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TECHNICAL NOTE

TOXICOLOGY

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Detection of Amitriptyline, Citalopram, and Metabolites in Porcine Bones Following Extended Outdoor Decomposition*

ABSTRACT: Skeletal remains of a domestic pig were assessed for relative distribution of amitriptyline, citalopram, and metabolites. Following acute exposure and outdoor decomposition for 2 years, drugs and metabolites were analyzed in 13 different bones. Bones were pulverized following a simple wash procedure, and drugs were extracted by passive incubation in methanol, followed by solid-phase extraction. Samples were analyzed by ultra-high performance liquid chromatography (UHPLC) and confirmed with gas chromatography–mass spectrometry. The Kruskall–Wallis test showed that bone type was a main effect with respect to drug level for all analytes, with levels varying from 33- to 166-fold. Ratios of levels of drug to that of the corresponding metabolite were less variable, varying roughly one- to eightfold. This suggests limitations in the interpretive value of drug measurements in bone and that relative levels of drug and metabolites should be further investigated in terms of forensic value.

KEYWORDS: forensic science, toxicology, amitriptyline, citalopram, bone, ultra-performance liquid chromatography

Toxicological analysis can be one of the most important tools in elucidating cause and manner of death. Typically, blood is preferred for toxicological analysis as parent drug levels or the total and relative amounts of parent drug and the pharmacologically active metabolite(s) can be related to the extent of toxicity. However, when significant bodily decomposition occurs, conventional toxicological samples such as blood, urine, and vitreous humor are often no longer available for analysis. Bones and hair may be the only tissues available. Although a growing number of articles are being published on the subject (1–17), the understanding of the implications of positive drug screen results in bones is still quite limited.

Many factors may affect levels of drugs in the skeletal tissues. Factors such as environmental conditions and duration of exposure to those conditions influence the rate of decomposition and could potentially influence analyte levels in skeletal tissues in a drug-dependent manner. The specific effects of different conditions of drug administration and postmortem environment on the relative distribution of drugs in different bones remains poorly understood. More comprehensive research needs to be conducted in the area.

A wide array of drugs have been detected in bone tissues, including antidepressants (1,2), antipsychotics (1,2), benzodiazepines (1,3–6), barbiturates (4,7), opioids (1,6,9–13), and various other drugs (1,4,6,14–18). Nonetheless, the information is still quite limited. Most of the information is based on single case studies or animal research, and there is a lack of standard sample preparation methods. Various bones have been examined, including femora (1,3,7–14), vertebrae (8,9,12), pelvises (8–10,12), and humeri (7). It is unclear what differences can be expected from different bones. However, it does appear that trabecular bone may be a better site for drug analysis than cortical bone (3,8,9,12–14). To date there have been very few studies that have examined drug distribution throughout the skeleton, so the magnitude of any site dependence in drug level remains unclear.

In this study, the spatial distribution of selected antidepressants (amitriptyline, citalopram) and their metabolites (nortriptyline and desmethylcitalopram) were evaluated in 13 different bones. Amitriptyline is a dibenzocyclopetadine derivative drug from the class of the tricyclic antidepressants (18,19). Tricyclic antidepressants can be used for a wide variety of reasons, including the treatment of depression, anxiety, eating disorders, attention deficit hyperactivity disorder, enuresis in children or nocturia (inability to control urination and nighttime urination), and for neuropathic pain (19,20). Side effects of amitriptyline use include cardiac conduction abnormalities and anticholinergic effects such as dry mouth, blurred vision, constipation, urinary retention, and decreased sweating (19). Citalopram is a selective serotonin reuptake inhibitor, commonly prescribed for the treatment of depression and some anxiety disorders. Symptoms of citalopram toxicity may include central nervous system depression, serotonin syndrome, seizures, and cardiac abnormalities (21).

