Thor Hilberg,[1] M.D.; Åse Ripel;[1] Adrian J. Smith, Ph.D.,[2] D.V.M.; Lars Slørdal,[1] Ph.D., M.D.; Jørg Mørland,[1] Ph.D., M.D.; and Anders Bjørneboe,[1] Ph.D, M.D.

Postmortem Amitriptyline Pharmacokinetics in Pigs after Oral and Intravenous Routes of Administration

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ABSTRACT: In this study we have evaluated the postmortem pharmacokinetics of amitriptyline (Ami) and metabolites in pigs after oral and intravenous administration, and the results are compared with previous studies in rats and humans. In addition a meticulous investigation of blood and tissue concentrations after postmortem intravenous infusion of Ami was undertaken. Of a total of 9 overnight fasted pigs, 3 were given 25 mg/Kg Ami orally, and another 3 pigs received an intravenous infusion lasting 1 h of 3.3 mg/Kg Ami prior to death. The final 3 pigs were sacrificed and then given the intravenous infusion after death. After ~5 h at room temperature, all carcasses were subsequently stored at 4–5°C. Postmortem blood samples were collected at 0.25, 1, 2, 4, 8, 24, 48, and 96 h through an indwelling intracardial needle. Postmortem examination with blood and tissue sampling was performed 96 h after death. Analysis was carried out by high performance liquid chromatography with ultraviolet detection. Postmortem blood samples from the heart of the orally dosed animals revealed large and variable concentration increases of 99(30–243)% for Ami and 96(52–429)% for the main metabolite 10-OH-Ami at 96 h. In the intravenously infused live pigs heart blood Ami increased by 55(33–69)% and 10-OH-Ami increased by 232(76–240)%. Blood from the atria had significantly higher Ami concentrations than blood from both ventricles in the animals dosed while alive, and the drug concentration in femoral blood was higher than in heart blood ($p < 0.01$). In the orally dosed pigs the left lobe of the liver had significantly higher Ami levels than the right lobe.

Tissue/blood Ami concentration ratios were generally lower than previously reported in rats and approximating the levels reported in humans. The animals infused intravenously after death demonstrated high drug levels in blood samples from central vessels, heart, lungs as well as cerebrospinal fluid and vitreous humour. This implies that the presence of a lethal concentration of a drug in just one sample of heart blood can prove worthless in a case where agonal drug infusion may have occurred.

KEYWORDS: forensic science, postmortem redistribution, tricyclic antidepressants, amitriptyline pharmacokinetics, forensic toxicology, swine, amitriptyline metabolism, tissue distribution

In autopsies one has to consider the possibility that the drug concentration found may not be representative for the concentration while still alive, and postmortem artefacts such as drug redistribution between tissues and blood can seriously hamper interpretation in such cases (1,2). In previous experiments rats have been used to establish the relationship between postmortem interval and postmortem drug concentration changes (3–5). However, because of the size of the animal compared to man the representativeness of such results has been questioned, and we therefore wanted to study postmortem pharmacokinetics in a larger animal (6).

Hospital deaths and medical mishaps frequently instigate forensic investigations (7). If a patient dies while receiving an intravenous infusion, the infusion may continue and thereby cause a high drug concentration in the venous blood in proximity to the infusion site. If toxicological analysis reveals high drug concentrations in blood and tissue samples taken at autopsy, this could have serious consequences for the staff involved.

The aim of this study was to observe the pharmacokinetics of amitriptyline and its metabolites after oral and intravenous administration, both before and after death. Furthermore, we wanted to examine the distribution of the drug to tissues, to establish which tissue samples, if any, were most representative of the antemortem blood drug concentrations, and to compare the results with previous experiments in rats. The experimental design was chosen to investigate the relative contribution from one set of animals with a gastrointestinal depot of drug, another group of animals without such a depot, and finally one set of animals with solely an intravenous depot of drug.

