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Diffusion as a mechanism of postmortem drug redistribution: an experimental study in rats

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Summary. In some cases of drug overdose there is a reservoir of unabsorbed drug in the stomach and gut. Furthermore, agonal aspiration might establish a second reservoir in the lungs. Two experimental rat models were used to study if diffusion from these reservoirs could contribute to the phenomenon of postmortem drug redistribution. Overnight fasted rats were sacrificed by CO_2 and 75 mg of amitriptyline (AMI) was administered by a gastric tube. In the first series ($n = 19$), the tubes were removed after AMI administration. In the second series ($n = 17$), the trachea was ligated and cut prior to drug administration to prevent airways contamination. The rats were left at room temperature on their back for a period of 5, 10, 24, 48, 96 up to 192 h and samples of heart blood, blood from the inferior vena cava, tissue samples from heart, lungs, different liver lobes, kidney and psoas muscle were taken. In both series of rats we observed that as early as 5 h postmortem increasing concentrations of amitriptyline were found in the liver lobes lying closest to the stomach. In rats where the trachea was not ligated, drug contamination of the lungs also resulted in an increase in drug concentration within 5 h in heart blood and heart muscle. In rats where the trachea had been ligated, amitriptyline was found in the lungs after 96 h postmortem. The main metabolite nortriptyline was also detected. We concluded that postmortem diffusion from the gastrointestinal tract could be a major mechanism behind the phenomenon of postmortem redistribution of drugs in human case material, and that agonal aspiration may be followed by a rapid increase in postmortem drug concentration in autopsy samples.

Key words: Postmortem – Diffusion – Blood-drug concentration – Tissue-drug concentration – Tricyclic antidepressants

Zusammenfassung. In einigen Fällen von Medikamenten-Dosierung gibt es ein Reservoir nicht-resorbierter Wirkstoffe im Magen und im Darm. Außerdem könnte eine agonale Aspiration zur Entstehung eines weiteren Reservoirs in den Lungen führen. Zwei experimentelle Tiermodelle mit Ratten wurden benutzt, um zu untersuchen, ob die Diffusion von diesen Reservoirs einen Beitrag leistet zu dem Phänomen der postmortalen Wirkstoff-Umverteilung. Während der Nacht hatten die Ratten keine Nahrung und wurden mit CO_2 getötet, und zusätzlich wurde eine Dosis von 75 mg Amitriptylin (AMI) durch einen Magenschlauch gegeben. In der ersten Serie ($n = 19$) wurden die Schläuche nach der AMI-Zuführung entfernt. In der zweiten Serie ($n = 17$) wurde die Trachea vor der Zuführung des Wirkstoffs ligiert und durchtrennt, um eine Kontaminierung der Luftwege zu verhindern. Die Ratten wurden bei Raumtemperatur auf dem Rücken liegen gelassen für Zeiträume von 5, 10, 24, 48, 96 bis zu 192 Stunden, und Proben des Herzblutes, von der unteren Hohlvene, Gewebsproben vom Herzen, den Lungen, verschiedenen Leberlappen, von der Niere und vom Psoasmuskel wurden entnommen. In beiden Serien beobachteten wir, daß bereits 5 Stunden postmortem ansteigende Konzentrationen von Amitriptylin in den Leberlappen gefunden wurden, welche am nächsten zum Magen liegen. Bei den Ratten, bei welchen die Trachea nicht ligiert wurde, führte die Kontamination der Lungen mit Wirkstoff innerhalb von 5 Stunden zu einem Anstieg des Medikamentes im Herzblut und im Herzmuskel. Bei Ratten, bei welchen die Trachea ligiert worden war, wurde Amitriptylin nach 96 Stunden in den Lungen gefunden. Der Hauptmetabolit Nortriptylin wurde auch gefunden. Wir schlußfolgern, daß die postmortale Diffusion vom Gastrointestinaltrakt ein Hauptmechanismus sein könnte, welcher verantwortlich ist für die postmortale Umverteilung von Wirkstoffen in menschlichen Todesfällen und daß eine agonale Aspiration von einem raschen Anstieg der postmortalen Wirkstoffkonzentration in Autopsieproben gefolgt wird.

Schlüsselwörter: Postmortal – Diffusion – Wirkstoffkonzentration im Blut – Wirkstoffkonzentration in Geweben – Trizyklische Antidepressiva

Introduction

Forensic toxicologists must daily interpret the results of drug analyses performed on biological samples ob-

tained postmortem. However, postmortem concentration changes have been reported for several drugs both in man and experimental animals [1–3].

