Raymond C. Pohland,[1] *Ph.D. and Norman R. Bernhard,*[2] *B.S.*

Postmortem Serum and Tissue Redistribution of Fluoxetine and Norfluoxetine in Dogs Following Oral Administration of Fluoxetine Hydrochloride (Prozac®)

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ABSTRACT: Antemortem serum and postmortem serum and tissues were evaluated to determine if postmortem redistribution of the antidepressant, fluoxetine (Prozac®) and its major active metabolite, norfluoxetine, occurred in dogs following oral administration of fluoxetine hydrochloride. Beagle dogs (four males) received daily oral doses of 10 mg fluoxetine/kg for five days. Antemortem serum concentrations of fluoxetine and norfluoxetine were determined 3 and 24 h following administration of the first four daily doses of fluoxetine and 3 h after the fifth dose in order to monitor for steady-state serum concentrations of parent and metabolite prior to postmortem serum concentration determinations. Antemortem serum concentrations of fluoxetine and norfluoxetine 3 h postdose on Day 5 ranged from 530 to 1210 ng/mL and 1460 to 1980 ng/mL, respectively. Immediately following the 3 h blood sample on Day 5, each dog was euthanized. Serum concentrations of fluoxetine and norfluoxetine were determined from blood samples collected from the vena cava, heart, and clotted blood within the heart at 2 h after death in two dogs and 12 h after death in the remaining two dogs. Similarly, tissue concentrations of fluoxetine and norfluoxetine in heart, liver, and lung were determined 2 and 12 h postmortem.

Serum concentrations of fluoxetine and norfluoxetine from the vena cava and heart 2 h postmortem were 2.2- to 6.0-fold and 2.3- to 3.6-fold higher, respectively, than antemortem serum concentrations. Similarly, serum concentrations of fluoxetine and norfluoxetine from vena cava and heart 12 h postmortem were 1.3- to 3.5-fold and 1.7- to 3.3-fold higher, respectively, than antemortem serum concentrations. However, 2-h and 12-h postmortem serum concentrations of fluoxetine and norfluoxetine from clotted blood within the heart were equal to or less than levels determined in antemortem serum. Results from 2-h and 12-h postmortem average tissue concentrations of fluoxetine and norfluoxetine in heart, liver, and lung demonstrated that fluoxetine and norfluoxetine concentrations in myocardium were similar 2 h and 12 h postmortem. However, in liver, fluoxetine concentrations decreased 39% and norfluoxetine concentrations decreased 23% from 2 h to 12 h postmortem. Even greater postmortem decreases in fluoxetine and norfluoxetine concentrations were observed in lung. Fluoxetine and norfluoxetine concentrations in lung decreased 49% and 39%, respectively, from 2 h to 12 h postmortem.

In conclusion, this study demonstrated that fluoxetine and norfluoxetine undergo postmortem redistribution in the dog. Furthermore, postmortem serum concentrations appear to be dependent on the sample site and the degree of coagulation of the blood. Finally, postmortem decreases in concentrations of fluoxetine and norfluoxetine in liver and lung may, in part, explain the observed increase in serum concentrations at 2 and 12 h postmortem.

KEYWORDS: forensic science, forensic toxicology, fluoxetine, norfluoxetine, Prozac, postmortem redistribution

The site and time dependent variability of postmortem blood and tissue sampling and the phenomenon of postmortem redistribution have been well documented and reviewed (1) in the literature. Several controlled animal studies as well as case reports have been published addressing these issues. Studies in rabbits with acetaminophen demonstrated that postmortem drug concentrations in blood increased significantly with time for central sampling sites compared to peripheral blood (2). The postmortem redistribution of amitriptyline was demonstrated in rats which suggested the postmortem drug release from lungs and other drug-rich tissues into the blood (3,4). Postmortem redistribution has also been demonstrated in rats following administration of morphine (5,6), digoxin (7), and secobarbital (8). Conversely, rats given cimetidine showed no statistical significant difference in postmortem changes in cimetidine concentrations in tissues compared to blood (9).