Bones were examined from an adolescent domestic pig (*Sus domesticus*) that was dosed with an oral cocktail of amitriptyline, citalopram, diazepam, and morphine, and euthanized by intraperitoneal (and subsequently intracardiac) administration of pentobarbital. The pig remains were subjected to decomposition

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between the summer of 2007 and the fall of 2009 in a rural Ohio setting, thereby replicating the situation where bones would be most likely chosen for toxicological analysis (i.e., when conventional fluids or tissues are not available). In our initial work with these remains (22), we analyzed bones for amitriptyline, diazepam, nordiazepam, and pentobarbital by gas chromatography–mass spectrometry (GC–MS). In that work, a substantial site dependence in drug level was observed for all drugs examined, with levels between the most concentrated and the least concentrated varying by a factor of roughly 20–40. However, the ratio of levels of diazepam to nordiazepam (the only metabolite analyzed in that work) displayed substantially lower site dependence, varying only by a factor of nine. Consequently, we undertook this work to examine whether the trend of lower site-dependent variability in ratios of levels of parent drug and metabolites would extend to other drugs to which the pig was exposed. This work represents one of the first occasions that such a comprehensive analysis of skeletal tissues has been undertaken in highly decomposed remains of an animal with comparable physiology to humans.

Methods

Chemicals

Drug standards were purchased from Cerilliant (Round Rock, TX) and were obtained as 1 mg/mL methanolic solutions and diluted as required. Methanol and acetonitrile were high-performance liquid chromatography grade and purchased from EMD Chemicals (Gibbstown, NJ). Trifluoroacetic acid anhydride (TFAA) was purchased from United Chemical Technologies (Bristol, PA). Water (18 MΩ) for ultra-high performance liquid chromatography (UHPLC) mobile phase was purified with Barnstead Easypure® RoDI (Waltham, MA). All other chemicals were reagent grade and were obtained from EMD Chemicals.

Drug Administration

The bones were obtained from a domestic pig that had been used to examine the fate of drugs in liver and muscle (23). The original study was performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and was approved by the ILACUC, Ohio State University, Columbus, OH. Pigs from that study were a Yorkshire/Hampshire cross-breed, and females and/or male castrates (as available). A cocktail of drugs, including morphine, amitriptyline, citalopram and diazepam was administered to the pig by gavage. Amitriptyline was administered at a dose of 75 mg/kg and citalopram was administered at a dose of 7 mg/kg. Four hours elapsed before sacrifice, in order to allow for drug absorption and distribution. The pig was sacrificed with pentobarbital (intracardiac). The pig was then allowed to decompose between the summer 2007 and fall 2009, at which point the bones were analyzed.

Treatment of Porcine Bone

Skeletal remains were recovered with no traces of soft tissue observable. The bones of interest were separated according to anatomical location, and included cervical vertebrae (C1 and C2), thoracic vertebrae (T7 and T8), lumbar vertebra (L5), pelvis, scapula, ribs, tibial diaphyses, tibial epiphyses, femoral diaphyses, femoral epiphyses, humeral diaphyses, humeral epiphyses, and ulna. Bones were rinsed twice in distilled water and once in acetone. The bones were left to dry overnight. Bones were initially crushed manually with a mallet, and then ground in an all-purpose household grinder. Samples of each type of bone (3 g, $n = 3$) were weighed into threaded glass test tubes and incubated in 10 mL of methanol at 50°C for 96 h. Supernatants were then recovered. Bones were rinsed with 5 mL of methanol, which was then recovered and pooled with the original methanol. Extracts were centrifuged at $1100 \times g$ for 3 min and supernatants were recovered. Clomipramine (250 ng) was added to each sample. Methanol was evaporated under a gentle stream of air at 70°C. Samples were reconstituted in 2 mL of phosphate buffered saline (PBS; 0.1 M, pH = 6). Concentrated acetic acid (100 µL) was added to each sample. Methanol:acetonitrile (1:1, v/v) (3 mL) was then added to each sample, and the samples were placed in a freezer (−18°C) for approximately 1 h. Samples were then centrifuged for 10 min at $1100 \times g$ and the supernatant was recovered. Samples were blown down to 2 mL under a gentle stream of air at 70°C. Sample volume was then adjusted to 5 mL with PBS.

Solid-Phase Extraction

All samples were then extracted with Strata-XC mixed-mode solid-phase extraction (SPE) cartridges (3 cc, 60 mg; Phenomenex, Torrance, CA). Cartridges were conditioned with 3 mL methanol, 3 mL distilled water, and 2 mL PBS (all at 1 mL/min). Samples (4.5 mL) were then loaded onto the SPE cartridges at 0.5 mL/min. Cartridges were washed with 3 mL 0.1 M acetic acid (HOAc), 3 mL MeOH:H_2O:HOAc (25:73:2, v/v), and 3 mL MeOH:H_2O (3:1 v/v) (all at 1 mL/min). Cartridges were then dried under vacuum at approximately 125 mmHg for 5 min. All samples were eluted with approximately 3.5 mL (1 column volume) 2% NH_4OH in ethyl acetate:isopropanol (80:20 v/v). Cartridges were dried under vacuum at approximately 125 mmHg for 5 min. Extracts were then evaporated under a gentle stream of air at 70°C.