This phenomenon of postmortem drug redistribution may create difficulties when estimating the concentration of drug at the time of death and the amount of drug that was taken, and might also lead to erroneous conclusions with regards to the cause of death [4, 5]. The exact mechanisms for these changes have not been clearly established [2]. During our work with an experimental animal model using rats fed amitriptyline by a gastric tube in vivo, we observed marked postmortem drug concentration increases in blood and several organs. In the liver, the highest drug concentrations were found in the samples that were taken from regions close to the stomach [6]. In that study, as in human suicidal cases, a rather large drug dose was given perorally shortly before death. This might lead to the establishment of a drug reservoir in the stomach and/or gut which is not fully absorbed at the time of death. The aim of the present study was to observe the extent of passive drug diffusion from a drug reservoir in the stomach and duodenum during the postmortem period in rats. Furthermore, we wanted to see if postmortem contamination of airways could contribute to the postmortem increase in drug concentration.

Materials and methods

Two series of experiments were performed. Male Wistar rats, body weight 300–450 g, were fasted overnight and sacrificed with CO_2. Within 10 min after death 75 mg amitriptyline hydrochloride dissolved in 2 ml of water was administered by a gastric tube. In the first series of experiments ($n = 19$), the tube was withdrawn and the animals were left lying horizontally on their back at room temperature for 5, 10, 24, 48, or 96 h. After clamping the vena cava just above the diaphragm, heart blood samples (≈ 300 µl divided into 3 aliquots were taken. Blood (≈ 100 µl) from the inferior vena cava was taken after clamping the inferior vena cava below the liver. The following tissue samples were also collected: Right and left lungs, the heart, 4 samples from different lobes of the liver, right kidney and psoas muscle.

In the second series of experiments ($n = 17$), the same procedure was followed, except that after careful dissection of the neck, the trachea was ligated at 2 sites and cut in between before administration, and the gastric tube was left in place to further prevent regurgitation and aspiration. The data are given as median and range, and statistics have been performed by linear regression analysis using Minitab release 7.1, Minitab Statistical Software, USA, unless otherwise specified. Results with $P < 0.05$ were considered to be significant.

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 67% w/v in water. 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.

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 autosampler, 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. The flow rate 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 concentration of 0.2 g tissue/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 standard curve obtained using liver tissue as this was found satisfactory within ± 20%.

To 100 µl blood or 100 µl 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 minutes 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 under vacuum (Buchler Vortex Evaporator, USA), and the residue was reconstituted with 100 µl of the mobile phase. Recovery obtained from blood was 78% for amitriptyline and 80% for nortriptyline at a concentration of 5 µmole/l. The detection limit in blood was 0.3 µmole/l for both amitriptyline and nortriptyline.

Results

Rats without ligation of trachea ($n = 19$)

Amitriptyline was detected in both heart blood, heart muscle and blood from the inferior vena cava 5 h after administration (Tables 1 and 2). There was a significant correlation between the average drug concentration in the lungs and the drug concentration in heart blood ($R^2 = 0.23$, $P < 0.05$) and heart muscle ($R^2 = 0.23$, $P < 0.05$). The ratio between the concentration of amitriptyline in heart muscle and heart blood ranged from 0.03 to 10.4, and did not show any significant change with time. In the lungs, the concentration of amitriptyline was high, probably as a consequence of drug aspiration. There were no consistent differences between both lungs. In the liver, amitriptyline was detected 5 h after administration in the lobes adjoining the stomach, namely the caudate lobe (Lobe 7) and the left lateral part of the left lobe (Lobe 4) (Fig. 1). The highest drug concentrations were also found in these lobes. The right section of the right lobe (Lobe 1) situated furthest away from the stomach, had significantly lower drug concentrations than the caudate lobe ($P < 0.01$, sign test). When considering the average drug concentration in the different liver lobes, there was a significant increase with time ($R^2 = 0.44$, $P < 0.01$).

In the kidney, amitriptyline was detected from 10 h postadministration, but the concentrations varied considerably, ranging from 0 to 626 µmole/kg. In the psoas muscle, amitriptyline was detected in variable amounts from 48 h after administration, being detectable in 4 out of 7 animals.

