Federal Aviation Administration

DOT/FAA/AM-10/11
Office of Aerospace Medicine
Washington, DC 20591

Distribution of Oxycodone in Postmortem Fluids and Tissues

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June 2010

Final Report

NOTICE

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-10/11		

4. Title and Subtitle	5. Report Date
Distribution of Oxycodone in Postmortem Fluids and Tissues	June 2010
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Botch SR, Johnson RD, Chaturvedi AK, Lewis RL	

9. Performing Organization Name and Address	10. Work Unit No. (TRAIS)
FAA Civil Aerospace Medical Institute P.O. Box 25082 Oklahoma City, OK 73125	11. Contract or Grant No.

12. Sponsoring Agency Name and Address	13. Type of Report and Period Covered
Office of Aerospace Medicine Federal Aviation Administration 800 Independence Ave., S.W. Washington, DC 20591	14. Sponsoring Agency Code

15. Supplemental Notes

This work was accomplished under the approved task AM-B-10-TOX-204.

16. Abstract

Introduction: Oxycodone is a heavily used and abused analgesic agent. Its pharmacological effects, including euphoria, respiratory depression, nausea, and drowsiness, have the potential to adversely affect performance. The postmortem distribution of oxycodone has not been well characterized, particularly at sub-lethal levels. Therefore, an attempt was made to evaluate the distribution of oxycodone in postmortem specimens collected from aviation accidents.

Methods: A search of our database identified 4 oxycodone-positive fatalities from separate civil aviation accidents that occurred during a period of 6 years that had numerous biological tissues and fluids available (blood, urine, vitreous humor, liver, kidney, skeletal muscle, lung, spleen, heart muscle, and brain). These specimens were extracted using solid-phase extraction and were analyzed for oxycodone by GC/MS.

Results: Oxycodone concentration ranges (μg/mL, μg/g) found in the different tissues and fluids were: blood 0.027-0.742, urine 2.20 - 12.5, vitreous humor 0.048 - 0.118, liver 0.103-3.35, lung 0.047-1.35, kidney 0.045-3.12, spleen 0.115-2.43, muscle 0.017-0.400, brain 0.032-1.36, and heart 0.038-3.19.

Conclusion: The blood concentrations found indicate that the oxycodone in these cases ranged from therapeutic to above therapeutic, but all were below lethal levels. Tissue/fluid to blood distribution coefficients were found to have large coefficients of variation (ranging from 26-128%), thereby rendering them unreliable for estimating a blood oxycodone concentration from a tissue value when no blood is available for analysis.

17. Key Words	18. Distribution Statement
Forensic Toxicology, Oxycodone, Distribution, GC/MS, Aircraft Accident Investigation	Document is available to the public through the Defense Technical Information Center, Ft. Belvoir, VA 22060; and the National Technical Information Service, Springfield, VA 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	9	

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

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Distribution of Oxycodone in Postmortem Fluids and Tissues

INTRODUCTION

Oxycodone, a semi-synthetic opioid agonist, is available in several formulations, including controlled release (OxyContin®), immediate release (OxyIR®, OxyFast®), or in combination with other non-narcotic analgesics such as acetaminophen (Percocet®, Tylox®, Roxicet®) and aspirin (Percodan®). It is used for the treatment of postoperative pain, pain associated with cancer, and other pain management.[1-3] Oxycodone is commonly employed as a valuable alternative to morphine due to its longer analgesic action.[4,5] Abuse of this semi-synthetic opiod and its role in fatal overdoses has been documented in the literature.[6-11] In addition to the analgesic effects, numerous side effects have been associated with the use of oxycodone. Dizziness, drowsiness, headache, nausea, vomiting, and muscle weakness have been linked with the use of this drug.

Oxycodone is readily absorbed after oral administration and has pharmacokinetics that differs from that of morphine.[5] The volume of distribution (Vd) of oxycodone is 277 ± 187 L/kg and it is a highly protein-bound drug (45%).[4,12,13] The large Vd for oxycodone suggests substantial distribution among all tissues and fluids in the body. The half-life ($t_{1/2}$) for the controlled-release oral formulation of oxycodone has been documented to range from approximately 3.5 to 8 hours,[1,4,5] and peak therapeutic plasma concentrations typically occur within 1 to 3 hours, depending on inter-individual variation.[14-16] The duration of action of immediate release oxycodone is ~4 hours.[5] Up to 19% of ingested oxycodone is excreted unchanged after oral administration.[1] Within 24 hours, 30 to 60% of the original dose has been reported to be excreted in the urine as free and/or conjugated oxycodone, conjugated oxymorphone, and noroxycodone.[14]

Scientific information concerning the distribution of oxycodone in various body compartments is limited.[7,9] Postmortem samples from aviation accident victims are analyzed for drugs at the Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI). A search of our laboratory accident database identified four aviation fatalities that were positive for oxycodone in blood. These cases also had a full complement of other tissue and fluid types available for additional analysis. Therefore, this study was pursued to determine the distribution of oxycodone in postmortem tissues and fluids of the four aviation accident victims.

MATERIALS AND METHODS

Chemicals and Reagents

All aqueous solutions were prepared using double deionized water (DDW), which was obtained using a water purification system (Elga Water Systems; High Wycombe, UK). All chemicals used were purchased in the highest possible purity and used without any further purification. Oxycodone and oxycodone-d_3 were obtained from Cerilliant Corporation (Round Rock, TX) as methanolic standards at concentrations of 1.00 mg/mL and 0.100 mg/mL, respectively, in sealed glass ampoules. Propionic anhydride was obtained from Sigma-Aldrich (St. Louis, MO). The pH of all solutions was measured using a Corning Life Sciences model 430 pH meter (Acton, MA), connected to a Corning 3-in-1 model pH electrode.