Materials and Methods

Animal Experiments

Three sets of experiments were performed in a total of nine overnight fasted pigs (Norwegian Landrace) with a median body weight of 38 (31–62) Kg. In experiment A, three pigs received 25 mg amitriptyline hydrochloride per Kg body weight dissolved in 30 mL water through an oropharyngeal tube. Blood samples were collected every 15 minutes for the first hour and then every 30 minutes for the next three hours from either an indwelling catheter in the ear or from the jugular vein. After 4 hours, alphaxalone/alphadolone anaesthesia was administered and the animals were sacrificed with 10 mL of 3 M potassium chloride solution injected through an indwelling intravenous cannula in the ear. After death an intracardial needle with mandrin (spinal needle) was introduced through the chest wall ~10 cm inferior to the clavicle just to the left of the sternum. Dual postmortem intracardial blood samples of 0.2 mL were collected at 0.25, 1, 2, 4, 8, 24, 48, and 96 h postmortem in Eppendorf tubes containing 50 μL potassium fluoride. In addition, serial samples of about 0.5 g were taken at 4, 24, 48, and 96 h from thigh muscle and subcutaneous

[1]National Institute of Forensic Toxicology, PO Box 495 Sentrum, N-0105 Oslo, Norway.
[2]Laboratory Animal Unit, Norwegian College of Veterinary Medicine, PO Box 8146 Dep., N-0033 Oslo, Norway.
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fat. Sternal bone marrow was sampled at 4 h postmortem using a trochar and cannula. About two hours after sacrifice, the pigs were transported and placed in dorsal recumbency in a cooling box with a temperature of 4–5°C. A complete postmortem examination was performed at 96 h, and samples were collected meticulously from the sites listed in Table 1. Cerebrospinal fluid was collected from the cisterna magna by posterior midline puncture just beneath the skull base.

In experiment B three pigs received an intravenous infusion of 3.3 mg/Kg amitriptyline in 100 mL 0.9% sodium chloride administered through an ear vein cannula and slowly infused over one hour. Blood were sampled every half hour until sacrifice at two hours. Postmortem blood and tissue samples were obtained as in experiment A.

In experiment C three pigs were sacrificed as above and within 3 minutes, an intravenous infusion similar to experiment B was administered in the right earlobe while the animal was lying in the left lateral position. Samples of heart blood were collected at 8, 24, 48, and 96 h postmortem, sternal bone marrow at 8 h and postmortem examination was undertaken at 96 h as above.

Extraction, Chromatography and Analytical Procedure

The acetonitrile was of HPLC grade (Fisons, UK), and all the other chemicals were of analytical grade. Amitriptyline hydrochloride, nortriptyline hydrochloride, desmethyl-nortriptyline hydrochloride, Z- (cis-) and E- (trans-) 10-OH-amitriptyline, and Z- and E-10-OH-nortriptyline maleate were all supplied by H. Lundbeck A/S, Denmark and trimipramine maleate by Rhône-Poulenc Rorer, France. Borate buffer was made of saturated sodium tetraborate in water adjusted to pH 11 with 6 M sodium hydroxide. Potassium fluoride solution was made of 67% w/v of KF in water.

All samples were weighed after sampling and stored at −20°C until analysis. Tissue samples were homogenized with an Ultra-Turrax T5 homogenizer (IKA, Janke & Kunkel, Germany) in borate buffer pH 11 to a final concentration of 0.2 g tissue/mL homogenate. Standard curves were prepared by spiking drug-free blood or homogenized liver with amitriptyline hydrochloride and metabolites. Gastric contents were diluted (1:9) with water. To 200 μL blood was added 200 μL borate buffer and 40 μL of an aqueous solution of the internal standard trimipramine, and to 200 μL of tissue homogenate only the internal standard solution was added. The mixture was extracted on a mixer for 10 minutes with 800 μL of 2% 2-butanol in hexane (v/v), and centrifuged for 10 min at 740 g. The organic phase was transferred to 1.1 mL autosampler vials, evaporated at 40°C under vacuum (Buchler Vortex Evaporator, USA), and the residue was reconstituted with 100 μL of the mobile phase. The liquid chromatograph consisted of a Hewlett Packard, USA, Series 1050 pump and the following units from Shimadzu Corp., Japan: SIL 9A autosampler, SPD 10A variable UV detector, and C-R4AX Chromatopac integrator. Chromatographic separation was performed at ambient temperature by a 25 cm × 4.6 mm ID column packed with 5 μm LC-Si (Supelco, USA). The mobile phase consisted of 10% v/v 0.025 M ammonium acetate in acetonitrile. The flow rate was 3 mL/min, and the injection volume 20 μL. The UV-detector was set at 230 nm. The calibration curves were linear and determined from 4 standards analyzed in duplicate at the beginning and in the end of each series with a minimum correlation coefficient of 0.99. Calibration curves were prepared for each run of analyses. Drug concentrations in blood, fluids and bone marrow samples were determined from a calibration curve prepared from blood, while tissue concentrations were determined from a calibration curve prepared from liver

