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An Animal Model of Postmortem Amitriptyline Redistribution

REFERENCE: Hilberg, T., Bugge, A., Beylich, K-M., Ingum, J., Bjørneboe, A., and Mørland, J., **"An Animal Model of Postmortem Amitriptyline Redistribution,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 1, January 1993, pp. 81–90.

ABSTRACT: An experimental rat model was developed to study postmortem changes of drug concentration after an acute overdose. Overnight fasted rats were fed 75 mg of amitriptyline (AMI). Two h after dosing, the rats were anaesthetized and blood samples were drawn from the femoral vein (peripheral blood—PB) and the heart (HB). The rats were sacrificed by CO_2 and left at room temperature for either 0.1, 0.5, 1, 2, 5, 10, 24, 48, or 96 hours, when samples of heart blood, blood from the inferior vena cava (PB) and tissue samples from different liver lobes, heart, lungs, kidney, thigh muscle, and brain were taken. Samples were analyzed by high performance liquid chromatography. The AMI concentration in HB increased fairly rapidly within the first 2 h postmortem and from then the average ratio was 6.4 ± 0.8 (mean ± sem) (n = 31). In PB, the post/antemortem AMI concentration ratio followed an approximately exponential rise; at 2 h postmortem the ratio was 1.6 ± 0.3 (n = 5), and at 96 h 55.1 ± 23.8 (n = 4). For the main metabolite nortriptyline (NOR), the concentration changes followed the same pattern, but to a lesser extent. Among the tissues, the liver lobes had high, but variable drug concentrations; lobes lying closest to the stomach had the highest drug concentrations. The drug concentration in the lungs declined significantly. This animal model demonstrates postmortem drug concentration changes similar to those described in humans. Probable mechanisms include drug diffusion from the stomach and GI tract to the surrounding tissues and blood; and postmortem drug release from the lungs and possibly other drug-rich tissues into the blood.

KEYWORDS: toxicology, toxicology postmortem, blood-drug concentration, tissue-drug concentration, tricyclic antidepressants

When there is a suspicion of a drug overdose, determination of drug concentration in whole blood is commonly used to verify the cause of death. In 1980, Bandt reported that the postmortem blood tricyclic antidepressant drug concentration might be several-fold higher than at the time of death [*1*]. Several other reports have confirmed this finding [*2*,*3*], and the phenomenon has been referred to as postmortem changes of drug concentration or postmortem drug redistribution. The postmortem change of drug concentration may create difficulties for the forensic toxicologist in the interpretation of how

Received for publication 17 March 1992; accepted for publication 1 June 1992.

Presented in part at the 28th Meeting of the International Association of Forensic Toxicologists, Perth, Australia, October 1990.

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much drug the decedent took. However, it has been suggested that taking several blood samples from different sites, and also tissue samples, will make the interpretation more reliable [*4–6*].

To study this phenomenon in more detail, we examined postmortem redistribution of amitriptyline in an animal model using rats fed amitriptyline by intragastric tube. The purpose was to evaluate when and to what extent these changes in amitriptyline concentration occurred, if the site of blood sampling affected the result, if there was a change in the concentration of the main metabolite nortriptyline, and if the changes also occurred in tissues such as liver, heart, lungs and skeletal muscle.

Materials and Methods

Chemicals and Reagents

Glycine buffer was made of 51.2 parts of an aqueous solution of 1 mole/L glycine (Sigma) and 1 mole/L NaCl and 48.8 parts of 1 M NaOH, adjusted to pH = 11. Potassium fluoride solution was made of 67% w/v of KF in water. The acetonitrile was of HPLC grade (Fisons, UK), all the other chemicals were of analytical grade. Amitriptyline hydrochloride and nortriptyline hydrochloride were obtained from H. Lundbeck A/S, Denmark and trimipramine maleate from Rhône-Poulenc, France.

