampling

Autopsy was performed two (1 to 7) days postmortem (median (range)). The human femoral blood was collected by making an incision in the groin and elevating the lower limb, draining blood into a clean scoop and transferring to a Sterilin® tube containing 0.25 mL 67% w/v potassium fluoride solution. Care was taken to avoid blood from the iliac veins above. The samples of heart blood were from unspecified heart chamber. Samples were stored at −20°C until analyses.

Animal Studies

The experimental rat study was performed to serve as a comparison for the data from the human cases. Only tetrahydrocannabinol was investigated in rats in the present study, as the other drugs were compared with previous animal studies using a similar design (8,9).

Male Wistar rats with body weight 260 to 300 g were fasted overnight and fed 30 mg tetrahydrocannabinol (Sigma) ($n = 6$) mixed with 1 mL water by a gastric tube. Ninety minutes after dosing, the rats were anaesthetized and blood samples of 500 μL were drawn from the heart and transferred to glass tubes containing 25 μL potassium fluoride solution 67% w/v. The rats were then sacrificed with CO_2 and were left at room temperature for two hours, when autopsy was performed. A postmortem heart blood sample was drawn after having clamped the inferior vena cava just above the diaphragm. Blood from the inferior vena cava was sampled after clamping just superior to the renal veins, and vitreous humour was collected from both eyes.

Drug Analyses

Amitriptyline, nortriptyline, imipramine, desipramine and verapamil were analyzed by high performance liquid chromatography according to a slight modification of a previously reported method (6). In the present study the extraction was performed using 0.8 mL ethylacetate/heptane (4:1) and the column was a 250 × 4.6 mm ID column, packed with 5 mm LC-CN (Supelco, USA). The mobile phase consisted of 10% v/v 0.05 M ammonium acetate in acetonitrile, and the ultraviolet detector was set at 254 nm. Recovery from blood exceeded 80% for these substances at concentrations of 1 and 10 μmol/L. The limit of detection was 0.2 μmol/L, and the calibration range was from 0.2 to 10 μmol/L, and there was linearity within this range ($R^2 > 0.99$). The coefficient of variation (CV) of within-run precision for these substances was less than 10%.

The samples containing chloroquine were kept in iced silanized glass tubes to which 100 μL of an aqueous solution of the internal standard promazin and 300 μL 1 M Tris (pH = 10.7) were added. The mixture was extracted for 10 min with 400 μL of butylacetate, and centrifuged for 10 min at 740 g. The organic phase was transferred to 100 μL autosampler vials. The gas chromatograph was HP 5890, and the column was a polar 15 m ∗ 0.32 mm DB 1701, 0.32 μm film thickness (J&W Scientific, USA). The injection volume was 5 μL and detection was nitrogen-phosphor. Injector temperature was 250°C, and detector temperature was 300°C. The temperature program started at 150°C for 1 min and increased by 20°C per min to 250°C for 0.5 min with further increments of 5°C per min to 300°C for 5 min. Carrier gas was helium with flow 1.5 mL/min and make up gas to a total of 30 mL/min. Recovery from blood exceeded 90% for chloroquine at concentrations of 1 and 10 μmol/L, and the limit of detection was 0.2 μmol/L in blood. The calibration range was from 0.2 to 30 μmol/L in blood, and there was linearity within this range ($R^2 \geq 0.99$). The coefficient of variation (CV) of within-run precision was better than 6% (9) at concentrations of 1 and 10 μmol/L.

Blood samples with amphetamine were extracted with cyclohexane, and the extracts were derivatized with perfluorooctanoyl chloride prior to gas chromatography/mass spectrometry according to a previously reported method (10). Tetrahydrocannabinol was quantified by capillary column gas chromatography/electron impact mass spectrometry combined with selected ion monitoring. Deuterium-labeled THC was used as the internal standard and was added to the samples prior to extraction according to a previously reported method (11). The calibration range was from 0.001 to 0.305 μmol/L in blood, and there was linearity within this range ($R^2 \geq 0.997$). The method was found to be unsuitable for tissue sample analyses, and accordingly only results from blood and vitreous humour are presented from the rat study.