TABLE 1—*The concentration of amitriptyline in samples taken at autopsy 96 h after sacrifice expressed as median values and ranges (μmol/L or μmol/Kg).*

	Orally dosed live pigs		Intravenously dosed live pigs		Intravenously dosed dead pigs	
	Median	(Range)	Median	(Range)	Median	(Range)
Blood right atrium	1.3	(1.0–2.3)	0.7	(0.6–0.7)	174	(82.8–198)
Blood right ventricle	0.8	(0.4–1.0)	0.6	(0.4–0.6)	74.8	(50.7–209)
Blood left atrium	1.3	(0.6–1.6)	0.6	(0.5–0.8)	95.2	(2.1–195)
Blood left ventricle	0.3	(0.3–0.4)	0.3	(0.3–0.7)	0.5	(0.3–1.4)
Blood right jugular vein	1.6	(1.0–2.8)	0.7	(0.5–1.2)	266	(178–366)
Blood left jugular vein	1.4	(1.2–2.1)	1.0	(0.3–2.6)	10.3	(2.2–31.9)
Blood both femoral veins	2.7	(1.7–6.3)	1.2	(1.1–1.5)	0.3	(0–0.7)
Blood inferior vena cava	2.0	(1.6–2.4)	1.1	(0.7–1.9)	100	(51.7–149)
Cerebrospinal fluid	1.1	(0.6–1.1)	0.9	(0.4–1.0)	22.6	(11.2–24.9)
Vitreous humour both eyes	0.8	(0.5–1.5)	0.4	(0.2–0.7)	0.2	(0–0.8)
Pericardial fluid	1.4	(0.9–2.1)	1.2	(1.0–1.3)	65.8	(33.5–68.6)
Pleural fluid	3.2	(1.7–3.6)	2.1	(1.7–2.5)	8.2	(4.5–46.0)
Peritoneal fluid	10.1	(7.8–12.3)	1.7	(1.5–1.9)	0.2	(0.2–0.3)
Bone marrow femur	2.2	(1.2–7.5)	0.5	(0.1–2.2)	0	(0–0)
Bone marrow sternum	1.9	(0.4–1.9)	0.2	(0.2–3.6)	1.5	(0.7–2.7)
Right lung apex	8.4	(6.6–16.8)	7.9	(7.1–14.2)	33.2	(28.9–73.7)
Right lung base	7.9	(7.1–10.2)	7.1	(2.0–21.7)	10.7	(5.2–11.5)
Left lung apex	7.6	(4.7–14.4)	9.0	(8.8–10.9)	6.7	(1.6–17.5)
Left lung base	10.6	(6.8–23.3)	6.7	(6.3–30.8)	3.5	(1.2–5.5)
Myocardium right ventricle	1.4	(0.9–3.0)	1.1	(<0.2–1.9)	92.9	(55.6–194)
Myocardium left ventricle	1.4	(0.6–1.5)	0.9	(0.5–1.6)	6.5	(2.3–9.5)
Liver right lobe	5.3	(4.4–5.4)	3.5	(2.8–5.5)	3.2	(0.3–7.4)
Liver left lobe	13.6	(6.4–61.8)	3.6	(3.4–6.0)	0	(0–3.1)
Kidney left	6.3	(2.7–12.2)	3.8	(3.4–8.8)	0	(0–0)
Brain occipital lobe	2.8	(2.3–5.6)	2.6	(2.2–7.6)	1.8	(0.8–3.1)
Gastric contents	304	(107–413)	4.8	(4.3–19.9)	0	(0–0)
Thigh muscle	0.7	(0.2–1.8)	0.2	(<0.2–0.9)	0	(0–0)
Subcutaneous fat thigh	0	(0–0)	<0.2	(0–<0.2)	0	(0–0)

tissue. Recovery from blood exceeded 88% for amitriptyline, 68% for N-desmethyl-nortriptyline, 81% for nortriptyline, 80% for Z- and E-10-OH-amitriptyline and 67% for 10-OH-nortriptyline (whose Z- and E-enantiomers were not separated) at concentrations of 1.0 and 10 μmol/L.[3] Recovery from liver tissue exceeded 91% for amitriptyline, 66% for N-desmethyl-nortriptyline, 89% for nortriptyline, 79% for 10-OH-amitriptyline and 70% for 10-OH-nortriptyline at concentrations of 1.0 and 10 μmol/Kg. The detection limit for these substances were 0.04 μmol/L in blood and 0.2 μmol/Kg in liver tissue. The calibration ranges were from 0.24 to 30 μmol/L in blood and from 1.2 to 150 μmol/Kg in tissue. The within-run coefficient of variation (CV) for amitriptyline was less than 5% in blood and liver tissue, and the corresponding CVs for the metabolites were typically around 10%. Linearity was excellent up to 1000 μmol/L ($R^2 \geqslant 0.999$). All samples were analyzed in duplicate and the mean value is reported. Results are presented as median (range) or as mean ± standard error of the mean in μmol/L or μmol/Kg.