Animal Studies

Male Wistar rats, body weight of 250 to 350 g, were fasted overnight and fed 75 mg of amitriptyline hydrochloride dissolved in 2 mL of water by a gastric tube. Two hours after dosing, the rats were anaesthetized with a subcutaneous injection of fluanisone, fentanyl and midazolam (5 mg, 0.1 mg and 2.5 mg per kg rat, respectively). Two blood samples (≈100 μL) were drawn from the femoral vein and transferred to Eppendorf tubes containing 25 μL of KF solution. Then a sample of heart blood (≈300 μL) was obtained and divided into three aliquots. Additional blood samples obtained before sacrifice were taken in some experiments and kept in tubes at room temperature as controls. The rats were then sacrificed with CO_2 and were left at room temperature for one of the following time periods: 0.1, 0.5, 1, 2, 5, 10, 24, 48, or 96 h. Postmortem heart blood samples (≈300 μL divided into three aliquots) were drawn after having clamped off the inferior vena cava just above the diaphragm. Peripheral blood (≈100 μL) from the inferior vena cava was then taken after clamping off just superior to the renal veins. The following tissue samples were also collected. From all animals (n = 41): The heart as well as a piece (≈0.4 g) from the inferior part of the left lateral lobe of the liver were taken. From 17 animals: seven samples from all lobes of the liver as in Fig. 1. Samples from right and left lungs (n = 20), right kidney (n = 13), thigh muscle (n = 15) and brain (n = 10) were also taken.

Extraction, Chromatography and Analytical Procedure

The liquid chromatograph used was an integrated system from Shimadzu, Japan, consisting of a LC 7A pump, a SIL 6B auto-sampler, a SPD 6A variable UV detector, a SCL-6B system controller, and a C-R4AX Chromatopac integrator. Chromatography was performed at room temperature on 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. Flow was 3 mL/min isocratic, and the injection volume 20 μL. The UV-detector was set at 230 nm. Tissue samples were homogenized with a Ultra-Turrax T5 homogenizer (IKA, Janke & Kunkel, FRG) in glycine buffer to a final con-

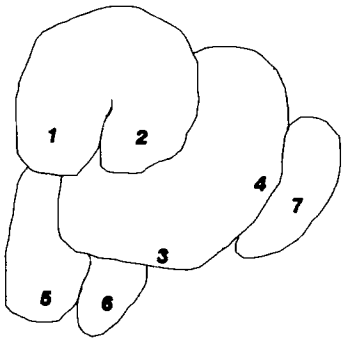

FIG. 1—*Schematic drawing of the rat liver. The numbers correspond to the samples taken.*

centration of 0.2 g tissue per mL homogenate. Standard curves were prepared by spiking drug free blood or homogenized liver with ami- and nortriptyline hydrochloride. All tissue concentrations were determined from a liver standard curve as this was found satisfactory within ±20%. To 100 μL of blood or 100 μL of tissue homogenate was added 100 μL of an aqueous solution of the internal standard trimipramine and 75 μL glycine buffer. The mixture was extracted on a tilt mixer for 10 min with 800 μL of 2% 2-butanol in hexane, and centrifuged for 10 min at 740 g. The organic phase was transferred to 1.1 mL autosampler vials, evaporated at 40°C in vacuum (Buchler Vortex Evaporator, USA), and the residue was reconstituted with 100 μL of the mobile phase. Recovery obtained in blood was 78% for amitriptyline and 80% for nortriptyline at a concentration of 5 μmoles/L. The detection limit in blood was 0.3 μmol/L for both amitriptyline and nortriptyline. The data are given as mean ± sem and statistics have been performed by regression analysis using Minitab release 7.1, Minitab Statistical Software, USA, unless otherwise specified. Results with $P < 0.05$ has been considered significant.

Results

The blood concentrations of amitriptyline and nortriptyline are outlined in Table 1. The ratios between postmortem inferior vena cava concentration and antemortem femoral vein concentration (both representing peripheral blood) at various times after death for amitriptyline and nortriptyline gives a highly significant concentration increase with time ($P < 0.01$) (Fig. 2). The ratios between postmortem and antemortem heart blood drug concentrations also show a significant increase with time ($P < 0.01$) (Fig. 3). The corresponding plots for nortriptyline were analogous, but the values were 36 ± 7% and 27 ± 5% lower than for amitriptyline, respectively (data not shown). The shape of the curves are different in that the drug concentration increase in heart blood occurs mainly within the first 2 h postmortem and thereafter shows a flattening, whilst in peripheral blood the increase occurs mainly after 5 h postmortem.