Ultra-High Performance Liquid Chromatography

UHPLC was used for the analysis of amitriptyline, nortriptyline citalopram, and desmethylcitralopram in pig bones. The UHPLC used was an Acquity™ Ultra Performance LC from Waters (Milford, MA). The column used was a Kinetex 2.6 µm C18 column (100 × 2.1 mm) from Phenomenex. Mobile phase A consisted of 0.1% (v/v) concentrated formic acid, 10% (v/v) acetonitrile, and 90% (v/v) water, and mobile phase B was acetonitrile. Samples were run with an isocratic mobile phase, consisting of 84% mobile phase A and 16% mobile phase B. The flow rate was 0.300 mL/min. The sample temperature was set to 25°C, and the column temperature was set to 50°C. The photodiode array detector range was set from 220 to 400 nm, and quantitative comparisons were made using the response ratio (i.e., RR—the ratio of peak areas for drug and internal standard) measured at 240 nm. The entire run time was 14 min.

Dry extracted samples were reconstituted in 300 µL of mobile phase A. Samples were micro centrifuged for 10 min at $10{,}500 \times g$. All samples were then transferred to autosampler vials, and 15 µL of sample was injected into the UHPLC. After UHPLC, the samples were dried under a gentle stream of air at 70°C, so they could be derivatized and analyzed by GC–MS for confirmation of UHPLC results.

Ultra-High Performance Liquid Chromatography Validation

Samples of drug-free decomposed bone tissue were sonicated in PBS to create a solution which contained extract of decomposed

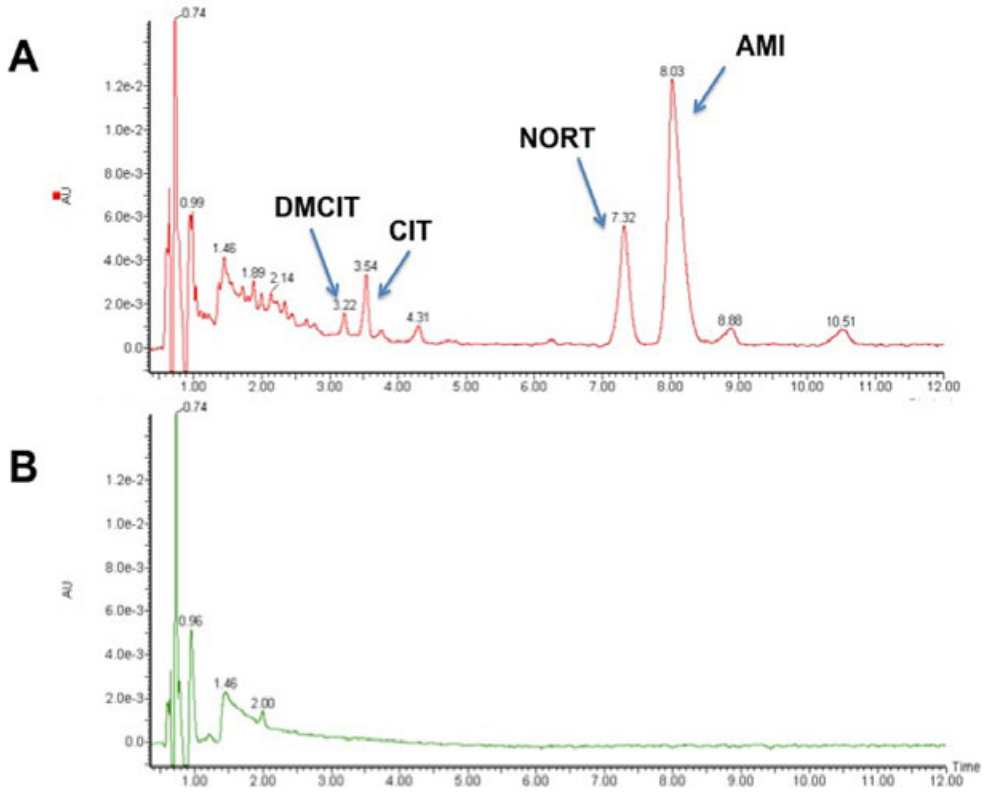

FIG. 1—*Ultra-high performance liquid chromatography chromatogram of extract of porcine rib bone derived from (A) drug-exposed animal and (B) drug-free animal. Labeled peaks correspond to desmethylcitalopram (DMCIT), citalopram (CIT), nortriptyline (NORT), and amitriptyline (AMI).*