Results

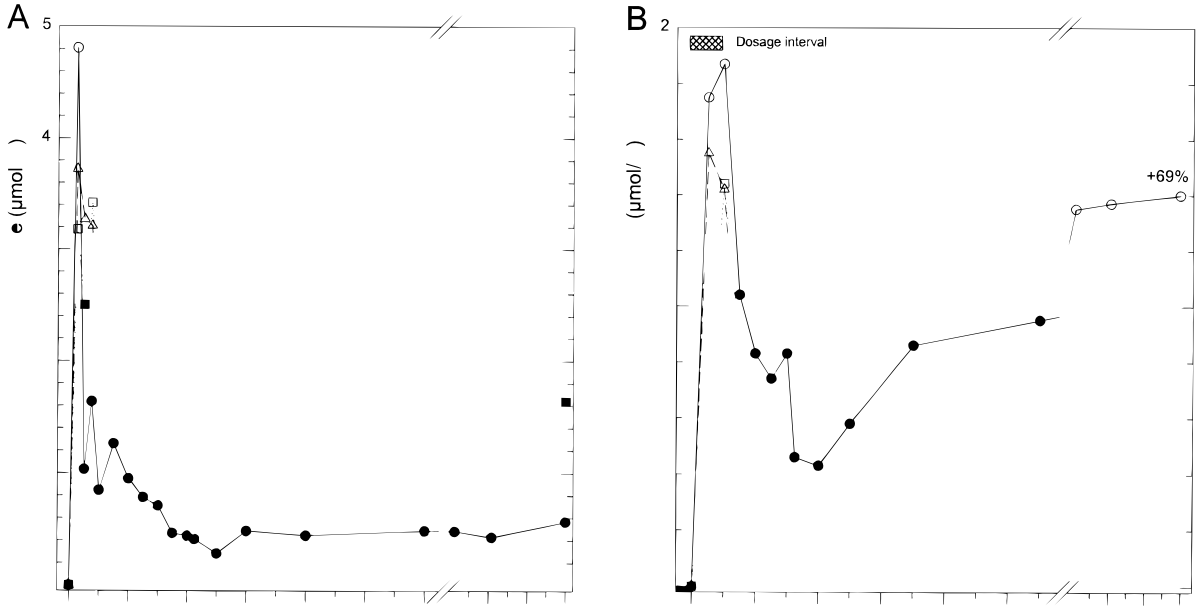
The live dosed pigs became moderately sedated after drug administration, but seemed otherwise unaffected by the drug.

In the orally dosed pigs, maximum blood concentration was reached early and within 15 minutes in two animals (Fig. 1A). In the samples obtained postmortem through the intracardial needle, there was a variable concentration rise and at 96 h postmortem the concentration was 99(30–243)% higher than the last corresponding antemortem blood value (0.8(0.4–1.0) μmol/L). The concentration of 10-OH-amitriptyline in blood increased fairly rapidly after drug administration (Fig. 2A) and at time of sacrifice was 189(95–193)% of the concentration of amitriptyline. At 96 h postmortem the concentration was 96(52–429)% higher than the last antemortem value (0.9(0.8–1.5) μmol/L).

Table 1 demonstrates that after oral dosing the highest amitriptyline concentrations were found in the gastric contents, corresponding to a total of 32(10–49) mg amitriptyline hydrochloride, representing 1–5% of the total dose. The highest blood drug concentrations were found in femoral blood, while the lowest concentrations were in the cardiac ventricles. Among the tissues, the left lobe of the liver, the lungs and kidney together with the peritoneal fluid had the highest concentrations. The left lobe of the liver had significantly higher concentrations than the right lobe in the orally dosed pigs ($p < 0.05$, Mann-Whitney test). The lower lobe of the left lung had higher drug levels than the upper lobe in all of the three orally dosed animals, but the differences did not reach statistical significance.