The liver had the highest drug concentration of the tissues investigated (Table 2). There was however a striking difference in drug concentration within the liver; the lobes lying closest to the stomach, the left lateral part of the left lobe and the caudate lobe, having the highest concentrations (Fig. 1 and Table 3). We also observed large variations within the same liver lobe. Furthermore, the concentration of amitriptyline found in the liver lobes shows a significant rise ($P < 0.01$) with increasing time after death (Fig. 4). There was no significant rise in the concentration of nortriptyline in the liver (data not shown). The concentration of both amitriptyline and nortriptyline in the lungs showed a significant

TABLE 1—*The concentration of amitriptyline and nortriptyline in heart blood and femoral vein blood antemortem and heart blood and blood from the inferior vena cava at different postmortem intervals in μmoles/liter.*

Time after death (h)	*n*	Amitriptyline				Nortriptyline			
		Heart blood concentration		Inferior vena cava concentration		Heart blood concentration		Inferior vena cava concentration	
		Median	(Range)	Median	(Range)	Median	(Range)	Median	(Range)
Antemortem	39	3.3	(0.5–49.4)	2.7	(0.6–13.8)[a]	1.6	(0.3–9.7)	1.3	(0.4–12.5)
0.1	3	10.8	(6.9–13.4)	5.5	(3.1–9.4)	2.4	(2.1–2.5)	1.7	(1.1–2.0)
0.5	3	8.1	(2.0–33.5)	4.9	(1.6–8.9)	2.6	(1.3–3.1)	1.5	(1.1–2.2)
1	3	11.1	(4.3–68.9)	3.6	(2.8–16.6)	3.9	(1.6–7.1)	1.8	(0.7–1.9)
2	6	18.7	(2.2–98.1)	8.9	(3.5–15.2)	3.2	(1.2–9.4)	1.7	(0.9–4.7)
5	6	8.9	(2.8–36.8)	5.0	(2.4–32.4)	2.5	(1.2–5.5)	1.4	(0.6–4.0)
10	5	11.0	(1.7–54.1)	12.3	(3.7–100)	3.1	(0.8–15.1)	3.9	(1.2–10.3)
24	4	14.4	(4.0–28.3)	16.8	(2.7–30.9)	3.6	(2.7–7.0)	3.3	(0.9–4.9)
48	3	15.6	(10.1–104)	14.8	(5.2–23.7)	5.8	(1.9–8.6)	2.3	(1.4–3.1)
96	4	44.3	(11.7–132)	140	(45–1160)	10.5	(4.4–19.5)	13.2	(2.9–104)

[a]Femoral blood.

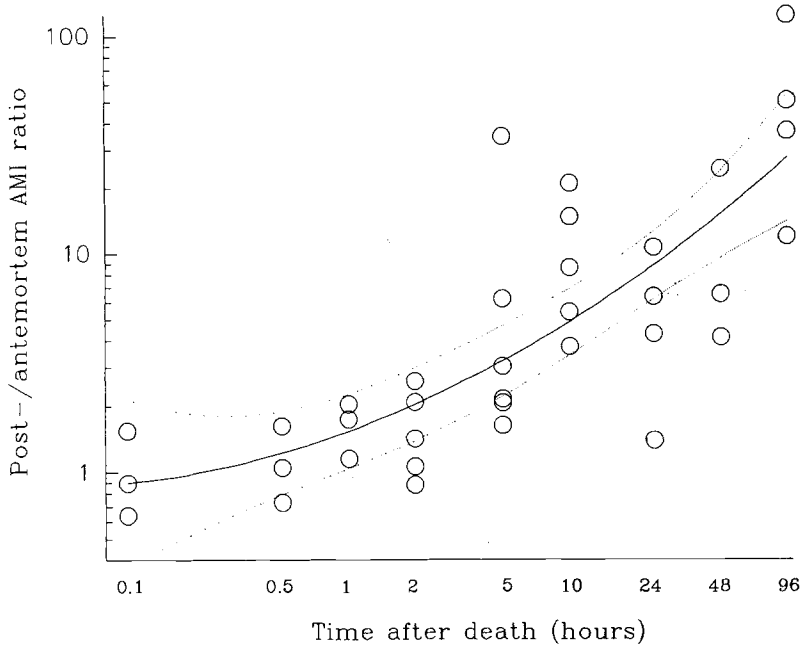

FIG. 2—*The ratio between postmortem inferior vena cava and antemortem femoral vein blood concentration of amitriptyline vs. time after death. Note double logarithmic scale. Regression line with 95% confidence interval (c.i.).*