soft tissue, marrow, and bone for use in analytical method. The response ratio was linear ($R^2 \geq 0.99$) from 10 ng/mL to at least 5000 ng/mL for all drugs. The precision (%CV) of duplicate analyses of standards over the linear range was <20% on each of four different days. The %CV for the average measured concentration of a prepared drug standard in bone extract, over 4 days, was 9.3%. The average percent difference for blind trials ranged from 3.2 to 6.5%.

Gas Chromatography–Mass Spectrometry Confirmation

GC–MS was used for mass confirmation. Pig bone samples were derivatized with TFAA. To each dry sample, 100 µL of ethyl acetate and 100 µL of TFAA were added. The samples were then transferred to test tubes (as they were still in the UHPLC vials). All tubes were capped and heated at 70°C for 30 min. The samples were blown down under a gentle stream of air at 70°C. All samples were then reconstituted in 100 µL of ethyl acetate for GC–MS analysis.

All samples were analyzed on a PekinElmer Clarus 600 GC–MS (Perkin Elmer LAS, Shelton, CT), equipped with a ZB-Drug-1 column (15 m × 0.25 mm × 0.25 µm; Phenomenex). GC–MS was run in the electron impact ionization mode, using selected ion monitoring mode. The mass spectrometer source and transfer line temperatures were 250°C, and the electron energy was 70 eV. For each sample, a 2-µL extract was injected in the GC–MS. Helium was used as the carrier gas at a flow rate of 1 mL/min. Temperature ramps were as follows: 60°C for 3 min, 100°C/min ramp to 160°C and held at this temperature for 1 min, followed by a 12°C ramp to 300°C and held at this temperature for 3 min. Ions monitored were 189.1, 202.2, and 215.0 for amitriptyline, 202.2, 219.0, and 232.0 for nortriptyline, 208, 238, and 324 for citalopram, 208, 238, and 239 for desmethylcitalopram, and 228.1, 268.0, and 314.0 for clomipramine.

Statistics

All statistical analyses were performed using StatPlus 2009 (AnalystSoft Inc. [http://analystsoft.com/en/]). The Kruskall–Wallis test was utilized to compare the variability within replicate extractions of a given bone type to the variability in analytical response across all tissues assayed, in order to determine whether any significant tissue-dependent distribution existed. Statistical significance was attributed at $p < 0.01$.

Results

Examples of UHPLC chromatogram of porcine bone extracts from drug-positive and drug-negative animals are shown in Fig. 1. All samples were compared using the mass corrected analyte response ratio (i.e., RR/*m*—response ratio divided by mass of bone extracted). The average RR/*m* value for the triplicate analysis of a given bone, along with the standard deviation, is plotted in Fig. 2 for amitriptyline and nortriptyline, and in Fig. 3 for citalopram and desmethylcitalopram.

All four drugs were detected by both UHPLC and GC–MS in all tissues except for desmethylcitalopram in the tibial diaphyses. Measured RR/*m* values ranged from 0.44 to 24.6, 0.20 to 8.4, 0.08 to 17.4, and 0.02 to 6.0 for amitriptyline, nortriptyline, citalopram, and desmethylcitalopram, respectively. The %CV of the measured RR/*m* values ($n = 3$) of a given bone type ranged from 3 to 17% for amitriptyline, from 8 to 34% for nortriptyline, from 4 to 120%