The intravenously infused live pigs had lower maximum blood amitriptyline concentrations than the orally dosed pigs (Fig. 1B), and the concentrations of amitriptyline in the blood obtained through the intracardial needles at 96 h postmortem was 55(33–69)% higher than the last live blood sample (0.4(0.4–0.8) μmol/L). There was a drop in the level of amitriptyline in the first postmortem sample in these animals of 42(33–45)%, but this occurred only to a minor extent with the metabolites. Figure 2B demonstrates that the concentration of 10-OH-amitriptyline was 77(31–92)% of amitriptyline at time of sacrifice. At 96 h postmortem the concentrations of this 10-hydroxylated metabolite had risen to 232(76–240)% higher than the last antemortem value (0.3(0.3–0.4) μmol/L). At autopsy, the highest blood drug levels also in this series of animals were measured in specimens from the femoral veins (Table 1). Among the tissues, the lungs, liver and kidney had the highest levels.

When comparing the two series of live dosed animals, both atria had significantly higher amitriptyline concentrations than the respective ventricles in all six animals ($p < 0.05$, Mann-Whitney



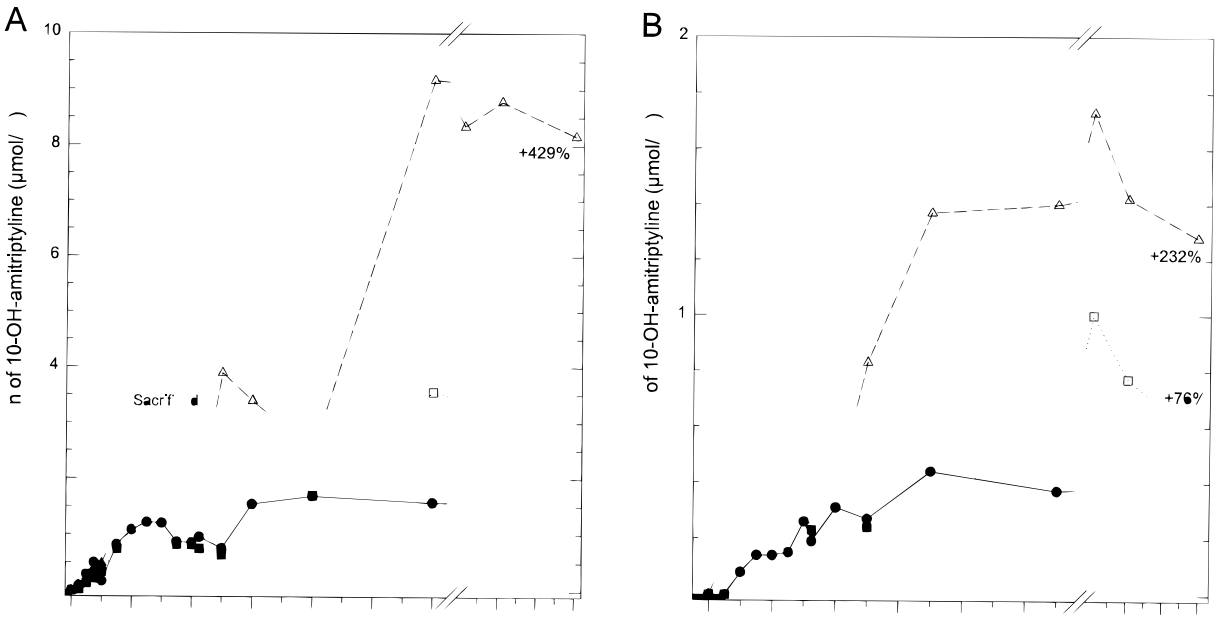

A
10
8
6
4
n of 10-OH-amitriptyline (µmol/)
+429%
Sacrif
B
2
1
of 10-OH-amitriptyline (µmol/)
+232%
+76%

TABLE 2—*The postmortem tissue to antemortem (last sample before sacrifice) blood amitriptyline and 10-OH-amitriptyline concentration ratios in declining order of representativeness for the antemortem blood amitriptyline concentration for both orally and intravenously dosed live pigs (pooled data) unless otherwise stated (mean ± standard error of the mean).*