Blood and plasma concentrations are presented in μmol/L, and the results from the present and previous rat studies are presented as mean ± standard error of the mean. The concentration ratios are presented as median, lower and upper quartiles and 95% reference range, the latter representing the 2.5 and 97.5 percentile (12). Statistical analysis was performed using the nonparametrical Wilcoxon test, and compared results with $p < 0.05$ were considered statistically significant.

Results

Human Case Reports

Case 1—A 49-year-old male (body height 188 cm, body weight 108 kg) took an overdose of allegedly 150 tablets of 25 mg amitriptyline (Sarotex®, Lundbeck) and 50 tablets of 25 mg chlorpromazine (Hibanil®) 3 to 4 h prior to admission. He was unconscious when admitted to hospital, and died three days afterwards. Serum samples were obtained on admission, the following day and the day of death (Day 1, 2 and Antemortem in Table 1). A medicolegal

TABLE 1—*Drug concentrations in cases 1 to 8 in μmol/L.*

		Day 1 Serum	Day 2 Serum	Ante- or Perimortem Serum	Heart Blood from Autopsy	Femoral Blood from Autopsy	Postmortem Heart Blood to Antemortem Serum Drug Concentration Ratio	Postmortem Femoral Blood to Antemortem Serum Drug Concentration Ratio	Postmortem Interval (days)
Case 1	Amitriptyline	2.3	3.1	1.4	. . .	4.3	. . .	3.1	3
	Nortriptyline	1.6	2.2	2.3	. . .	8.1	. . .	3.5	
Case 2	Amitriptyline	. . .	. . .	2.0	. . .	12	. . .	6.0	2
	Nortriptyline	. . .	. . .	1.3	. . .	6.0	. . .	4.6	
Case 3	Amitriptyline	. . .	. . .	2.3	. . .	3.6	. . .	1.6	1
	Nortriptyline	. . .	. . .	2.0	. . .	1.8	. . .	0.9	
Case 4	Amitriptyline	. . .	. . .	2.0	12	. . .	6.0	. . .	4
	Nortriptyline	. . .	. . .	3.6	24	. . .	6.7	. . .	
Case 5	Nortriptyline	. . .	. . .	7.0	. . .	23	. . .	3.3	2
Case 6	Imipramine	. . .	. . .	11	. . .	33	. . .	3.0	1
	Desipramine	. . .	. . .	2.3	. . .	6.0	. . .	2.6	
Case 7	Imipramine	. . .	. . .	0.2	2.7	1.2	13.5	6.0	3
	Desipramine	. . .	. . .	0.6	4.0	2.0	6.7	3.3	
	Verapamil	. . .	. . .	2.8	15.0	4.2	5.4	1.5	
Case 8	Chloroquine	4.0	2.3	1.3	. . .	64	. . .	49	2

autopsy three days postmortem revealed pulmonary congestion and hypertrophy and dilation of the heart. The concentrations of amitriptyline and nortriptyline from the serial plasma samples and femoral blood from the autopsy are outlined in Table 1. The postmortem to antemortem drug concentration ratios were 3.1 for amitriptyline and 3.5 for nortriptyline. No chlorpromazine was detected. Death was considered to have been caused by amitriptyline intoxication.

Case 2—A 38-year-old female (body height 161 cm, body weight 54 kg) using Sarotex® was found unconscious at home. Just prior to admission there was an episode of respiratory arrest. On admission she had ventricular tachycardia, but asystole ensued in spite of resuscitation. A medicolegal autopsy two days postmortem revealed pulmonary oedema and dark sanguinous gastric contents. In the antemortem serum sample the concentration of amitriptyline was 2.0 μmol/L and nortriptyline 1.3 μmol/L. The peripheral blood sample taken at autopsy revealed a concentration of amitriptyline of 12 μmol/L and nortriptyline of 6 μmol/L, giving postmortem to antemortem drug concentration ratios of 6 and 4.6, respectively. The cause of death was considered to be intoxication with amitriptyline.

Case 3—A 45-year-old female (body height 168 cm, body weight 60 kg) with a previous history of alcohol abuse and depressions took an unknown amount of amitriptyline (Sarotex®). She was dead when admitted to hospital, even though resuscitation had been attempted. A medicolegal autopsy one day postmortem revealed pulmonary embolisms, and this was considered to be the cause of death, with a possible contribution of amitriptyline intoxication. A serum sample obtained immediately after admission was analyzed and had concentrations of amitriptyline of 2.3 μmol/L and nortriptyline of 2.0 μmol/L. The femoral blood sample obtained at autopsy revealed concentrations of amitriptyline of 3.6 μmol/L and nortriptyline of 1.8 μmol/L, giving postmortem to perimortem concentration ratios of 1.6 and 0.9 for amitriptyline and nortriptyline, respectively.

