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## Drug Residues after Intravenous Anesthesia and Intrathecal Lidocaine Hydrochloride Euthanasia in Horses

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**Background:** Intrathecal lidocaine hydrochloride under general anesthesia has been used as an alternative method of euthanasia in equids. Carnivore, scavenger, and even human consumption of horse meat from carcasses have been anecdotally reported in rural areas after this method of euthanasia. The presence of drug residues in horse meat has not been investigated.

**Hypothesis/Objectives:** To investigate if drug residues are found in horse tissues and determine their concentrations.

**Animals:** Of 11 horses requiring euthanasia for medical reasons.

**Methods:** Prospective descriptive study. Horses were anesthetized with total IV dose of xylazine (mean, 2.5 mg/kg), midazolam (0.1 mg/kg), and ketamine hydrochloride (mean, 5.8 mg/kg). An atlanto-occipital cisterna centesis for the collection of cerebrospinal fluid (CSF) and administration of lidocaine hydrochloride (4 mg/kg) was performed. Blood samples for both serum and plasma, skeletal muscle (*triceps brachii*, *gluteus medius*), and CSF were collected for the determination of drug residues. Frozen skeletal muscle available from 5 additional horses that received standard dosages of drugs for short-term anesthesia (xylazine 1.1 mg/kg, midazolam 0.1 mg/kg, and ketamine 2.2 mg/kg) also were analyzed.

**Results:** Drug residues were found in the tissues of all horses, but at extremely low concentrations.

**Conclusions and Clinical Importance:** Euthanasia by administration of lidocaine intrathecally to horses under IV anesthesia poses a low risk of toxicity to carnivores and scavengers that might consume muscle tissue from a carcass in which this protocol has been used.

**Key words:** Death; Equine; Pharmacology; Toxicology.

Consumption of carrion from horses that have been euthanized with pharmacological agents could represent an important health hazard for humans, domestic animals, and wildlife.<sup>1–5</sup> Inappropriate disposal of animal remains might result in exposure to these hazards.<sup>6</sup> Drugs used for euthanasia of animals such as barbiturates, phenytoin, and others can contaminate animals' tissues posing a danger if consumed.<sup>3,7</sup> In remote rural communities, rendering or burial facilities are unavailable, and the carcasses of horses euthanized with barbiturates are considered toxic,<sup>6</sup> and might be eaten by scavengers or carnivores. Furthermore, human consumption of animals' remains occurs in poor communities on which sources of food are scarce.<sup>4</sup>

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The study was performed at the University of California at Davis.

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### Abbreviations:

CSF	cerebrospinal fluid
LCMS	liquid chromatography tandem-mass spectrometry
LOQ	limit of quantitation
MTBE	methyl tert-butyl ether
TFC	turbulent flow chromatography system

Intrathecal 2% lidocaine hydrochloride has been used to euthanize horses under IV anesthesia<sup>a</sup> as an alternative method as a result of lack of availability, expense, or government regulations of approved methods of euthanasia. Lidocaine hydrochloride is widely available and inexpensive. However, because the efficacy, safety, and welfare aspects of using this method of euthanasia in equids remain unknown, we recently investigated if this method induced loss of electroencephalographic activity, and cardiac and respiratory arrest.<sup>8</sup> This method of euthanasia proved to be an effective alternative if barbiturates are not available.<sup>8</sup> In countries in which this method has been used, animals are premedicated with xylazine hydrochloride and ketamine hydrochloride is used to induce anesthesia before intrathecal administration of lidocaine.<sup>a</sup> Therefore, the purpose of this study was to investigate if drug residues (those used for intravenous anesthesia and lidocaine) are found in various tissues of horses undergoing euthanasia.

## Materials and Methods

### Animals

This prospective descriptive study included 11 horses for which euthanasia was elected based on published guidelines during a study period from September to December 2014.<sup>6,8</sup> Reasons for euthanasia included poor quality of life, intractable pain, or chronic progressive debilitating or incapacitating disease with a

poor prognosis. Horses were sourced from a research herd from the University of California at Davis. The study was approved by an institutional animal care and use committee.

### Intravenous Anesthesia and Euthanasia Protocol

All horses had an IV catheter placed in the jugular vein for the administration of xylazine hydrochloride at a dosage of 1.1 mg/kg followed 5 minutes later by midazolam hydrochloride at 0.05 mg/kg and ketamine hydrochloride at 2.2 mg/kg. Instrumentation for electrophysiologic studies and catheterization of the facial artery were time consuming. Several horses required repeated doses of xylazine (0.3 mg/kg) and ketamine (0.7 mg/kg) to maintain the complete immobilization required for these procedures, as well as the recording of the actual electrophysiologic data. This anesthetic maintenance resulted in higher total doses for xylazine and ketamine in the instrumented horses as shown in Table 1.