Postmortem Sample	Postmortem Tissue/Antemortem Blood Amitriptyline Concentration Ratio (Mean ± s.e.m.)	Postmortem Tissue/Antemortem Blood 10-OH-Amitriptyline Concentration Ratio (Mean ± s.e.m.)
Vitreous humour	1.0 ± 0.1	2.1 ± 0.4
Blood from right ventricle	1.0 ± 0.1	1.7 ± 0.3
Thigh muscle	0.9 ± 0.2	1.1 ± 0.2
Blood from left ventricle	0.7 ± 0.2	1.5 ± 0.3
Blood from left atrium	1.4 ± 0.2	2.7 ± 0.3
Cerebrospinal fluid	1.5 ± 0.3	1.7 ± 0.1
Blood from right atrium	1.8 ± 0.2	2.9 ± 0.4
Myocardium	2.1 ± 0.2	1.9 ± 0.5
Pericardial effusion fluid	2.2 ± 0.2	2.6 ± 0.3
Blood from jugular veins (mean)	2.2 ± 0.4	1.8 ± 0.3
Blood from thoracic inf. vena cava	2.5 ± 0.6	4.1 ± 1.2
Bone marrow from shaft of femur	3.1 ± 1.2	1.7 ± 0.6
Peritoneal fluid pigs infused iv.	3.5 ± 1.0	8.0 ± 2.4
Blood from femoral veins (mean)	4.0 ± 0.9	5.8 ± 1.5
Pleural effusion fluid	4.1 ± 0.5	4.5 ± 0.5
Cerebrum	6.0 ± 0.8	4.1 ± 1.3
Liver—right lobe	7.6 ± 1.0	17 ± 6.5
Liver—left lobe, pigs infused iv.	8.1 ± 0.5	27 ± 9.6
Left kidney	9.2 ± 0.9	13 ± 4.8
Peritoneal fluid, orally dosed pigs	11 ± 1.3	5.4 ± 2.8
Lungs (mean of 4—upper and lower, right and left)	17 ± 1.7	11 ± 4.1
Liver—left lobe, pigs dosed orally	36 ± 22	13 ± 7.2

TABLE 3—*The concentration of the different metabolites in blood and sampled tissues at autopsy from the six live-dosed animals expressed as percentage of the total concentration of the main metabolite 10-OH-amitriptyline (mean ± standard error of the mean).*

	E-10-OH-amitriptyline	10-OH-nortriptyline	Nortriptyline
Blood	14 ± 2%	14 ± 1%	3 ± 1%
Tissues	24 ± 4%	41 ± 6%	15 ± 4%

TABLE 4—*The concentration of amitriptyline in heart blood from three pigs infused intravenously after death, sampled through an indwelling intracardial needle expressed as median values and ranges (μmol/L).*

Hours Postmortem	Median	(Range)
8	111	(32.7–113)
24	71.9	(45.6–83.4)
48	39.0	(25.1–61.8)
96	51.8	(49.8–65.3)

The anaesthesia was administered of ethical reasons, because the potassium injection could be painful. The anaesthetic agents were chosen because of considerable experience with these agents in pigs and they have been tested for adverse responses in pigs (12). The possibility of pharmacokinetic interactions with amitriptyline was considered small. Potassium was chosen for sacrifice as it induces cardiac death rapidly. The heart stops in diastole (13), and it is possible that, e.g., death by ventricular fibrillation could have caused blood shifts that would have given different results, as postmortem left ventricular action has been shown to be of importance in postmortem blood flow in rabbits (14). Agonal and postmortem cardiac activity are unknown factors in most human deaths, and death due to asystole is certainly a possible death mechanism also in man.

At postmortem examination the intracardial needles were found to be situated in the right ventricles, and the blood inside the heart chambers was found to be mainly clotted. The blood obtained through the intracardial needles gradually became more serous and the samples collected at 8 h and later appeared like sanguineous serum. The concentration of amitriptyline and metabolites found in the intracardial samples at 96 h corresponded relatively poorly to the levels found in the blood sampled from the right ventricles. The blood clotted relatively early in the postmortem period and this is probably why the clotted blood is more representative for the antemortem blood concentrations. Accordingly, we made no observations to suggest that the samples have been contaminated with pericardial effusion fluid. During sampling of blood at autopsy some coagulated samples were also taken, and there was a tendency that these samples had a lower and more representative drug level with respect to the antemortem situation than the fluid blood, but this was not always the case. The potassium chloride solution used for sacrifice delayed blood clotting *in vitro*, but it is not known if this affects clotting in the body after death. If so, an alternative method of sacrifice should be recommended. The lower drug concentrations found in the ventricles could imply that when sampling blood from the heart one should try to get a clean ventricular sample.