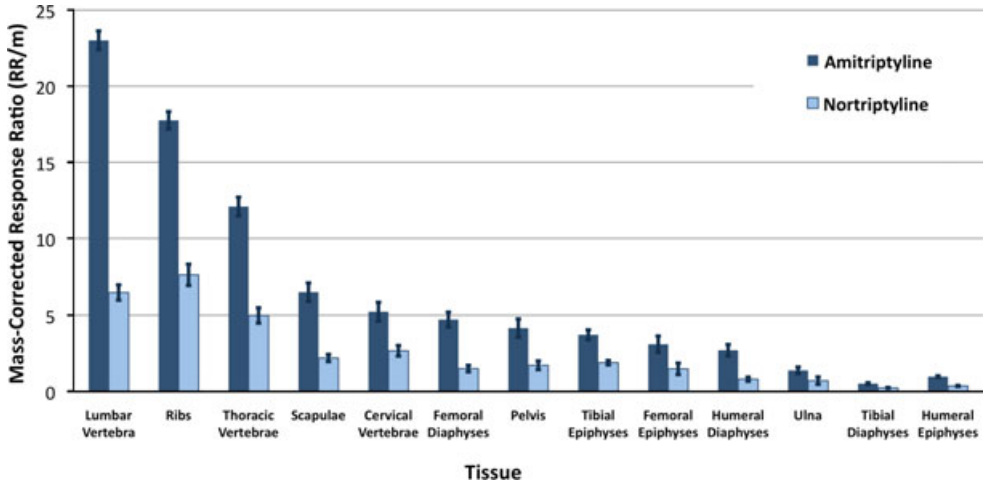

FIG. 2—*Distribution of mass normalized response ratio (RR/*m*) values for amitriptyline and nortriptyline in various bones.*

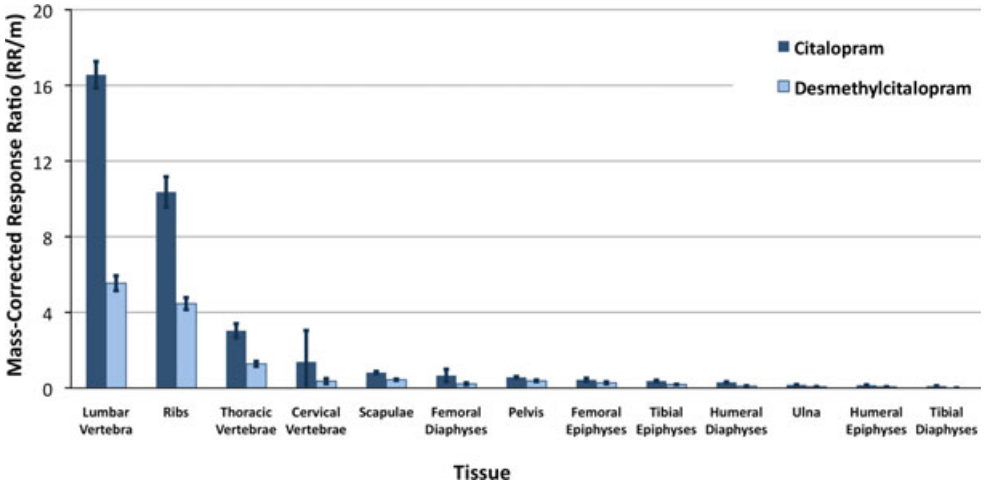

FIG. 3—*Distribution of mass normalized response ratio (RR/*m*) values for citalopram and desmethylcitalopram in various bones.*

for citalopram, and from 5 to 40% for desmethylcitalopram. The ratio of RR/*m* values for amitriptyline/nortriptyline in a given bone ranged from 1.7 to 3.8 and from 1.1 to 7.7 for citalopram/desmethylcitalopram. These data are summarized in Fig. 4. The Kruskall–Wallis test was used to assess the variability in RR/*m* values between and within bone types and showed that bone type was a main effect with respect to RR/*m* for all four drugs ($p < 0.01$). Bone type was also shown to be a main effect for the ratio of RR/*m* values for amitriptyline relative to nortriptyline ($p < 0.01$), and the ratio of RR/*m* values for citalopram relative to desmethylcitalopram approached the cutoff used for statistical significance ($p = 0.01$).

Discussion

Representation of the Data

In this study, we examine the distribution of amitriptyline, citalopram, and their *N*-desmethyl-metabolites in 13 different pig bones. We compare levels of drug in terms of response ratio (peak area of given drug over peak area of internal standard) divided by the mass of bone extracted. This allows for a linear comparison of the drug levels in each tissue, but does not ascribe an actual concentration. Given the fact that, at this point, it is impossible to quantify the extent of the drug extracted from the bone, reporting drug concentration as drug mass per gram of bone is inappropriate. Further studies examining radiolabeled drugs in skeletal tissues may help further understand the extent of drug extraction from bones.