Rats with ligated trachea ($n = 17$)

Amitriptyline appeared much later in the heart blood of rats with tracheal ligation than in those without. Only low concentrations were found 48–96 h postadministration (Table 1). Even after 8 days these rats had not

Table 1. The concentration of amitriptyline (in μmole/l) in heart blood and blood from the inferior vena cava at different postdosage intervals

Time after dosage (h)	Heart blood concentration						Inferior vena cava concentration					
	Non-ligated trachea			Ligated trachea			Non-ligated trachea			Ligated trachea		
	n	Median	(Range)	*n*	Median	(Range)	*n*	Median	(Range)	*n*	Median	(Range)
5	4	27	(20–610)	2	0	(0)	4	22	(0–66)	2	0	(0)
10	3	430	(0–560)	2	0	(0)	3	5.0	(0–460)	2	0	(0)
24	4	240	(20–900)	3	0	(0)	4	18	(15–36)	3	0	(0–0.7)
48	3	47	(18–400)	3	0.4	(0–2.2)	3	5.2	(0–16)	3	0.2	(0–7.7)
96	5	290	(13–1470)	4	0.7	(0–9.4)	5	260	(6–720)	4	3.5	(0–230)
192				3	3.0	(2.1–3.6)				3	104	(104)[a]

[a] Only possible to obtain blood from one animal in this group

Table 2. The concentration of amitriptyline (in μmole/kg) in heart muscle and lungs at different postdosage intervals

Time after dosage (h)	Heart muscle						Lungs (average)					
	Non-ligated trachea			Ligated trachea			Non-ligated trachea			Ligated trachea		
	n	Median	(Range)	*n*	Median	(Range)	*n*	Median	(Range)	*n*	Median	(Range)
5	4	4.0	(2.8–1140)	2	0	(0)	4	201	(1.8–9510)	2	0	(0)
10	3	62	(0–430)	2	0	(0)	3	20	(1.2–7750)	2	0	(0)
24	4	91	(1.0–1360)	3	0	(0)	4	240	(2.2–7940)	3	0	(0)
48	3	180	(1.5–860)	3	0	(0–2.7)	3	44	(16–1840)	3	0	(0–33)
96	5	290	(14–2630)	4	4.9	(0–7.0)	5	1200	(33–2420)	4	2.9	(0–9.9)
192				3	9.6	(2.4–13)				3	26	(8.4–53)

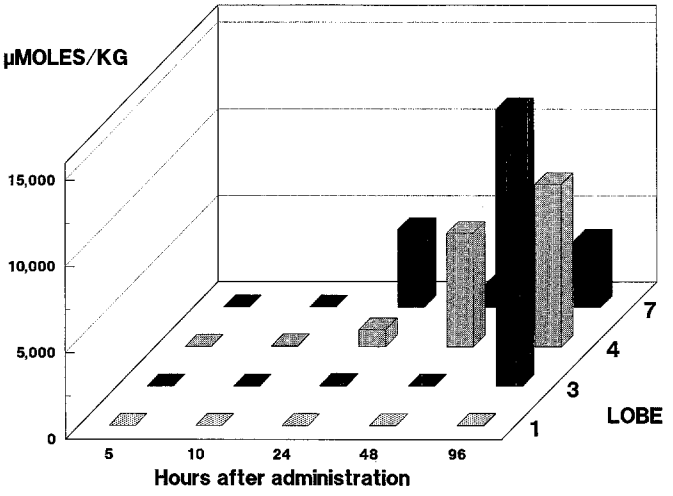

Fig. 1. The median concentration of amitriptyline in the different liver lobes from the non-ligated series of rats vs. hours after drug administration. Lobe 1 = Right part of right lobe; Lobe 3 = Inferior part of left lobe; Lobe 4 = Left lateral part of left lobe; Lobe 7 = Caudate lobe

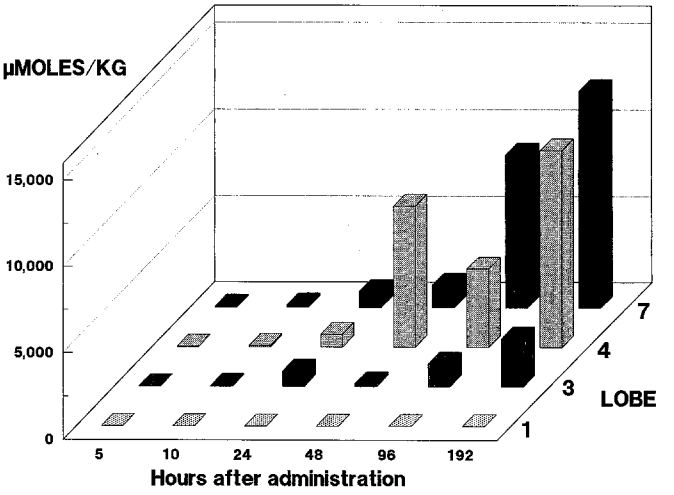

Fig. 2. The median concentration of amitriptyline in the different liver lobes from the ligated series of rats vs. hours after drug administration