We have previously presented data suggesting that a main cause of postmortem drug redistribution to central blood is release of drug, mainly from the lungs (5). The extent of drug redistribution has consequently been related to the tissue to blood drug concentration ratio, particularly in pulmonary tissue, which again is related

to the time between drug administration and death. In this study, no significant correlation was found between the lung to blood amitriptyline concentration ratio and the postmortem drug level increase. In previous experiments with rats that were given amitriptyline orally or subcutaneously, the postmortem drug level increase was more pronounced and occurred earlier than in the present study (3,5). In those rat experiments the lung to blood amitriptyline concentration ratio was in the order of 50 to 80. Accordingly, the more modest postmortem level increase seen in the present study could be due to the lower tissue to blood ratio in pigs (Table 2). There may also be other species differences (15). Another important difference is the storage temperature. Recently Pounder and Smith found that cadavers kept at 4°C had a slower diffusion of alcohol than those kept at room temperature, and this is probably also the case for larger molecules like antidepressant drugs (16). When collecting blood from a rat heart, blood is obviously also withdrawn from anatomically related vessels and lungs, due to a siphon effect. To avoid this, only small sample volumes (0.2 mL) were withdrawn from the heart in the present study. In smaller animals diffusion of drug directly from the gastric depot to surrounding tissues is pronounced and may lead to misinterpretations (4,17,18). This effect has also been reported in humans (19). In the orally dosed pigs this effect is only evident in the left lobe of the liver, and possibly to a limited extent in the lower left lobe of the lungs.

The mean heart to femoral blood concentration ratio in the six pigs dosed while alive were 0.37 ± 0.05. This is contrary to previous findings in both experimental animals and human cases (1,3,5,20). However, Teige and Kveseth found that the drug level was higher in femoral than atrial heart blood in 39 of a total of 115 human cases (21). Other reasons for high femoral blood drug levels could be postmortem diffusion of drug from vessel walls or the kidneys. A control experiment was performed in one of the orally dosed pigs by ligating an earlobe vein at two sites 5 cm apart immediately after death. The blood contents of this vein was sampled at autopsy and the amitriptyline concentration was found to have increased by 38% compared to the last antemortem venous sample. Previously it has been reported that vessel walls in humans do not contribute to the postmortem concentration increase seen for digoxin, however the two drugs may well behave differently (22). The kidneys have a relatively high drug concentration, are highly vascular (23) and could be a major source of drug for postmortem release. In this study there was no significant correlation between the postmortem drug concentration increase in femoral blood and the drug concentration in the kidney. Consequently, we do not know the exact reason for the high femoral blood drug level seen in these experiments.

Among the samples obtained at autopsy, the vitreous humour proved to be the most representative of the last antemortem blood amitriptyline concentration and having the least variability of all samples in animals dosed orally and intravenously while alive (Table 2). However, it is noteworthy that the mean level of 10-OH-amitriptyline in vitreous humour was more than twice the antemortem blood level. Also in rats injected with amitriptyline subcutaneously vitreous humour was a reasonably representative tissue for the antemortem situation, but the drug concentration was on average more than twice the concentration in antemortem blood (5). Tricyclic antidepressant concentrations in vitreous humour has been found to constitute about 10% of those in serum at steady state (24). Similarly, the concentration of amitriptyline in cerebrospinal fluid in nine human cases surviving intoxication corresponded with the unbound fraction of drug in plasma, which was 3–8% of total blood amitriptyline (25). Vitreous humour and cerebrospinal fluid imipramine concentrations of about 60 to 80% of the concentrations in blood have been reported in two cases of suicidal overdose (2,26), while a vitreous humour to blood ratio of more than 7 has been reported for amitriptyline (27). Increased levels of digoxin in vitreous humour after death has been found in dogs, due to dissociation from its primary binding sites in the choroid-retina (28). These reports corroborate our results, indicating extensive postmortem diffusion to both vitreous humour and cerebrospinal fluid. The drug concentration ratio between brain tissue and cerebrospinal fluid are probably in the order of 30 to 60, indicative of an obvious potential for postmortem diffusion. The results from the pigs infused after death confirm that postmortem diffusion to these compartments may well occur.

The liver to blood amitriptyline concentration ratios for the right lobe were not significantly different between the intravenously and orally dosed animals. This is in contrast to previous findings in rats where subcutaneously injected rats had relatively low liver to blood amitriptyline concentration ratios in the magnitude of 2 to 3 and the orally dosed rats had very high liver to blood concentration ratios in the order of 330 to 590 (3,5).