In addition, given the fact that there is no standard method of drug extraction from bones, interlaboratory analysis of skeletal tissues may yield different drug concentrations. It could be expected that different levels of drug could be extracted based on the surface area of bone that is exposed to the extraction solvent. Hence, ascribing a given drug concentration in skeletal tissue could be misleading. However, comparison of drug extracted from different tissues within a given extraction protocol can provide semi-quantitative information about relative distribution that provides useful forensic data. To give an approximate idea of the level of drug observed in the extracts obtained here, amitriptyline concentrations observed ranged from roughly 40 to 2200 ng/g, nortriptyline concentrations ranged from <10 to 660 ng/g, citalopram concentrations ranged from <10 to 2000 ng/g, desmethylcitalopram concentrations ranged from <10 to 550 ng/g. Again, these values should be

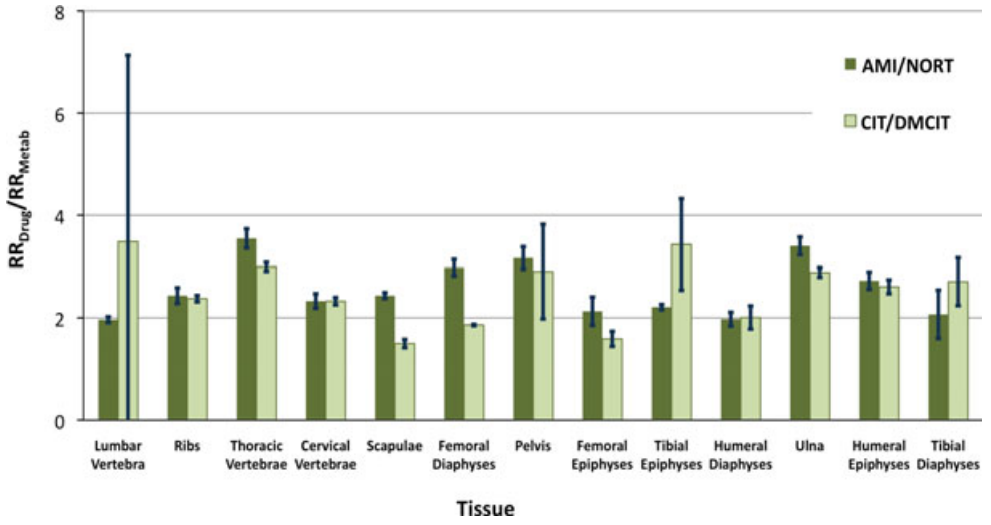

FIG. 4—*Ratio of assay response for drug to that of metabolite ($RR_{Drug}/RR_{Metabolite}$) in various bones.*

treated cautiously in terms of interpretive value, as drug recovery could not be properly characterized and because our sample preparation methods differ from those used in other laboratories (1,2,6).

Significance of the Results and Interpretation

In this study, a wide range of drug levels was observed. For amitriptyline, there was a 45-fold difference between the mean RR/*m* value associated with the tissue showing the lowest drug level (tibial diaphyses) and that showing the highest level (lumbar vertebra). This difference was 33-fold for nortriptyline, where the lowest assay response was found in the tibial diaphyses and the largest response was found in the rib. The difference in response was 166-fold for citalopram and 95-fold for desmethylcitalopram, where the lowest assay responses were found in tibial diaphyses and the largest responses were found in lumbar vertebra. At this moment, it is unclear why there is such a wide variation in the different bones and for the different drugs. However, it is clear that the bones with the highest levels of drug were located near the trunk of the body (i.e., lumbar vertebra, ribs, thoracic vertebrae). Given the fact that most of the soft tissue is located in the trunk, it is possible that during the decomposition process, drugs partitioned from the liquefied tissues into the semi-porous bones. This process will inevitably influence the apparent distribution of the drug in different bones. For example, those tissues where drugs may concentrate (e.g., lungs, liver) may generate a larger drug level in adjacent bone tissues. Further, the extent of drug distribution may depend on the type of bone (i.e., trabecular or cortical bone), because of structural features that can lead to different rates of mass transfer into and out of the mineralized bone.

In addition, the type of marrow surrounding bones may have an effect of the way drugs in circulation distribute within the bone. Humans have both red and yellow marrow: red marrow is in contact with blood, while yellow marrow acts as a storage compartment (24). In adult humans, red marrow is mainly located in the body of vertebrae, ribs, pelvis, scapulae, and sternum. As red marrow is in direct contact with circulating blood, it may be expected that bones with red marrow would have higher levels of drug. In this study, it was seen that vertebrae, ribs, and scapula had the highest levels of drug, while the pelvis was located in the middle of the range. It is possible that the mechanism by which drugs enter the bones is a combination of both the decomposition process and the circulation process.