Case 4—A 37-year-old female (body height 158 cm, body weight 49 kg) had a history of drug and alcohol abuse and depressions. She was found unconscious after having taken allegedly 15 tablets of unknown kind over the last two days. She was alive when admitted to hospital, but died after three days due to irreversible brain damage. A serum sample was obtained the day before she died. A medicolegal autopsy four days postmortem showed left-sided pneumonia. In the antemortem serum sample the concentration of amitriptyline was 2.0 μmol/L and nortriptyline 3.6 μmol/L. The cardiac blood sample taken at autopsy had a concentration of amitriptyline of 12 μmol/L and nortriptyline 24 μmol/L, giving postmortem to antemortem heart blood concentration ratios of 6 and 6.7, respectively.

Case 5—A 30-year-old female (body height 167 cm, body weight 60 kg) was treated in hospital for depression with nortriptyline (Noritren®, Lundbeck). She was found unconscious in the basement of the hospital with severe cardiac arrhythmia and died within 3 h. A medicolegal autopsy performed two days afterwards revealed no pre-existing diseases. Antemortem serum concentration of nortriptyline was 7 μmol/L, while in postmortem femoral blood the concentration was 23 μmol/L, giving a postmortem to antemortem concentration ratio of 3.3. The cause of death was considered to be nortriptyline poisoning.

Case 6—A 19-year-old male (body height 167 cm, body weight 73 kg) had a previous history of diabetes since he was 8 years old. He had been depressed and was considered to be suicidal the last few weeks. He came home and went to bed, but less than 3 h afterwards he got up because he was vomiting. He was noticed to have unsteady gait and he complained of headache. On admission to hospital he was unconscious. An empty container was missing 180 tablets of 25 mg Tofranil® (Geigy). Blood sugar was found to be normal. Resuscitation was attempted, but he died within 2 h after admission. A medicolegal autopsy performed the day after exposed congested lungs and superficial gastritis. An antemortem serum sample obtained during resuscitation revealed a concentration of imipramine of 11 μmol/L and desipramine of 2.3 μmol/L. The postmortem femoral blood concentration of imipramine was 33 μmol/L and desipramine of 6.0 μmol/L, giving postmortem to antemortem concentration ratios of 3.0 and 2.6, respectively (Table 1). The cause of death was considered to be imipramine poisoning.

Case 7—A 48-year-old male (body height 173 cm, body weight 87 kg) with a history of depressions and alcohol abuse was found unconscious with respiratory distress at home. Just after admission to hospital cardiac arrest occurred and resuscitation was started, but he died soon after. A moderately elevated blood glucose, a blood pH of 7.13 and base excess of −18.5 mmol/L were detected prior to death. A serum sample was obtained during resuscitation, and at the medicolegal autopsy three days postmortem both cardiac and femoral blood samples were taken. Drug concentrations are outlined in Table 1. The postmortem femoral blood to antemortem serum drug concentration ratios were 6.0 for imipramine, 3.3 for desipramine and 1.5 for verapamil, while the drug concentrations in heart blood were higher. Death was considered to be due to a combination of diabetic ketoacidosis and verapamil poisoning.

Case 8—A 15-year-old previously healthy Asian female (body height 156 cm, body weight 50 kg) became unwell with vomiting and was soon thereafter unconscious. Cardio-respiratory arrest ensued and resuscitation was performed. On admission to hospital she was hypothermic with dilated pupils unresponsive to light. Laboratory investigations showed hypokalaemia with a serum potassium of 1.5 mmol/L. She was treated in the intensive care unit with mechanical ventilation, but was pronounced dead after six days due to irreversible hypoxic brain damage. Toxicological analysis of a serum sample from admission revealed a chloroquine concentration of 4.0 μmol/L, while the concentration in a serum sample taken 8 h later was 2.3. Four days later the serum chloroquine concentration was 1.3 μmol/L. A medicolegal autopsy performed two days postmortem was consistent with Adult Respiratory Distress Syndrome and hypoxic brain damage. Analysis of femoral blood from the autopsy revealed a chloroquine concentration of 64 μmol/L, giving a postmortem femoral blood to antemortem serum chloroquine concentration ratio of 49.