An area over the atlanto-occipital space was clipped and cleaned aseptically with betadine solution for cerebrospinal fluid (CSF) centesis as previously described.<sup>9</sup> An 18-gauge 10.62-cm needle was used for the collection of 60 mL of CSF (only 30 mL in an Arabian colt) and administration of 2% lidocaine hydrochloride at a dosage of 4 mg/kg over 30 seconds (a timer was used).

Physical, neurological, and electrophysiological variables to investigate events such as cortical electrical silence, lack of brainstem activity, and loss of cardiovascular variables including loss of electrocardiographic (ECG) activity were recorded and reported in a previous study.<sup>8</sup>

### Tissue Collection

Immediately after confirmation of death by loss of respiration, brain electrical activity, and electrocardiogram, tissue samples were collected to investigate drug residues. Samples collected included whole blood for serum (red top tube) and plasma (EDTA tube) extraction, CSF from the atlanto-occipital space and skeletal

muscle. A 2 × 2 cm muscle sample was collected from the *triceps brachii* and *gluteus medius* muscles. All samples were immediately frozen in liquid nitrogen, and stored at -80°C until further processing. The CSF fluid was refrigerated for use in unrelated research and teaching.

### Sample Preparation

For analysis, lidocaine,<sup>b</sup> ketamine,<sup>c</sup> midazolam,<sup>c</sup> and xylazine<sup>b</sup> reference standards were combined into one working solution. Working solutions were prepared by dilution of the stock solution with methanol to concentrations of 0.01, 0.1, 1, and 10 ng/μL. Calibrators were prepared by dilution of the working standard solutions with drug-free equine matrix to concentrations of 0.1–1,000 ng/mL for serum and plasma (12 calibrators) and from 0.5 to 5,000 ng/mL for CSF (11 calibrators). Calibration curves were prepared fresh for the quantitative assay. In addition, quality control samples (2 or 3 concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Before analysis, equine control serum and plasma (0.5 mL) and equine control CSF (0.2 mL) were diluted with 100 μL of water containing d10-lidocaine<sup>d</sup> and d6-xylazine<sup>b</sup> to a concentration of 0.025 ng/μL and d4-midazolam<sup>c</sup> and d4-ketamine<sup>c</sup> to 0.0025 ng/μL. The samples were vortexed briefly to mix, and 5 mL of methyl tert-butyl ether (MTBE)<sup>e</sup> was added to each serum sample. Samples subsequently were mixed by rotation<sup>f</sup> for 20 minutes at 40 rpm. After rotation, samples were centrifuged at 3300 rpm (2260 g) for 5 minutes at 4°C and the top organic layer was transferred to a 12 × 75 mm glass tube.<sup>g</sup> Samples were dried under nitrogen and dissolved in 120 μL of 5% acetonitrile<sup>h</sup> in water<sup>h</sup> with 0.2% formic acid.<sup>i</sup> The injection volume was 20 μL. For tissue quantitation, hard tissue homogenizer tubes were spiked with analyte from 1 to 2,000 ng/tube (8 calibrators). Before analysis, the homogenizer sample tubes were weighed, 0.1–0.5 mg tissue was added, and the tubes were reweighed to obtain the tissue weight. The internal standard mixture (100 μL) and acetonitrile (3.9 mL) then were added and the tissue was homogenized for

**Table 1.** Drug dosages (mg/kg) and tissue concentrations [ng/mL for plasma, serum, and CSF; ng/g for skeletal muscle (*gluteus medius*, *triceps brachii*)].