Several case reports have described similar findings. Site dependent postmortem changes in blood and tissue concentrations of digoxin (10,11), cocaine (12), methadone (13), and methamphetamine (14) have been reported. Postmortem redistribution of chloroquine (15), dothiepin (16), amitriptyline (17), tranylcypromine (18), and co-proxamol (19) have also been reported. In contrast, other case reports have determined that there is little evidence that postmortem redistribution occurs with tricyclic antidepressants (20), trazodone (21), and zopiclone (22).

Clearly, the site and time dependent variability of postmortem blood and tissue sampling and the phenomenon of postmortem redistribution is not consistent or drug class specific and must therefore be evaluated on an individual basis. Accordingly, the present study was designed to determine if postmortem redistribution of the antidepressant, fluoxetine, and its major active metabolite, norfluoxetine, occurred in dogs following oral administration of 10 mg fluoxetine/kg.

Materials and Methods

Animals—Four male beagle dogs, obtained for Marshall Research Animals, Inc. (North Rose, NY) were housed in individual cages and allowed to acclimate to caging, feeding, and watering

[1]Senior research scientist, Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, IN.

[2]Deceased.

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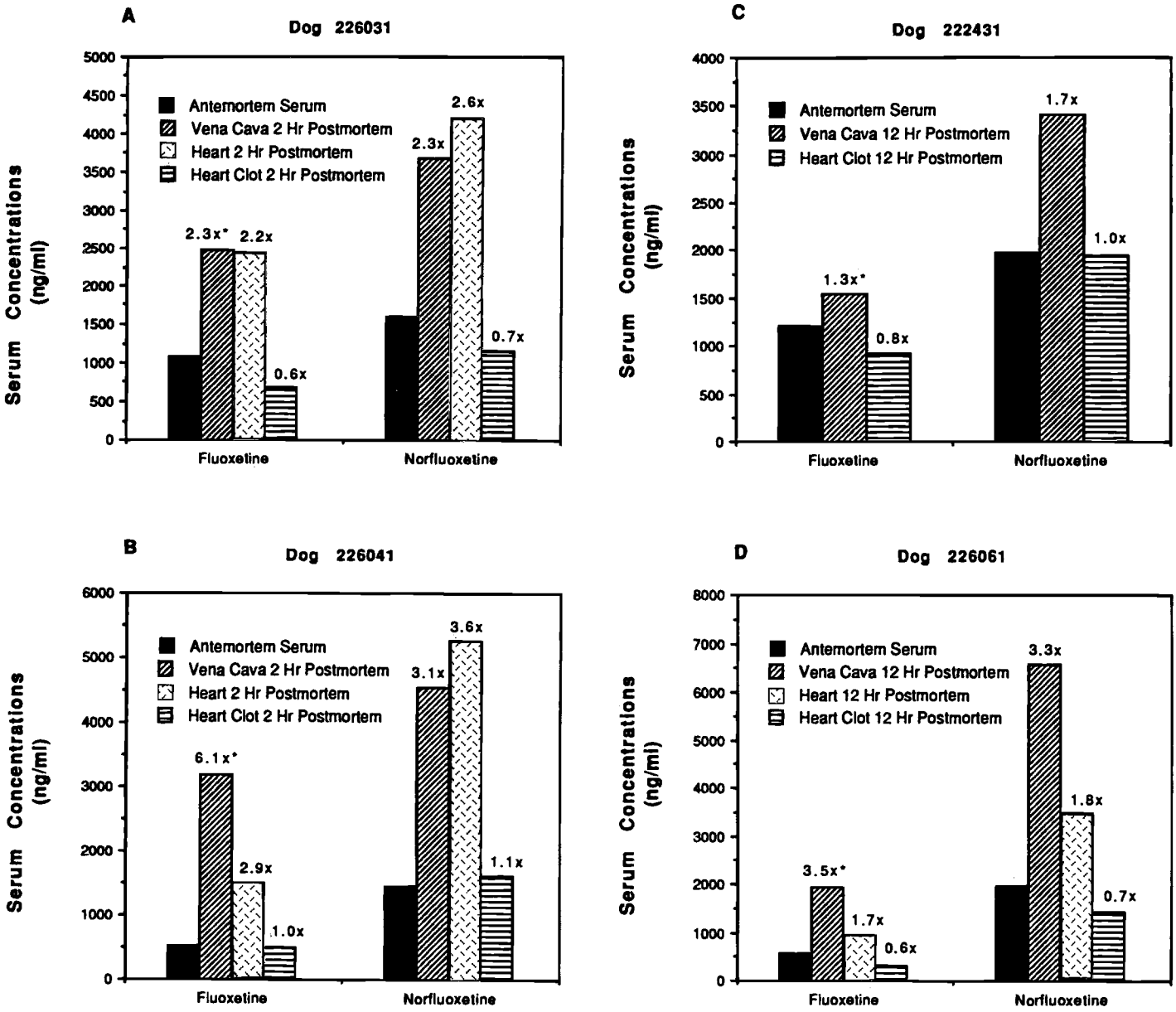