Because of the heterogeneous anatomic structure of a given bone and the structural variability between different bones, it cannot be assumed that drug or metabolite is uniformly distributed throughout the tissue, both within a given bone type and between different bone types. Thus, it is critical to ascertain whether the observed variability in measured RR/*m* values across different bone types was greater than the variability resulting from replicate extractions of a given bone type. Accordingly, this comparison was made using the Kruskall–Wallis test, which does not presume that the RR/*m* values within a given bone type are normally distributed. For all four drugs, this analysis showed that the bone type was a main effect on the level of drug detected, demonstrating that the variability in RR/*m* values between the different bones was indeed significant. Typically, there was good reproducibility between the triplicate analyses of a certain bone type. In the 52 sample sets analyzed (13 bone types × four drugs), only seven had %CV above 20%. As drug distribution within a certain bone may not be uniform, poor precision in replicate extraction may complicate reanalysis.

Despite the high level of variability observed in the level of a given drug or metabolite as a function of bone type, one potential source of information may be the ratio of RR/*m* values for parent drug relative to metabolite (i.e., $RR_{drug}/RR_{metabolite}$). The mean ratio of $RR_{drug}/RR_{metabolite}$ values for amitriptyline to nortriptyline was 2.6 ± 0.6, and 2.5 ± 1.1 for citalopram/desmethylcitalopram. Thus, the relationship between the parent drug and the metabolite was relatively constant regardless of the bone type, unlike the levels of the individual drugs.

Implications

Based on the data provided here, it appears that substantial site-dependent variability may be observed in drug levels in bones derived from different anatomical sites. It is important to note that the data presented here are based on the analysis of tissues from a single animal, and so the extent of site-dependent distribution is limited to this single case. In this animal, bones located near the central cavity displayed higher levels of all drugs assayed, which suggests the possibility of the influence of drug transfer from visceral tissues during the decomposition and liquefaction of those tissues. On one hand, these bones would clearly offer a greater

chance of drug detection. However, such dramatic interbone variability in measured drug levels poses very significant complications for toxicological interpretation of any given measurement. Thus, the comparative stability in the ratio of levels of parent drug and metabolite (i.e., $RR_{drug}/RR_{metabolite}$) may provide a valuable tool in assessing the circumstances of drug exposure (e.g., fatal acute overdose vs. delayed death following chronic exposure). It is very likely that the ability to estimate the circumstances of drug exposure will depend on the drug(s) in question, where phenomena such as stability and the relative kinetics and volumes of distribution of the drug and its metabolites will have to be considered. It should be expected that this approach may require complex modeling of relative levels of a given drug and multiple metabolites in order to be successful. However, until such data may be collected, it would remain prudent to report drug-positive skeletal tissues in a qualitative manner (i.e., detected or not detected).

Conclusions

In this research, we examined the distribution of amitriptyline, nortriptyline, citalopram, and desmethylcitalopram in 13 different porcine skeletal tissues, after a period of decomposition of approximately 2 years. The data showed that bone type main effect with respect to the level of drug detected, with levels of a given drug varying from 33- to 166-fold across the different bone types assayed. However, the ratio of assay response of drug to that of the corresponding metabolite showed significantly less variability, varying only by roughly one- to eightfold. Therefore, while site-dependent variability and limitations in terms of analytical calibration limit the interpretive value of individual drug measurements in bone, relative levels of drug and metabolite(s) may prove useful in discriminating different patterns of drug exposure.