	Dosage (mg/kg)	Plasma (ng/mL)	Serum (ng/mL)	CSF (ng/mL)	Gluteus (ng/g)	Triceps (ng/g)
<b>Xylazine</b>						
Mean	2.5	1179.4	3162.2	395.8	103.1	112.4
SD	0.8	408.8	4,030	163.2	44.2	36.9
Median	2.5	1166.5	1379.8	427.1	98.7	95.5
Range	0.8–3.6	490.1–2096.9	928.7–12447.4	167.8–666.1	55.4–167.4	88–177.2
<b>Midazolam</b>						
Mean	0.1	23.2	23.2	1.2	36.6	50.2
SD	0	8.6	8.6	0.7	19.8	56.3
Median	0.05	21.8	21.8	1.2	35.6	37.5
Range	0.05–0.1	12.5–44.5	12.5–44.5	0.6–3	9.6–77.3	0–205.3
<b>Ketamine</b>						
Mean	5.8	2293.4	2298.3	1004.6	1176.5	1756.6
SD	2.5	1230.4	1051.7	334.9	644.3	741.6
Median	6.3	2531.2	2329.3	1008.4	1096.9	1643.8
Range	2.5–7.4	491–5691.7	581.4–4718.5	431.8–1513.3	539.5–2649.8	316.2–2886
<b>Lidocaine</b>						
Mean	4	476.6	259.2	NA	368.9	168.8
SD	0	914.5	496.2	NA	420.8	293.3
Median	4	26	14.1	NA	285.2	28
Range	4	7.4–2632.8	2.2–1497.2	NA	3.3–1380	4.3–925.6

NA, not applicable.

60 seconds at 3000 g in a Precellys 24-tissue homogenizer.<sup>h</sup> After centrifugation, 2 mL of the supernatant was transferred to 16 × 125 mm glass tubes and dried under nitrogen. The residue was redissolved in 1 mL of 95:5 water:acetonitrile and extracted with MTBE as described above. The final residue was dissolved in 200 µL and analyzed as described below.

### Measurement of Drug Concentrations

The concentrations of lidocaine, ketamine, midazolam, and xylazine were measured in serum, plasma, CSF, and tissue by liquid chromatography tandem-mass spectrometry (LC-MS/MS) with positive heated electrospray ionization (HESI[+]) at 300°C. Quantitative analysis was performed on a TSQ Vantage triple quadrupole mass spectrometer<sup>h</sup> coupled with a turbulent flow chromatography system (TFC TLX2)<sup>j</sup> having LC-10ADvp liquid chromatography systems<sup>k</sup> and operated in laminar flow mode. The spray voltage was 3500V, the vaporizer temperature was 210°C, and the sheath and auxiliary gas were 45 and 30, respectively (arbitrary units). Product masses and collision energies of each analyte were optimized by infusing the analytes into the mass spectrometer. Chromatography employed an ACE 3 C18 10 cm × 2.1 mm 3 µm column<sup>l</sup> and a linear gradient of acetonitrile in water with a constant 0.2% formic acid at a flow rate of 0.40 mL/min. The initial acetonitrile concentration was held at 10% for 0.50 minutes, ramped to 90% over 7 minutes, and subsequently reequilibrated for 3.83 minutes at initial conditions.

Detection and quantification was conducted by Selective Reaction Monitoring (SRM) of initial precursor ion for lidocaine (mass-to-charge ratio [m/z] 235.16), ketamine (m/z 238.071), midazolam (m/z 326.043), xylazine (m/z 221.092), and internal standards d10-lidocaine (m/z 245.288), d4-ketamine (m/z 242.095), d4-midazolam (m/z 330.075), and d6-xylazine (m/z 227.127). The responses for the product ions for analytes and internal standards were plotted and peaks at the proper retention time integrated by Quanbrowser software.<sup>h</sup> Quanbrowser software was used to generate calibration curves and quantitate analytes in all samples by linear regression. A weighting factor of 1/X was used for all calibration curves. All calibration curves had a correlation coefficient ( $R^2$ ) of 0.99 or higher.

### Additional Tissue Samples

Frozen skeletal muscle (*gluteus medius*) from 5 additional horses euthanized with intrathecal lidocaine was available for drug analysis. These horses were premedicated and anesthetized in the field situation with routine dosages of IV xylazine (1.1 mg/g), midazolam (0.1 mg/kg), and ketamine hydrochloride (2.2 mg/kg). Intrathecal lidocaine (4 mg/kg) administration was as described previously in Materials and Methods.

### Statistical Analysis

Data were summarized and shown as mean, standard deviation (SD), median, and range values.

## Results

### Animals

Eleven horses of Thoroughbred (n = 10) and Arabian (n = 1) breeds were included in the study. There were 4 males (3 castrated; 1 intact) and 7 females. The mean age was 13.2 years (median, 10; range, 10 months to 24 years). Seven horses had chronic multiple orthopedic

**Table 2.** Skeletal muscle concentrations (ng/g) of drug residues in 5 additional horses at routine dosages of intravenous anesthesia in field situations.