FIG. 1—*Antemortem and postmortem serum concentrations (ng/mL) of fluoxetine and norfluoxetine (A and B—2 h postmortem; C and D—12 h postmortem). *Difference from antemortem serum concentrations.*

conditions. Dogs were 10 to 12 months old and weighed 9.8 ± 1.7 kg (mean ± SD).

Test Chemical—Fluoxetine hydrochloride (Prozac) is chemically identified as dL-N-methyl-γ-[4-(trifluoromethyl)phenoxy]-benzenepropanamine hydrochloride. Analytical characterization of this material indicated a purity of >99%.

Dose Preparation and Administration—Fluoxetine was weighed into individual gelatin capsules for oral administration. Each dog received a daily oral dose of 10 mg/kg for 5 consecutive days.

Serum and Tissue Collection and Analysis for Fluoxetine and Norfluoxetine—Antemortem venous blood samples were collected from each dog in 5-mL tubes at 3 and 24 h after dose administration on Days 1, 2, 3, and 4 and at 3 h after dosing on Day 5. Eachdog was euthanized with a barbituate immediately after collection of the 3-h blood sample and remained at room temperature during postmortem sampling.

Postmortem blood samples were collected from the heart and the vena cava of two dogs at 2 h, and from the remaining two dogs at 12 h after death (5 and 15 h after the final dose administration). Blood was collected using a Vacutainer®, and/or by incising the heart and the vena cava and removing clots of blood which were placed into appropriately labeled tubes. Following collection, the samples were placed on ice until processed. Serum was obtained from the clotted blood via low speed centrifugation and analyzed for fluoxetine and norfluoxetine as described below.

Postmortem tissue samples were collected from two dogs at 2 h and from the remaining two dogs at 12 h after death. Sections of liver, lung, and heart were removed from each dog and approxi-mately 3 g of each organ were weighed and placed on ice. Each of the three tissues was homogenized with Milli-Q® water using a ratio of 1.0 g tissue to 3-mL water.

TABLE 1—*Antemortem and postmortem serum concentrations of Fluoxetine (F) and Norfluoxetine (N) in dogs following oral administration of fluoxetine hydrochloride (Prozac).*

		Antemortem Serum Concentrations (ng/mL)							
	Hour	Dog 226031		Dog 226041		Dog 222431		Dog 226061	
Day	Postdose	F	N	F	N	F	N	F	N
1	3	170	170	120	160	150	110	40	40
	24	130	570	70	560	170	780	120	680
2	3	160	630	100	690	330	910	430	850
	24	350	910	230	910	310	1200	340	1060
3	3	1010	1090	890	1200	1080	1350	1100	1510
	24	460	1340	290	1150	350	1530	390	1370
4	3	460	1220	740	1400	910	1620	380	1470
	24	420	1240	380	1230	420	1750	470	1680
5	3	1090	1590	530	1460	1200	1980	560	1980

	2-H Postmortem Serum Concentrations (ng/mL)				12-H Postmortem Serum Concentrations (ng/mL)			
	Dog 226031		Dog 226041		Dog 222431		Dog 226061	
Sample Site	F	N	F	N	F	N	F	N
Vena Cava	24890	3680	3200	4540	1550	3420	1940	6580
Heart	2440	4200	1500	5270	No sample	No sample	940	3480
Heart Clot	670	1170	500	1620	920	1950	330	1440

Aliquots of serum and tissue homogenates were analyzed for fluoxetine and norfluoxetine concentrations using a gas chromatographic assay with electron-capture detection as described by Nash et al. (23).