Putrefactive fluids like pericardial, pleural and peritoneal effusions have also been suggested as possible specimens suitable for drug and alcohol analyses (16,29,30). In these experiments the pericardial fluid was more representative of the antemortem blood drug concentration than pleural effusion, probably reflecting the much lower drug concentration in the myocardium than in the lungs. Peritoneal effusion had high drug concentrations even in the intravenously infused animals, possibly due to diffusion from the liver. There was, however, no relationship between the drug concentration in liver and peritoneal effusion in this study. Myocardium is another tissue that may be used for drug analysis, and Bailey and Shaw have found a mean myocardium to blood amitriptyline ratio of 4.6 ± 4.6 (mean $\pm$ standard deviation) in 79 drug-related deaths (31). In the present experimental model myocardium appears to be fairly representative (Table 2). In the rat experiments, however, the mean myocardium to blood amitriptyline concentration ratios were in the order of 14 to 17 (3,5).

The drug concentrations in the serial muscle samples did not change significantly with time, and the variation seen in this study probably reflects more of a site-to-site variability. The mean drug concentration in muscle was close to antemortem blood, and muscle proved to be the most representative specimen in all groups in the present study. This is consistent with previous findings in humans (32). The mean muscle to antemortem blood drug concentration ratios in rats were, however, in the order of 7 to 10 (3,5). The very low concentrations found in adipose tissue is in accordance with a previous report in rats (33).

Bone marrow has also been suggested as a target tissue for postmortem drug analysis (34,35). Winek reported bone marrow to blood nortriptyline concentration ratios of 26 to 34 in rabbits after dosage for five days. The chronic dosage regimen and species differences could explain the different results obtained from the present study (Table 2). The early postmortem samples of sternal bone marrow correlated poorly with late sternal and femoral marrow samples. There was large variability in these samples and in the pigs dosed while alive bone marrow was not a good matrix neither for analysis nor interpretation.

The animals infused intravenously postmortem showed a fairly rapid distribution of drug to the heart as the concentration peaked

during the first 7 h after infusion (Table 4). The range of concentrations decreased as postmortem interval increased ($p < 0.05$, regression analysis), probably due to the drug being more evenly distributed with time and approaching a distribution equilibrium (5). Several reports have demonstrated postmortem blood flow or convection. Accordingly, it is not surprising that drug molecules originally present in drug-rich blood in a peripheral vein will appear in other parts of the cardiovascular system (14,36). However, the extent of drug diffusion into tissues such as lungs, liver, myocardium, brain, sternal bone marrow, cerebrospinal fluid and even vitreous humour was unexpected. Consequently, these tissues cannot be relied upon solely for toxicological analysis in cases where agonal transfusion is suspected (2,27,37,38). In this experiment the muscle samples from thigh, kidneys and bone marrow from femur appeared to be representative for the *in vivo* situation. The relatively low drug concentrations found in femoral blood samples compared to heart blood in this series of animals is in agreement with previous findings that the peripheral sample is a more representative specimen, and sampling should be as far as possible from the site of administration.

The hydrostatic pressure needed for the infusion to run postmortem was about 40 cm above heart level. Accordingly, if death occurs while an intravenous infusion is running, the infusion will probably continue after death. It could be argued that amitriptyline is not a drug that is given intravenously, nor are ear veins used for this purpose in man. However, corresponding results have been obtained in two pigs that received meperidine (pethidine), and where the infusion was given through a foreleg vein (unpublished data). We therefore think that the findings in this part of the experiment may well be representative for many drugs infused into a peripheral vein.

Conclusions

The concentration of drug in any blood or tissue sample postmortem will be a function of the antemortem value and the net result of blood and fluid convection and diffusion between the different tissues. These experiments shed light on the relative contribution of these factors in an experimental animal model which exhibits postmortem drug redistribution characteristics that in several respects are closer to man than what is suggested by previous rat experiments. Tissue concentrations in pigs are more representative for human cases than the rat model, and the rate of postmortem drug concentration increase is probably also closer to human cases. The wide variability between individuals seen in both rats and humans seems to be reproducible also in pigs. Thigh muscle represented the best overall sample in this study, but also in these specimens there were some variation. The high drug concentration found in the femoral blood samples in the animals administered amitriptyline while alive may not corroborate findings in rat and man. When dealing with cases where agonal infusion may have occurred, we recommend obtaining several blood and tissue samples such as femoral and heart blood, peripheral muscle, liver and kidney. Our results indicate that the presence of a lethal concentration of a drug in just one sample of heart blood can prove worthless in a case where agonal drug infusion may have occurred.

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Additional information and reprint requests:
Thor Hilberg, M.D.
National Institute of Forensic Toxicology
PO Box 495 Sentrum
N-0105 Oslo
Norway