N = 5 muscle	Xylazine (1.1 mg/kg) ng/g	Midazolam (0.1 mg/kg) ng/g	Ketamine (2.2 mg/kg) ng/g	Lidocaine (4 mg/kg) ng/g
Mean	77.6	32.4	1778.5	1.3
SD	38.6	22.9	1398.8	1
Median	87.6	31.1	1909.4	1.2
Range	51.3–133.8	11.4–69.9	603.9–3992.8	0.16–3

diseases, 2 had neurologic diseases (epilepsy, 1; progressive multifocal spinal cord disease, 1), and 2 had chronic progressive systemic disease (metastatic melanoma, 1; weight loss, 1).

### Intravenous Anesthesia and Euthanasia

Intravenous dosages administered for instrumentation were as follows: xylazine hydrochloride ranged from 1.8 to 3.6 mg/kg (mean, 2.5 ± 0.8 mg/kg; median, 2.5 mg/kg), ketamine hydrochloride from 2.8 to 7.4 mg/kg (mean, 5.8 ± 2.5 mg/kg; median, 6.3 mg/kg), and midazolam from 0.05 to 0.1 mg/kg (mean, 0.1 ± 0 mg/kg, median, 0.05 mg/kg). Intrathecal lidocaine hydrochloride dosage was 4 mg/kg.

### Determination of Drug Concentrations

The intraday accuracy (% nominal concentration) and precision (% relative standard deviation) ranged between 100 and 110% and 1 and 9%, respectively, for all drugs. The assay was optimized to provide a limit of quantitation (LOQ) of 0.1 ng/mL and a LOD of 0.01 ng/mL for all analytes in serum and plasma. The LOQ and LOD for CSF were 0.5 ng/mL and 0.05 ng/mL, respectively. The LOQ for muscle tissue was approximately 0.01 ng/mL. Drug residues were identified in the tissues examined, and concentrations are shown in Table 1. Table 1 also depicts the dosages used for each drug. Drug concentrations in skeletal muscle of 5 additional horses are shown on Table 2.

## Discussion

We showed that drug residues from IV anesthesia with xylazine hydrochloride, midazolam hydrochloride, and ketamine hydrochloride after euthanasia with intrathecal lidocaine hydrochloride administration were found in skeletal muscle, serum, and plasma. These residues could represent a health hazard, if horse meat was consumed by scavengers or carnivores. Proper disposal of animals' remains is essential to avoid environmental contamination and health hazards.<sup>4,6</sup> In our study, standard dosages of xylazine hydrochloride (1.1 mg/kg IV) and ketamine hydrochloride (2.2 mg/kg IV) were used for the induction of anesthesia. However, although considerably higher dosages of xylazine hydrochloride and

ketamine hydrochloride were used to maintain immobilization during electrophysiologic instrumentation than would be necessary for field situation euthanasia alone, the drug residues concentrations found in skeletal muscle, serum, and plasma were below the reported sedative or toxic concentrations.<sup>10,11</sup> Standard dosages used of IV xylazine, midazolam, and ketamine hydrochloride in the field situation resulted in lower drug concentrations in skeletal muscle than those found after higher dosages. Lidocaine hydrochloride also was found in skeletal muscle from horses anesthetized by routine dosages. Although all drugs including lidocaine were widely distributed throughout various tissues, the concentrations were far below the concentrations reported to have sedative, much less, toxic effect.<sup>10-14</sup> The muscle groups selected for sampling represent large skeletal muscle masses that likely would make up most of the mass consumed by a carnivore, scavenger, or scavenging human.

Limited information is available about the toxicity of xylazine, ketamine, midazolam, and lidocaine in various species.<sup>10-14</sup> Furthermore, no information is available about the effects of these drugs (such as sedation) on scavenging animals. Because such animals live in the wild, any changes in their level of consciousness could have serious consequences to their welfare,<sup>6</sup> which is the reason for concerns about residue drugs causing even a mild sedative effect. One study indicated that 1 mg/kg of xylazine administered PO will provide sedation in dogs and cats.<sup>11</sup> The therapeutic index of xylazine is relatively small and a 2- to 3-fold overdose could cause death as a result of cardiac and respiratory depression.<sup>12</sup> The concentration of xylazine found in the various tissues in our study was substantially lower than 1 mg/kg; therefore, unlikely to pose a risk if contaminated meat is consumed. Another study determined that the lethal dose of xylazine in mice is 150 mg/kg intraperitoneally.<sup>15</sup> Ketamine hydrochloride at 3 mg/kg provided adequate anesthesia in combination with xylazine with a good safety margin in cats.<sup>11</sup> The ketamine concentrations found in the tissues analyzed in our study were considerably lower than 3 mg/kg. The therapeutic range of midazolam is large, requiring dosages several-fold in excess of 0.5 mg/kg to cause toxicity.<sup>10</sup>