Results

Results of antemortem and postmortem serum concentrations of fluoxetine and norfluoxetine are presented in Fig. 1 and Table 1. Results of postmortem tissue concentrations of fluoxetine and norfluoxetine are presented in Fig. 2 and Table 2. Due to the small sample population, results were presented as individual data by animal number or as average data.

Antemortem serum concentrations of fluoxetine and norfluoxetine were determined 3 and 24 h following administration of the first four daily doses of fluoxetine and 3 h after the fifth dose in order to monitor for steady-state serum concentrations of parent and metabolite prior to postmortem serum concentration determinations. As indicated in Table 1, antemortem serum concentrations of fluoxetine were generally higher at 3 h postdose as compared to 24 h postdose on all days of dose administration. Furthermore, highest concentrations of fluoxetine at 3 and 24 h postdose occurred following the third daily dose. This is consistent with the plasma half-life of fluoxetine of approximately 1 day in the dog. Antemortem serum concentrations of fluoxetine 3 h postdose on Day 5 ranged from 530 to 1210 ng/mL.

Antemortem serum concentrations of norfluoxetine were, in almost all cases, higher at 24 h postdose as compared to 3 h postdose on all days of dose administration (Table 1). In addition, serum concentrations of norfluoxetine increased steadily following each dose, which is consistent with the plasma half-life in the dog of approximately 2 to 5 days. Antemortem serum concentrations of norfluoxetine 3 h postdose on Day 5 ranged from 1460 to 1980 ng/mL.

Immediately following the 3-h blood sample on Day 5, each dog was euthanized. Serum concentrations of fluoxetine and norfluoxetine were determined from samples collected from the vena

TABLE 2—*Postmortem tissue concentrations of Fluoxetine (F) and Norfluoxetine (N) in dogs following oral administration of Fluoxetine Hydrochloride (Prozac).*

	2-H Postmortem Tissue Concentration (μg/g)				12-H Postmortem Tissue Concentration (μg/g)			
	Dog 226031		Dog 226041		Dog 222431		Dog 226061	
Tissue	F	N	F	N	F	N	F	N
Heart	12.0	25.9	5.8	23.4	13.4	30.6	4.6	23.6
Liver	26.2	43.4	18.7	47.5	16.9	36.5	10.6	33.2
Lung	78.3	153.7	83.5	280.4	46.8	105.4	36.0	158.1

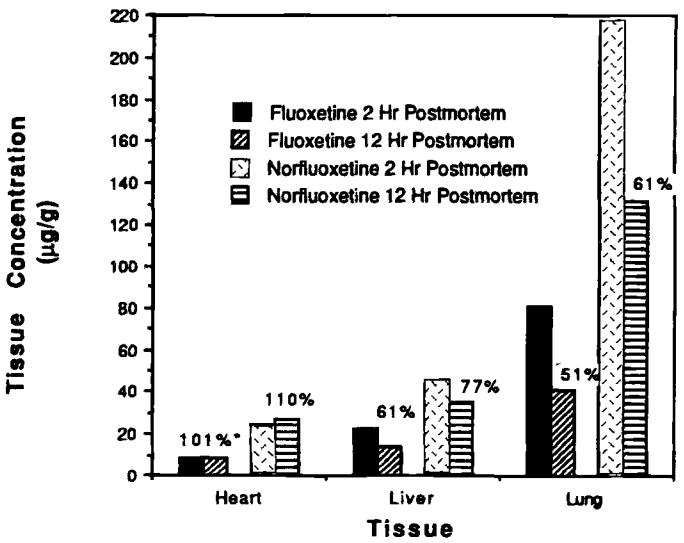

FIG. 2—*Average tissue concentrations (μg/g) of fluoxetine and norfluoxetine 2 and 12 h postmortem.*
**Percent of 2 h postmortem tissue concentration.*