reached the concentration which the first series had reached after 5 h. There was a significant increase with time (power regression $R^2 = 0.48$, $P < 0.05$). A similar picture was also seen in the blood from the inferior vena cava, with a significant increase with time ($R^2 = 0.27$, $P < 0.05$). In heart muscle, amitriptyline was detected only after 96 h (Table 2) ($R^2 = 0.61$, $P < 0.01$). The ratio between the concentration of amitriptyline in heart muscle and heart blood ranged in these animals from 0 to 14.9 and did not show any significant change with time. In the lungs, amitriptyline appeared only after 96 h postadministration (Table 2), and there were no significant differences between both lungs. In the liver, however, the pattern was much in accordance with the nonligated series of rats (Fig. 2) ($R^2 = 0.40$, $P < 0.01$). Amitriptyline was detected in the kidney from 24 h postadministration (0–102 μmole/kg), and in psoas muscle from 96 h

Table 3. The concentration of nortriptyline in blood and various organs as a percentage of the amitriptyline concentration

	Non-ligated trachea		Ligated trachea	
	n	Mean ± SEM	*n*	Mean ± SEM
Heart blood	15	1.3 ± 0.02	6	1.3 ± 0.01
Blood from inferior vena cava	7	1.7 ± 0.02	6	1.3 ± 0.01
Heart muscle	10	4.3 ± 1.0	7	–[a]
Lungs	13	6.2 ± 3.1	7	5.3 ± 3.0
Liver lobes (average)	14	2.2 ± 0.8	17	2.5 ± 0.5

Values are pooled for all postmortem intervals
SEM = standard error of the mean
[a] Below detection limit

(0–12 μmole/kg), in both cases somewhat later than in non-ligated rats.

The concentration of the main metabolite nortriptyline, when detectable (detection limit = 0.3 μmole/l in blood and 1.5 μmole/kg in solid tissues), exhibited a fairly constant proportion of the parent substance within the different tissues in both series (Table 3).

Discussion

Many mechanisms may underlie the postmortem increase in amitriptyline concentration observed previously [1, 2]. In these studies diffusion from a considerable reservoir of unabsorbed drug within the stomach und gut, and possibly also the lungs, could have contributed significantly to the gradual postmortem increase in the drug concentration observed. The present study was performed to test this possibility. By administering the drug to animals that were already dead, we could eliminate other major causes of postmortem drug concentration increase in blood and organs.

Our results thus demonstrated substantial redistribution of the drug. Previously little attention has been paid to the effects of passive diffusion of drugs postmortem. Parker et al. [7] reported a study of postmortem diffusion of secobarbital from the stomach to the liver in the rat. However, concentration gradients within the liver and diffusion to other parts of the animal were not studied. We were also able to demonstrate a marked difference between rats after ligation of the trachea and those without such ligation. The series of rats with non-ligated trachea served as an experimental model where there were 2 drug reservoirs; a primary source in the stomach and a variable secondary in the lungs. The results showed that postmortem contamination of the airways occurred in the present model, and that this process increased the postmortem drug concentration in most compartments. This confirms the findings of Pounder and Yonemitsu [8] on postmortem absorption of drugs and ethanol from the airways in an experimental human model. The lungs have a large surface area, thin diffusion membrane and a high vascularity that will favour diffusion of any aspirated drug. Therefore blood in the large central vessels and heart quickly equilibrates with the lungs. This is confirmed by the significant correlation between the concentration of amitriptyline in the lungs, heart blood ($P < 0.01$) and heart muscle ($P < 0.01$). The variable extent of airways contamination also explains the poorer correlation with time and the large variation in the non-ligated series.

Passive diffusion is described by Fick's first law of diffusion [9], which states that the rate of diffusion is proportional to the concentration gradient across the diffusion barrier. Tricyclic antidepressants have high but variable tissue binding affinities and have a high tissue to blood concentration ratio in vivo [10, 11], and also in vitro [12]. Accordingly, only the unbound fraction of the drug is available for diffusion and equilibration. One reason for the high amitriptyline concentrations found in these experiments, is that the permeability of the cell membranes increases in the postmortem period as the tissues and cellular membranes slowly disintegrate [13]. Furthermore, the rat is a small animal, consequently the diffusion distances between the different sampling sites are small.

The ligated series of rats served as a model where there was only one reservoir of drug. The drug concentration in the stomach was up to 135,000 μmole/l (37.5 mg/ml) and diffused from this site into the surrounding tissues and blood. Due to the thicker stomach wall compared to the alveolar membrane, the smaller surface area, the lower vascularity and possibly difference in the pH, the diffusion of drug from the stomach was much slower than from the lungs.