Case 9—A 29-year-old male (body height 187 cm, body weight 89 kg) with a history of narcotic abuse was shot in the right hemithorax. He was immediately brought to hospital, but died soon after. A perimortem blood sample was obtained from the heart within 1 h postmortem. A medicolegal autopsy was carried out one day postmortem, and the results are outlined in Table 2. The postmortem femoral to perimortem heart blood drug concentration ratios were 1.5 for amphetamine and 2.8 for tetrahydrocannabinol. The cause of death was considered to be due to the shotgun wound in the thorax.

TABLE 2—*Concentration of amphetamine and tetrahydrocannabinol in cases 9 and 10 in μmol/L. The perimortem blood samples were obtained from the heart and neck vein within 1 h postmortem, respectively.*

		<1 h Perimortem Blood	Postmortem Femoral Blood from Autopsy	Postmortem to Perimortem Concentration Ratio	Postmortem Interval (days)
Case 9	Concentration of amphetamine	5.4	8.1	1.5	7
	Concentration of tetrahydrocannabinol	0.004	0.011	2.8	
Case 10	Concentration of amphetamine	5.1	8.2	1.6	2
	Concentration of tetrahydrocannabinol	0.030	0.011	0.4	

TABLE 3—*Antemortem heart blood concentration of tetrahydrocannabinol in six rats in μmol/L, and postmortem blood and vitreous humour to antemortem blood concentration ratios. The results are expressed as mean ± standard error of the mean (s.e.m.).*

	Mean ± s.e.m.
Antemortem heart blood	0.164 ± 0.055
Postmortem/antemortem heart blood concentration ratio	1.2 ± 0.1 n.s.*
Postmortem vena cava/antemortem blood concentration ratio	0.6 ± 0.1†
Postmortem heart blood/vena cava concentration ratio	1.9 ± 0.4 n.s.*
Vitreous humour/antemortem blood drug concentration ratio	0.12 ± 0.04†

* n.s. = not significant.
† $p < 0.05$ (Wilcoxon).

Case 10—A 27-year-old male (body height 175 cm, body weight 65 kg) with a history of narcotic abuse was reported to be agitated and aggressive, possibly delirious. He died unexpectedly after having received moderate concussions during a tussle. A perimortem blood sample from a neck vein was obtained within 1 h after death. A medicolegal autopsy was carried out two days postmortem. No pathological lesions were revealed and only superficial skin abrasions were found. The results of toxicological analyses of femoral blood sampled at autopsy are outlined in Table 2. In addition buprenorphin and norbuprenorphin were found in the urine. The postmortem femoral to perimortem neck vein blood drug concentration ratios were 1.6 for amphetamine and 0.4 for tetrahydrocannabinol. The cause of death was considered to be due to amphetamine overdose.

Animal Studies

The six rats that were administered tetrahydrocannabinol had postmortem to antemortem tetrahydrocannabinol concentration ratios in heart blood of 1.2 ± 0.1 (mean ± s.e.m.), but the increase was not significant (Table 3). There was a significant fall in the drug concentration postmortem in blood from the inferior vena cava, and the mean inferior vena cava to antemortem heart blood concentration ratio was 0.6 ± 0.1 ($p < 0.05$, Wilcoxon).

Discussion

When comparing plasma and blood drug concentrations as in the present study it is important to keep in mind that several drugs distribute disproportionately in these two matrices (13). Chloroquine has been reported to have a plasma to blood concentration ratio of 0.1 to 0.2, and a platelet to plasma ratio of 400 (14,15). These ratios are variable, and consequently analysis of whole blood is recommended for this drug. Drugs like carbamazepine, diazepam and tetrahydrocannabinol are mainly found in the plasma fraction of blood, and hence are reported to have plasma to blood ratios of 1.6 to 1.8 (16). For tricyclic antidepressants the distribution in blood has been found to be influenced by temperature, pH, drug concentration (17), the concentration of α-1-glycoprotein (18), age, sex, disease, presence of other drugs, storage conditions and age of the specimen (19). Generally, the plasma to blood ratio for tricyclic antidepressants is relatively close to unity and possible inequalities can account for only a small fraction of the differences observed in this study.