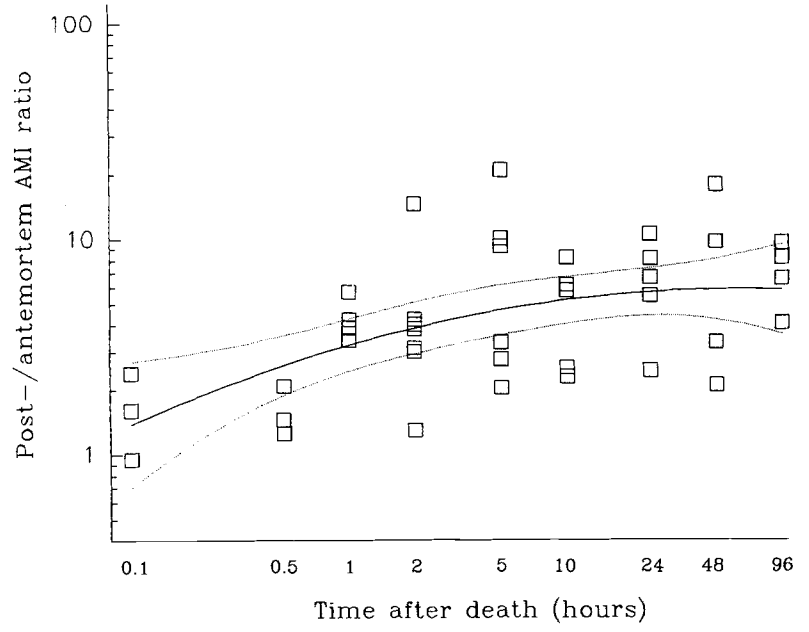

FIG. 3—*The ratio between postmortem and antemortem heart blood concentration of amitriptyline vs. time after death. Note double logarithmic scale. Regression line with 95% c.i.*

TABLE 2—*The ratio between postmortem tissue and antemortem femoral or heart blood drug concentration.*

Tissue/blood ratio	*n*	Ratio AMI (mean ± sem)	Ratio NOR (mean ± sem)
Brain/femoral blood	10	31.4 ± 10.0	11.8 ± 4.5
Heart/antemortem heart blood	41	15.5 ± 1.5	11.6 ± 0.9
Kidney/femoral blood	13	48.4 ± 8.9	36.0 ± 9.9
Liver[a]/femoral blood	41	460 ± 125	45.8 ± 5.9
Lungs/femoral blood	20	70.3 ± 13.9	47.0 ± 8.8
Thigh muscle/femoral blood	15	10.2 ± 2.6	7.5 ± 2.1

Values are pooled for all postmortem intervals.
[a]The inferior part of the left lobe.

TABLE 3—*Average concentration of amitriptyline and notriptyline in different liver lobes in μMoles/KG. Pooled data.*

Liver lobe	*n*	AMI (mean ± sem)	NOR (mean ± sem)
Median lobe, right part	17	814 ± 300	91.2 ± 22.3
Median lobe, left part	17	1308 ± 824	123.8 ± 23.7
Left lobe, inferior part	41	1700 ± 652	56.4 ± 7.5
Left lobe, left lat. part	17	8256 ± 2785	136.7 ± 31.2
Right lobe, right part	17	2532 ± 1171	97.5 ± 17.9
Right lobe, left part	17	759 ± 256	134.8 ± 52.8
Caudate lobe	17	6471 ± 1624	117.8 ± 22.4