However, several other factors complicate this simplified model. Postmortem blood flux, as described by Fallani [14], may explain the variable ratios between the drug concentration in the myocardium and the heart blood found in both series. When the heart blood has a higher drug concentration than the myocardium, blood flow or diffusion along and within the large vessels, e.g. from the lungs, must account for this. On the other hand, when the myocardium has the higher drug concentration, diffusion directly from the stomach or lungs through the myocardium to heart blood is a reasonable explanation.

As putrefaction develops, increasing pressure in the lumen of the gastro-intestinal tract might force the contents out, and increasing abdominal pressure will tend to force drug-rich blood from the abdomen into the peripheral parts of the body. Putrefactive drug-rich fluid will appear in the thoracic and abdominal cavities [15]. Depending on body position and the manner in which the

body is handled, this may lead to a further spread of drug in the body [7]. Interestingly, some concentration of the main metabolite, nortriptyline, was formed postmortem, and accordingly care should be taken when using the metabolite : parent substance ratio to interpret the cause of death [16, 17]. The higher concentration of the metabolite in the lungs compared to the liver (Table 3), may be because this tissue has a high level of enzymes which continue to metabolize amitriptyline postmortem [18].

Depending on the concentration of drug in the lungs and stomach, and the period between death and autopsy, a substantial amount of drug can be absorbed and redistributed in the body postmortem. The extent of this is largely unpredictable, so that interpretation of how much drug was taken will be difficult. Further studies will be needed to explore this phenomenon in man.

References

1. Hebb JH, Caplan YH, Crooks CR, Mergner WJ (1982) Blood and tissue concentrations of tricyclic antidepressant drugs in post mortem cases: literature survey and a study of forty deaths. J Anal Toxicol 6:209–216
2. Anderson WH, Prouty RW (1989) Postmortem redistribution of drugs. In: Baselt RC (ed) Advances in analytical toxicology, vol 2, 3rd edn. Year Book Medical Publishers, Chicago London Boca Raton, pp 70–102
3. Sawyer W, Forney R (1988) Postmortem disposition of morphine in rats. Forensic Sci Int 38:259–273
4. Pounder DJ, Jones GR (1990) Post-mortem drug redistribution – a toxicological nightmare. Forensic Sci Int 44:117–123
5. Prouty RW, Anderson WH (1990) The forensic science implications of site and temporal influences on postmortem blood-drug concentrations. J Forensic Sci 39:243–270
6. Hilberg T, Bugge A, Beylich KM, Ingum J, Bjørneboe A, Mørland J (1992) An animal model of postmortem amitriptyline redistribution. J Forensic Sci (in press)
7. Parker JM, Winek CL, Shanor SP (1971) Postmortem changes in tissue levels of sodium secobarbital. J Clin Toxicol 4:265–272
8. Pounder DJ, Yonemitsu K (1991) Postmortem absorption of drugs and ethanol from aspirated vomitus – an experimental model. Forensic Sci Int 51:189–195
9. Rowland M, Tozer NT (1989) Clinical pharmacokinetics, concepts and applications. Lea and Febiger, Philadelphia London
10. Kurz H, Fichtl B (1983) Binding of drugs to tissues. Drug Metab Rev 14:467–510
11. Stegmann R, Bickel MH (1977) Dominant role for tissue binding in the first-pass extraction of imipramine by the perfused rat liver. Xenobiotica 7:737–746
12. Bickel MH, Raaflaub RM, Hellmüller M, Stauffer EJ (1987) Characterization of drug distribution and binding competition by two-chamber and multichamber distribution dialysis. J Pharm Sci 76:68–74
13. Costantino AG, Caplan YH, Mergner WJ, Resau J (1990) The effect of hypoxia induced hepatocyte membrane injury on the release of amitriptyline from rabbit liver slices. In Vitro Toxicology 3:321–348
14. Fallani M (1961) Contributo allo studio della circulazione ematica postmortale. Minerva Medicolegale 81:108–115
15. Yonemitsu K, Pounder DJ (1992) Postmortem toxicokinetics of co-proxamol. Int J Leg Med 104:347–353
16. Apple F, Bandt C (1988) Liver and blood postmortem tricyclic antidepressant concentrations Am J Clin Pathol 794–796
17. Apple F (1989) Postmortem tricyclic antidepressant concentrations: assessing cause of death using parent drug to metabolite ratio. J Anal Toxicol 13:197–198
18. Gonzalez FJ (1989) The molecular biology of cytochrome P450s. Pharmacol Rev 40:243–288