When pooling the results of tricyclic antidepressants in the present study, the median postmortem heart blood to antemortem serum drug concentration ratio ($n = 4$ substances from cases 4 and 7) was 6.7, the 95% reference range being 6.1 to 13 (Table 4, first column). These figures, being based on only two cases, must obviously be interpreted with care. The median postmortem femoral blood to ante- or perimortem serum drug concentration ratio ($n = 11$ substances from case 1, 2, 3, 5, 6 and 7) was 3.3, the 95% reference range being 1.1 to 6.0 (Table 5, first column). The pooling of the results is based on the fact that tricyclic antidepressants are pharmacologically closely related with similar apparent volume of distribution and pharmacokinetic profiles. Studies on heart blood to femoral blood concentration ratios in man indicate that these substances have closely related postmortem redistribution characteristics (1,2). Amitriptyline and nortriptyline exhibited similar postmortem redistribution properties as many other basic lipophilic drugs with comparable apparent volume of distribution in previous rat studies (7,8).

The concentration changes postmortem in this study were compared with five human case reports from Prouty and Anderson (1) where the tricyclic antidepressant drug concentration in an early sample of heart blood (so-called field blood) is related to the concentration in heart blood and femoral blood from the autopsy. The drugs involved were amitriptyline, nortriptyline, doxepin and nordoxepin. From those data the median heart blood to field blood drug concentration ratio was 6.3, the 95% reference range being 1.6 to 16 ($n = 8$ substances) (Table 4, second column). The median femoral to field blood drug concentration ratio was 1.7, the 95% reference range being 1.2 to 2.4 ($n = 6$ substances) (Table 5, second column). The figures obtained in the present study corroborate reasonably well with that report (1). The main reason for the higher heart blood to antemortem serum concentration ratios seen in the present study could be due to the fact that postmortem redistribution from lungs to heart blood occurs early, and it is likely that the field blood obtained in at least some cases in the previous study has been subject to early changes by the time of sampling, as it may in case 3 in the present study. In cases 2, 3, 5, 6 and 7 in

TABLE 4—*The postmortem heart blood to antemortem serum drug concentration ratio found in cases 4 and 7 (*n = *4 tricyclic antidepressant substances) in the present study, postmortem heart blood to early field blood drug concentration ratio (*n = *8 tricyclic antidepressant substances in five cases) from Prouty and Anderson (1), postmortem heart blood to antemortem blood amitriptyline and nortriptyline concentration ratios from rats (*n = *54 substances in 27 rats) (8) and postmortem heart blood to antemortem blood amitriptyline and 10-hydroxy-amitriptyline concentration ratios in pigs (*n = *12 substances in six pigs) (6) expressed as median, lower and upper quartiles and 95% reference range (pooled data).*

	Postmortem Heart Blood to Antemortem Serum Drug Conc. Ratio in the Present Study	Postmortem Heart Blood to Early Field Blood Drug Conc. Ratio (1)	Postmortem Heart to Antemortem Blood Drug Conc. Ratios from Rats (8)	Postmortem Heart to Antemortem Blood Drug Conc. Ratios from Pigs (6)
2.5 percentile	6.1	1.6	1.5	1.3
25 percentile	6.5	3.4	2.4	1.5
Median	6.7	6.3	3.4	1.9
75 percentile	8.4	8.9	5.3	3.3
97.5 percentile	13	16	10	4.8

TABLE 5—*The postmortem femoral blood to ante- or perimortem serum drug concentration ratio for the tricyclic antidepressants found in cases 1, 2, 3, 5, 6 and 7 (*n = *11 tricyclic antidepressant substances) in the present study, postmortem femoral blood to early field blood drug concentration ratio (*n = *6 tricyclic antidepressant substances in four cases) from Prouty and Anderson (1), postmortem abdominal inferior vena cava to antemortem heart blood amitriptyline and nortriptyline concentration ratios from rats (*n = *54 substances in 27 rats) (8) and postmortem femoral blood to antemortem blood amitriptyline and 10-hydroxy-amitriptyline concentration ratios in pigs (*n = *12 substances in six pigs) (6) expressed as median, lower and upper quartiles and 95% reference range (pooled data).*