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References

1. McIntyre IM, King CV, Boratto M, Drummer OH. Post-mortem drug analyses in bone and bone marrow. Ther Drug Monit 2000;22(1):79–83.
2. Horak EL, Jenkins AJ. Postmortem tissue distribution of olanzapine and citalopram in a drug intoxication. J Forensic Sci 2005;50(3):1–3.
3. Watterson JH, Botman JE. Detection of acute diazepam exposure in bone and bone marrow: influence of tissue type and the dose-death interval on sensitivity of detection by ELISA and liquid chromatography tandem mass spectrometry confirmation. J Forensic Sci 2009;54(3):708–14.
4. Desrosiers NA, Betit CC, Watterson JH. Microwave-assisted extraction in toxicological screening of skeletal tissues. Forensic Sci Int 2009;188:23–30.
5. Gorczynski LY, Melbye FJ. Detection of benzodiazepines in different tissues, including bone, using a quantitative ELISA assay. J Forensic Sci 2001;46(4):916–8.
6. McGrath KK, Jenkins AJ. Detection of drugs of forensic importance in postmortem bone. Am J Forensic Med Pathol 2009;30(1):40–4.
7. Terazawa K, Takatori T. Determination of aminopyrine and cyclobarbital from a skeleton by radioimmunoassay. J Forensic Sci 1982;27(4):844–7.
8. Lafrenière NM, Watterson JH. Detection of acute fentanyl exposure in fresh and decomposed skeletal tissues. Forensic Sci Int 2009;185:100–6.
9. Lafrenière NM, Watterson JH. Detection of acute fentanyl exposure in fresh and decomposed skeletal tissues. Part II: the effect of dose-death interval. Forensic Sci Int 2010;94:60–6.
10. Guillot E, de Mazancourt P, Durigon M, Alvarez JC. Morphine and 6-acetylmorphine concentrations in blood, brain, spinal cord, bone marrow and bone after lethal acute or chronic diacetylmorphine administration to mice. Forensic Sci Int 2007;166:139–44.
11. Raikos N, Tsoukali H, Njau SN. Determination of opiates in postmortem bone and bone marrow. Forensic Sci Int 2001;123:140–1.
12. Watterson JH, Desrosiers NA. Microwave-assisted extraction in the study of the effect of dose-death interval on meperidine detection in skeletal tissue. Forensic Sci Int 2011;207:40–5.
13. VandenBoer TC, Grummett SA, Watterson JH. Utility of immunoassay in drug screening in skeletal tissues: sampling considerations in detection of ketamine exposure in femoral bone and bone marrow following acute administration using ELISA. J Forensic Sci 2008;53(6):1474–82.
14. Watterson JH, Vandenboer TC. Effects of tissue type and the dose-death interval on the detection of acute ketamine exposure in bone and marrow with solid-phase extraction and ELISA with liquid chromatography-tandem mass spectrometry confirmation. J Anal Toxicol 2008;3(8):631–9.
15. Österman T, Virtamo T, Kippo K, Laurén L, Pasanen I, Hannuniemi R, et al. Distribution of clodronate in the bone of adult rats and its effects on trabecular and cortical bone. J Pharmacol Exp Ther 1997;280(2):1051–6.
16. Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Res 1991;51:4837–44.
17. Ji AJ, Saunders JP, Wadgaonkar ND, Petersen PJ, O'Leary K, McWilliam WE, et al. A novel antibiotic bone assay by liquid chromatography/tandem mass spectrometry for quantitation of tigecycline in rat bone. J Pharm Biomed Anal 2007;44:970–9.
18. Baeck SK, Lim MA, Park SY, Lee JS, Lee HS, Koo KS. Blood concentrations of amitriptyline and its metabolite in rats after acute oral administration of amitriptyline. J Anal Toxicol 2000;24:271–4.
19. Melanson SEF, Lewandrowski EL, Griggs DA, Flood JG. Interpreting tricyclic antidepressant measurements in urine in an emergency department setting: comparison of two qualitative point-of-care urine tricyclic antidepressant drug immunoassays with quantitative serum chromatographic analysis. J Anal Toxicol 2007;31:270–5.
20. Goldman RH, Stason WB, Syun Park S, Kim R, Mudgal S, Davis RB, et al. Low-dose amitriptyline for treatment of persistent arm pain due to repetitive use. Pain 2010;149:117–23.
21. Hayes BD, Klein-Schwartz W, Clark RF, Muller AA, Miloradovich JE. Comparison of toxicity of acute overdoses with citalopram and escitalopram. J Emerg Med 2010;39:44–8.
22. Watterson JH, Desrosiers NA, Betit CC, Dean DE, Wyman JF. Relative distribution of drugs in decomposed skeletal tissues. J Anal Toxicol 2010;34(8):510–5.
23. Wyman J, Dean D, Yinger R, Simmons A, Brobst D, Bissel M, et al. The temporal fate of drugs in decomposing porcine tissue. J Forensic Sci 2011;56(3):694–9.
24. Kricun ME. Red-yellow marrow conversion: its effect on the location of some solitary bone lesions. Skeletal Radiol 1985;14:10–9.

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