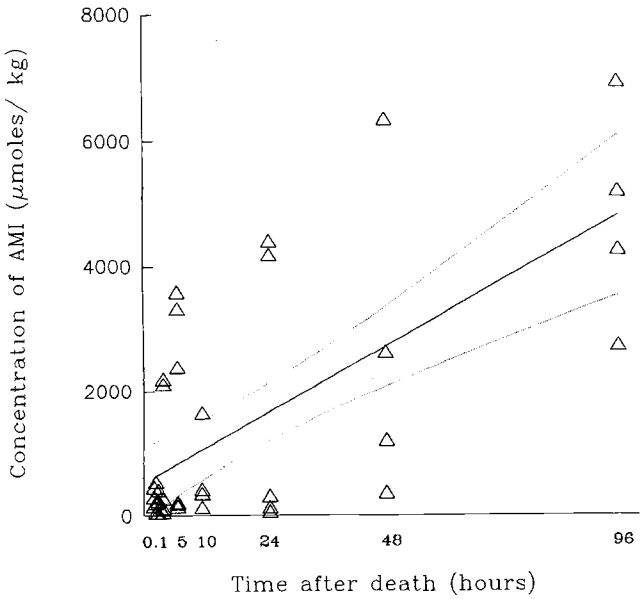

FIG. 4—*The concentration of amitriptyline in the liver (mean for all lobes) in μmoles/kg vs. time after death. Regression lines with 95% c.i.*

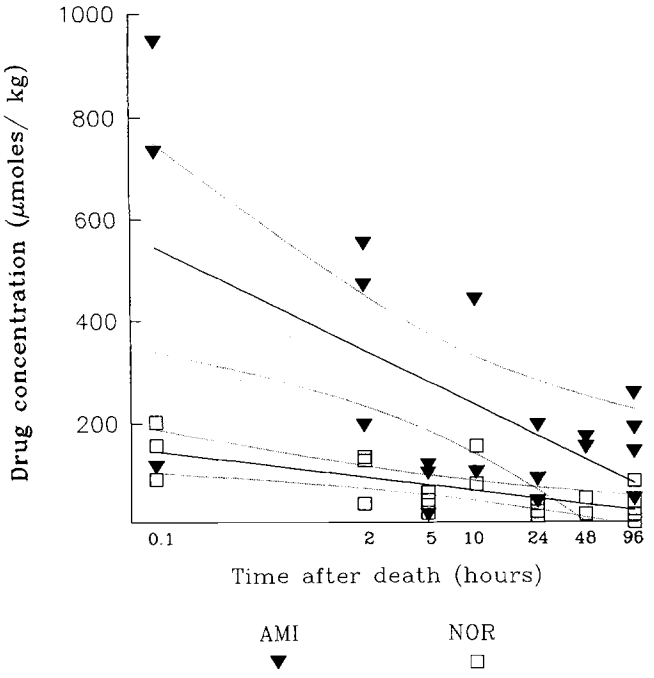

FIG. 5—*The average concentration of amitriptyline and nortriptyline in both lungs vs. time after death. Note semilogarithmic scale. Regression lines with 95% c.i.*

decrease with time ($P < 0.01$) (Fig. 5). For the other tissues; heart, brain, kidney and thigh muscle no significant changes with time were found (data not shown). Postmortem tissue to antemortem blood drug concentration are listed in Table 2.

Discussion

This animal model was designed to mimic an acute intoxication in humans. At time of death due to overdose, there is usually a variable amount of unabsorbed drug in the stomach [2]. The dosage chosen of 75 mg amitriptyline, corresponding to about 250 mg/kg, represents a half of LD_{50} for rats [7]. This is very high compared to the human therapeutic dosage of up to 300 mg daily [8]. Two h after dosing was chosen as time of sacrifice, because preliminary studies had demonstrated that the animals then were still in the absorption phase. Accordingly, at time of dissection, the stomach was noted to be large and filled with aqueous material.

At time of sacrifice, the clinical state of the animals was variable. Three animals died before the period of 2 h had passed and were omitted from the study. About one quarter of the animals were in respiratory distress. Adult respiratory distress syndrome has been described in some cases of tricyclic antidepressant intoxication [9], might have a bearing on the state of these animals. Most animals seemed lethargic, but some appeared unaffected by the drug. The clinical state of the animals has not been related to the concentrations measured.