	Postmortem Femoral Blood to Antemortem Serum Drug Conc. Ratio in the Present Study	Postmortem Femoral Blood to Early Field Blood Drug Conc. Ratio (1)	Postmortem Vena Cava to Antemortem Blood Drug Conc. Ratios from Rats (8)	Postmortem Femoral to Antemortem Blood Drug Conc. Ratios from Pigs (6)
2.5 percentile	1.1	1.2	0.7	1.5
25 percentile	2.8	1.2	1.4	2.7
Median	3.3	1.7	1.7	3.6
75 percentile	4.1	2.0	3.0	6.8
97.5 percentile	6.0	2.4	8.0	11

the present study resuscitation was attempted, and it has previously been shown that resuscitation reduces the postmortem heart to femoral drug concentration ratio (1). The reason for this is probably due to mixing of blood, and when done after drug release from lungs and other sources have started, resuscitation will probably cause both a reduction in the drug concentration in the heart blood and an elevation in peripheral blood. This fact may in part explain the lower postmortem heart to field blood concentration ratios seen in some of the cases in the previous study (1), as it may also explain the higher postmortem femoral blood to antemortem serum concentration ratios seen in the present study.

The postmortem concentration changes of amitriptyline and the main metabolite nortriptyline in a previous experimental rat study are included for comparison (8) (Table 4, third column). The rats were administered amitriptyline orally and heart blood and blood from the abdominal vena cava were investigated with postmortem intervals of 1, 2, 5, 10 and 24 h ($n = 22$). The postmortem drug concentration changes in heart blood observed in that study were close to what was found in the human studies, but the maximum changes observed were lower, possibly due to shorter postmortem interval and smaller body size. The concentration changes observed in the abdominal vena cava in rats were in the same order as the changes observed in human femoral blood, indicating that these samples in this experimental model are reasonably representative for human femoral blood samples (Table 5, third column).

The postmortem concentration changes reported for heart blood and femoral blood in six pigs (6) are also included for comparison (Tables 4 and 5, fourth column). In that experiment three pigs were administered amitriptyline orally and three intravenously, and changes were observed at 96 h postmortem for both amitriptyline and the main metabolite 10-hydroxy-amitriptyline. The concentration changes observed postmortem in the pig study differed from the other studies in that the drug concentration in femoral blood was significantly higher than the concentration in heart blood. The reason for this peculiarity is not known, but shows that the results obtained in the larger animal are not necessarily more representative for the changes observed in man.

Verapamil has an apparent volume of distribution of ~5.5 L/kg, and in a previous rat study the mean postmortem to antemortem heart blood verapamil concentration ratio was 2.7 ± 0.4, while the mean inferior vena cava to antemortem verapamil concentration ratio was 2.0 ± 0.3 (9). In case 7 the postmortem heart blood to antemortem serum verapamil concentration ratio of 5.4 is significantly higher, while the postmortem femoral blood to antemortem serum concentration ratio of 1.5 is comparable to the rat study.

In case 8 there is a striking postmortem concentration increase of chloroquine in the femoral blood sample. Chloroquine has an apparent volume of distribution of ~200 L/kg when estimated from whole blood data and up to 800 L/kg when estimated from plasma concentrations, and an elimination half-life of 20 to 60 days (14). The drug is highly concentrated in tissues like liver, kidney, lung and brain, and is highly toxic in overdoses (20). Due to extensive postmortem redistribution it is recommended that postmortem analyses and interpretations are done in liver tissue (21). Experiments in rats confirmed the high tissue concentrations, and chloroquine was found to redistribute significantly more than nortriptyline postmortem (9). Larger tissue to blood concentration

ratios are seen during chronic treatment (22), and hence more pronounced postmortem redistribution may be expected. The initial fall in plasma concentration in case 8 indicates distribution phase and at time of death high tissue concentrations were likely.

Amphetamine has an apparent volume of distribution of ~4.2 L/kg and has been reported to have higher heart blood than femoral blood concentrations (1). Such concentration differences are reported also for methamphetamine (23,24). In a previous rat experiment the mean postmortem vena cava to antemortem heart blood amphetamine concentration ratio was 2.3 ± 0.3 (9), in line with the findings in cases 9 and 10.