cava, heart, and clotted blood within the heart, 2-h postmortem (dogs 226031 and 226041) and 12 h postmortem (dogs 222431 and 226061). Similarly, tissue concentrations of fluoxetine and norfluoxetine in heart, liver, and lung were determined 2 and 12 h postmortem. Comparisons of postmortem serum concentrations of fluoxetine and norfluoxetine to Day 5 antemortem serum concentrations (3-h postdose) and presented in Fig. 1 and Table 1. Results indicated that 2-h postmortem serum concentrations of fluoxetine and norfluoxetine from the vena cava and heart were 2.2- to 6.0-fold and 2.3- to 3.6-fold higher, respectively, than antemortem serum concentrations. However, 2-h postmortem serum concentrations of fluoxetine and norfluoxetine from clotted blood within the heart were equal to or less than levels determined in antemortem serum. Similar results were observed from 12-h postmortem serum samples (Fig. 1 and Table 1). Postmortem serum concentrations of fluoxetine and norfluoxetine from vena cava and heart were 1.3- to 3.5-fold and 1.7- to 3.3-fold higher, respectively, than antemortem serum concentrations. Furthermore, 12-h postmortem serum concentrations of fluoxetine and norfluoxetine from clotted blood within the heart were equal to or less than levels determined in antemortem serum.

Results from 2-h and 12-h postmortem average tissue concentrations of fluoxetine and norfluoxetine in heart, liver, and lung are presented in Fig. 2 and Table 2. Results demonstrated that fluoxetine and norfluoxetine concentrations in myocardium were similar 2 h and 12 h postmortem. However, in liver, fluoxetine concentrations decreased 39% and norfluoxetine concentrations decreased 23% from 2 h to 12 h postmortem. Even greater postmortem decreases in fluoxetine and norfluoxetine concentrations were observed in lung. Fluoxetine and norfluoxetine concentrations in lung decreased 49% and 39%, respectively, from 2 to 12 h postmortem.

Discussion

The importance of the site and time dependent variability of postmortem blood and tissue sampling and the phenomenon of postmortem redistribution in the determination of the cause of death has been well documented. In fact, Pounder and Jones (24) have stated that the poorly studied phenomenon of postmortem redistribution creates major difficulties in interpretation and undermines the reference value data bases where the site of origin of postmortem blood samples is unknown. In the present study, elevated postmortem serum concentrations of fluoxetine and norfluoxetine as compared to antemortem serum concentrations indicated that fluoxetine and norfluoxetine undergo postmortem redistribution in the dog. Similar increases in fluoxetine and norfluoxetine serum concentrations in vena cava and heart samples at 2- and 12-h postmortem indicated that postmortem redistribution was established within 2 h after death.

This study also demonstrated that serum concentrations of fluoxetine and norfluoxetine were dependent on the sample site. Postmortem serum concentrations of fluoxetine and norfluoxetine were consistently higher in vena cava and heart samples as compared to serum concentrations obtained from clotted blood within the heart.

Finally, concentrations of fluoxetine and norfluoxetine in myocardium did not significantly change with time after death. However, concentrations of fluoxetine and norfluoxetine in liver and lung decreased with time after death. This pattern of redistribution of fluoxetine and norfluoxetine in certain tissues most likely accounts for the observed increase in serum levels at 2- and 12-h postmortem. Therefore, when determining postmortem serum and tissue concentrations of fluoxetine and norfluoxetine, it is important to consider the phenomenon of postmortem redistribution because postmortem drug concentrations may not be a true reflection of antemortem concentrations and as a result, inaccurate conclusions could be made in assessing the contribution of the drug to the death under investigation.

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Additional information and reprint requests:
Raymond C. Pohland, Ph.D.
Senior Research Scientist
Toxicology Research Laboratories
Lilly Research Laboratories
A Division of Eli Lilly and Company
Greenfield, IN 46140