Sacrifice by CO_2 asphyxiation was chosen, as it has previously been shown not to give histopathologic changes [10] or to affect the postmortem pH changes [11]. Furthermore, in liver slices pH changes have been shown to have a limited influence on the in vitro

loss of amitriptyline [*12*], indicating that pH change is not of crucial importance to the changes in drug concentration seen in this study.

We found that this experimental animal model demonstrates postmortem increases of the concentration of amitriptyline and the main metabolite nortriptyline in both heart blood and blood from the inferior vena cava, thus mimicking what has been found in humans. There was also a significant increase of amitriptyline concentration in the liver, while there was a significant decrease in the drug concentration in the lungs.

As reported in humans, there were wide interindividual variations in the drug concentrations both before and after death [*13,14*]. The earlier postmortem rise (within 2 h postmortem) in the heart blood of both the amitriptyline and nortriptyline concentration as compared to the blood from the inferior vena cava (after 5 h postmortem), is in our opinion due to close relationship of the heart to the lungs. When withdrawing 300 μL of blood from a rat heart, blood from the large central vessels are also obtained. The lungs were found to have high initial concentrations of both amitriptyline and nortriptyline (Fig. 5). The lungs in man have also been shown to have particularly high levels of tricyclic antidepressants [*5,15,16*]. The lungs are also the most vascular tissue in the body [*17*], and this might facilitate the release of drug from tissue to blood. To our knowledge this is the first report that demonstrates postmortem release of drugs from a tissue by demonstrating an actual decrease in tissue concentration. Another possible mechanism might be diffusion of drug from the myocardium to the heart blood. The ratio between the concentration of amitriptyline in myocardium and the postmortem heart blood was 4.2 ± 0.5 and was greater than one in all but one rat. There was no significant change in this ratio with time. Contamination from abdominal vessels can be ruled out due to the clamping of the inferior vena cava prior to the blood sampling.

The greater rise in concentration of amitriptyline in the inferior vena cava at 96 h postmortem compared to heart blood, suggests a mechanism that is not present in the heart blood at this stage. One possibility is diffusion of amitriptyline from the GI tract [*18*]. The close anatomical relationship between the small intestine and the inferior vena cava in the area where the blood sample was taken, underscores this [*19*].

As control, blood samples obtained before sacrifice and liver samples obtained immediately after sacrifice, were kept at room temperature up to 96 h. These samples did not demonstrate any change in amitriptyline or nortriptyline concentrations, thereby excluding the possibility of higher recovery from stored blood and tissue.

Redistribution of drug down a concentration gradient from solid organs into the blood, has been suggested as a mechanism for the blood concentration changes seen [*20*]. The significant increase in the concentration of amitriptyline in the liver mainly after 48 to 96 h, could challenge this theory. However, the late rise seen in these tissues might be due to passive diffusion, which would also explain the wide site-to-site concentration range seen in the liver, the lobes lying closest to the stomach having the highest drug concentration. This has not previously been reported (Table 3), and seems to occur mainly with amitriptyline, and not with the metabolite. Passive diffusion of amitriptyline directly from the stomach seems to occur also when the animal is alive, as these differences are also found in the animals dissected at 0.1 h after death. However, different rates of blood perfusion between the different liver lobes may possibly contribute to the differences seen. Apple and Bandt [*3*] have proposed using drug concentrations in the liver instead of blood to establish the manner of death. Using the ratio between a tricyclic antidepressant drug and its major metabolite in liver has also been suggested [*21*]. These approaches would be influenced very much by local tissue concentration differences within the liver. Skeletal muscle has also been suggested as a suitable specimen for drug analysis [*22*]. Our data confirms that there is little change between ante- and postmortem drug concentration in thigh muscle. However, the drug concentration in skeletal muscle is different (Table 2), and might have a different time profile [*23,24*] from that in blood, so that new reference values will have to be established.

If the findings in this experimental rat model mirror similar processes in man, one can draw the following conclusions: the blood sample for toxicological analysis should be a pure peripheral sample, that is, femoral blood without contamination from the abdominal cavity, blood from the inferior vena cava or the lungs; liver samples should be from a part that is not in close anatomical relationship to the GI tract, for example, the lateral part of the right lobe; peripheral skeletal muscle might be a useful specimen, but further studies should be undertaken to establish the toxicokinetics of this tissue.

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