To our knowledge there are no previous reports on postmortem concentration changes for tetrahydrocannabinol, the main psychoactive substance of cannabis. The apparent volume of distribution has been reported as high as 25–41 L/kg (25). After inhalation high blood concentrations are reached within minutes, and over the next few hours distribution to tissues occurs with a marked decrease in blood concentrations. Tissue concentrations are highest in lungs, liver, heart, brain and liver (26). The considerable postmortem fall observed in the blood concentration in case 10 may have several explanations. Tetrahydrocannabinol has been shown to be unstable in polystyrene containers (11), and enzymatic or bacterial substance degradation may also play a role (27). The antemortem interval could be of importance, as one of the main factors behind this phenomenon is thought to be the tissue to blood drug concentration ratio. During the distribution phase the arterial blood drug concentration can be appreciably higher than the venous, so also during life sampling site is of importance (28). Accordingly, the tissue to blood drug concentration ratio will increase until distribution equilibrium is achieved and the elimination phase ensues. Consequently, if death occurs early after drug administration, the tissue to blood drug concentration ratio is lower than at distribution equilibrium, and there is less potential for tissue drug release or even continued tissue uptake (6,29). The significant postmortem fall in the concentration of tetrahydrocannabinol in the blood from the inferior vena cava in the present rat study is in agreement with case 9, but has not been observed for amitriptyline or other drugs previously tested in similar experimental rat models (8,9,30). The reason for this is unclear, but the above-mentioned mechanisms probably play a role. Putrefaction is, however, less likely in the present rat study due to the short postmortem interval. In heart blood counteracting drug release may have caused the postmortem drug concentration to be relatively equal to the antemortem values. A similar phenomenon has previously been reported for tranylcypromine, where postmortem drug redistribution was counteracted by putrefactive breakdown of the drug (31). In man the most common route of tetrahydrocannabinol administration is by inhalation, while in the present animal study the drug was administered orally. This may affect the tissue distribution of the drug, especially to the lungs and liver, and accordingly the postmortem redistribution phenomena may also be affected. The relatively low concentration of tetrahydrocannabinol found in vitreous humour could well be due to the high plasma protein binding (94 to 99%) and the subsequent low free fraction available for equilibrating with this tissue (Table 3) (16).

The sampling technique is important, due to the large concentration gradients found in the blood. The so-called siphon effect causes blood from distant vessels to approach the sampling site during sampling, and clamping obviously will counteract this effect. Pounder has advocated clamping of the femoral vessels proximal to the sampling site, and sampling with a syringe and needle (4). Clamping was undertaken when sampling blood from the rats, but is not routinely done in medico-legal autopsies in Norway. Recently a Swedish compilation of reference drug concentrations has been published (32), and in that study the femoral blood sampling was performed by a fairly similar method to the one used in the present study, and our results should therefore be comparable.

The present study confirms that the drug concentration in femoral blood is closer to the antemortem level than cardiac blood. However, the concentration found in femoral blood cannot be considered representative for the antemortem value, but has to be compared to reference values from femoral blood (32) or corrected with values like those in Table 5. The wide ranges between the 2.5 and 97.5 percentile indicates with which level of precision one can estimate the antemortem concentration from a postmortem sample. In many cases circumstantial evidence indicates whether death was due to an intoxication. However, in rare cases, e.g., when there is a suspicion of a health worker having administered an overdose, great care must be taken. If the evidence is based on only one sample of postmortem blood, this could prove to be too scanty. In such cases we recommend that several blood and tissue samples be taken, as the confidence with which a diagnosis of overdose is made, will increase with the number of different samples analyzed. The drug concentration postmortem is probably more stable in tissues like skeletal muscle, and hence this may well be a suitable matrix for postmortem drug analysis (33).

We conclude that there is large variability in the concentration changes seen postmortem, also in femoral blood samples. Accordingly, the drug concentration found in femoral blood cannot be accepted unreserved as being representative for the antemortem level. The experimental rat model presented can be useful for estimating whether a drug is subject to postmortem redistribution, and the extent of changes observed are reasonably comparable to those observed in humans. If a diagnosis of drug intoxication is to be made with a high degree of certainty, mainly relying on the results of drug analysis, it is advisable to obtain several blood and tissue samples for analyses.

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