Lidocaine hydrochloride, a widely used and readily available drug, is a sodium channel blocker known to have effects on the cardiovascular and nervous systems.<sup>16-18</sup> Other mechanisms of action influencing neuronal transmission include inhibition of G-protein coupled receptor and N-methyl-D-aspartate receptors.<sup>19</sup> Epidural lidocaine administration has been used commonly during obstetric procedures to provide spinal anesthesia by causing sensory and motor block.<sup>16</sup> Lidocaine in CSF was not measured in our study, but its concentration is presumed to be high because of its route of administration. Although a scavenger might eat central nervous tissue, and thus be exposed to lidocaine, doing so would require opening the cranium or vertebral column, which would be unlikely to occur without considerable effort. The total toxic dosage of

lidocaine has been reported to be 11.1 mg/kg IV for dogs, but a wide range of values (4.6–19.4 mg/kg) has been given depending on rate and method of administration.<sup>20,21</sup> Although cats and goats reportedly are more sensitive, the range of total toxic dose for lidocaine is similar for other species.<sup>22</sup>

Based on the concentrations of drug residues found in our study, animals would have to eat approximately 9–10 kg of meat to ingest 1 mg total of xylazine, 20–27 kg for midazolam, 570–850 g for ketamine, and 2.7–6 kg for lidocaine. These results are different than those for pentobarbital sodium residues in horse meat after an IV overdose for euthanasia.<sup>23</sup> Reported sedative dosages varied from 0.6 to 23 mg of pentobarbital sodium per kg of horse meat.<sup>23</sup> In retrospect, organs other than skeletal muscle should have been sampled in our study. However, spleen, kidney, and liver make up a smaller portion of the carcass and would not be reached by a scavenger until the carcass had started to decompose. Such blood-rich organs might have higher concentrations of drugs. Table 1 shows that the means for all drugs in serum were higher than the mean concentrations in skeletal muscle. Even for xylazine, which showed the largest difference between mean serum and tissue concentrations, the serum concentrations were far below what could possibly cause a sedative and, presumably, a toxic effect when ingested. Carnivores might seek out organs such as liver or spleen, and these might contain drug concentrations approaching those found in blood. However, they still would have to ingest many kg of tissue, even at the higher concentrations of drug found in plasma in this study, to experience a clinical effect. Avian and reptile scavengers and carnivore species also might be affected by eating tissues containing anesthetic drugs. However, the few reports of PO dosages necessary to create sedation in these species suggest that the tissue concentrations found in our study would not cause an effect in nonmammalian scavengers and carnivores.<sup>24,25</sup>

Our study did not address drug decay in tissues over time, as would be expected in a carcass left in the field. Samples were immediately stored at  $-80^{\circ}\text{C}$ , a technique that is standard for preserving tissues for forensic testing of drug concentrations.<sup>26,27</sup> In a study of benzodiazepine drug concentrations over time, it was found that the most volatile agent, midazolam, decreased 5–12% in concentration over a year's time when stored in liquid nitrogen.<sup>28</sup> Loss of concentration of this order for the drugs analyzed in our study would not substantially affect the conclusions drawn.

In conclusion, our study emphasized the potential for sedation effects on the welfare of scavenger and carnivore species that might happen upon a cadaver euthanized by the intrathecal lidocaine technique. Because these sedative effects occur at dosages well below those required for acute toxicity, it can be presumed that ingestion of carrion from such a cadaver would be safe, at least in the short term. Longer-term effects of PO acquired sedative and anesthetic drugs at nanogram concentrations should be investigated in the future.

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## Footnotes

- <sup>a</sup> Cardenas J. Brazil: Personal communication, 2013  
<sup>b</sup> Sigma Aldrich, St Louis, MO  
<sup>c</sup> Cerilliant, Round Rock, TX  
<sup>d</sup> Toronto Research Chemicals, Toronto, ON  
<sup>e</sup> Fisher Scientific, Fair Lawn, NJ  
<sup>f</sup> Glas-Col, Terre Haute, IN  
<sup>g</sup> Burdick and Jackson, Muskegon, MI  
<sup>h</sup> Bertin Technologies, Saint Quentin en Yvelines, France  
<sup>i</sup> Alfa Aesar, Ward Hill, MA  
<sup>j</sup> Thermo Scientific, San Jose, CA  
<sup>k</sup> Shimadzu, Kyoto, Japan  
<sup>l</sup> Mac-Mod Analytical, Chadds Ford, PA
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*Conflict of Interest Declaration:* Authors declare no conflict of interest.

*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

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