Gas Chromatographic/Mass Spectroscopic Conditions

Analyses were performed using a bench-top gas chromatograph/mass spectrometer (GC/MS), which consisted of a Hewlett Packard (HP) 6890 series GC, interfaced with a HP 5973 quadrupole MS (Agilent; Palo Alto, CA). The GC/MS was operated with a transfer line temperature of 280°C and a source temperature of 250°C. The MS was tuned on a daily basis using perfluorotributylamine. The electron multiplier voltage was set at 106 eV above the tune value. Chromatographic separation was achieved using a Varian FactorFour® crosslinked 100% methyl siloxane capillary column 12 m x 0.2 mm i.d., 0.33 µm film thickness (Varian Co.; Harbor City, CA.). Helium was employed as the carrier gas at a flow rate of 1.0 mL/min. An HP 6890 autosampler was used to inject 1 µL of extract into the GC/MS. The GC was equipped with a split/splitless injection port operated at 250°C in the splitless mode with the purge time of 0.5 min. The oven temperature profile employed for the oxycodone analysis was: 130°C – 240°C @ 20°C/min, 240°C – 244°C @ 2°C/min, 244°C – 290°C @ 40°C/min, with a final hold time of 1.35 min. Quantitation and qualifier ions for each analyte were then selected based on both abundance and mass-to-charge ratio (m/z). To increase reproducibility and reduce interference, high-mass ions were selected when possible. The ions chosen for oxycodone were 371 (quant), 314, 372, and for oxycodone-D_3 were 374 (quant), 317, 375. Upon selection of unique ions, the MS was run in selected ion monitoring (SIM) mode with a dwell time of 30 msec for each recorded ion.

Sample Selection and Storage

A search of the CAMI ToxFlo™ database (DiscoverSoft Development, LLC; Oklahoma City, OK) identified four oxycodone-positive fatalities from separate civil aviation accidents that occurred during a period of 6 years (2003-2008). These four cases had a majority of the desired biological tissues and fluids (blood, urine, vitreous humor, liver, kidney, skeletal muscle, lung, spleen, heart muscle, and brain) were used for the study. In all cases, blood was stored at -20°C in tubes containing 1.00% (w/v) sodium fluoride/potassium oxalate until analysis. All other specimens were stored without preservation at -20°C until analysis.

Calibrator and Control Preparation

Calibration curves for oxycodone were prepared by serial dilution utilizing bovine whole blood as the diluent. Calibrators were prepared from one set of original stock standard solutions, while controls were prepared in a similar manner as calibrators but from a second set of unique stock solutions. The calibration curve was prepared at concentrations ranging from 3.13 – 800 ng/mL. A minimum of seven calibrators were used to construct the calibration curve. Controls were prepared at concentrations of 80 and 320 ng/mL and extracted with the unknowns to verify the accuracy of the calibration curve and the validity of the extraction. The internal standard solution, oxycodone-d_3, was prepared at a concentration of 400 ng/mL in DDW.

Quantitation was achieved via an internal standard calibration procedure. Response ratios for each compound were determined for every sample analyzed. The response ratio was calculated by dividing the area of the analyte peak by the area of the internal standard peak. Calibration curves were derived by plotting a linear regression of the analyte/internal standard response ratio versus the analyte concentration for each respective calibrator. These calibration curves were then used to determine the concentrations of each compound in the prepared controls and biological specimens. Acceptability criteria employed for analyte identification and quantitation were as follows: 1) ion ratios for a given analyte, measured as the peak area of a qualifier ion divided by the peak area of the quantitation ion, were required to be within ± 20% of the average of the ion ratios for each respective calibrator used to construct the calibration curve for that analyte; 2) each ion monitored was required to have a minimum signal-to-noise ratio (S/N) of 10; and 3) the analyte was required to have a retention time within ± 2.00% of the average retention time for each respective calibrator used to construct the calibration curve for that analyte. Analytes not meeting these criteria were reported as either negative or inconclusive.

Sample Preparation and Extraction Procedure

Postmortem specimens, calibrators, and controls were extracted in the following manner. Tissue specimens were homogenized in DDW (1.00% NaF) with a 1:2 dilution tissue:DDW, using an Omni post-mounted homogenizer (Marietta, GA). The generator used with this homogenizer was 30 mm in diameter and set to rotate at 22,000 rpm. Three-mL aliquots of fluid, calibrator, and control, and 3.0 g aliquots of each tissue homogenate (1.0 g tissue) were transferred to individual 16 x 150 mm screw-top tubes. To each specimen, calibrator, and control, 1.00 mL of the internal standard 400 ng solution was added. Samples were vortexed briefly and allowed to stand at room temperature for 10 min. Six mL of 0.10 M phosphate buffer, pH 6.00 was added to each sample, and the tubes were then shaken by hand for 2 min. Centrifugation at 1230χg for 45 min provided removal of cellular debris and proteins.

The extracts were transferred to Bond Elute Certify® solid-phase extraction (SPE) columns (Varian Co.; Harbor City, CA.), which had been pre-conditioned with 2.00 mL methanol, followed by 2.00 mL water. Care was taken not to dry the column prior to adding the extract. Column flow rates of 1-2 mL/min were maintained in each SPE step using a Varian 24 port Cerex® SPE processor (Varian Co.; Harbor City, CA.) with a nitrogen pressure of 3 psi. Once each sample had passed through its respective column, the columns were washed with 1.00 mL of water, followed by 1.00 mL of 0.10 M sodium acetate, pH 4.00. The columns were again washed by adding 2.00 mL methanol to each. Following the methanol wash, the columns were dried completely with 25 psi nitrogen for 5 min. The analytes were eluted off the columns with 3.00 mL of 2.00% ammonium hydroxide in an 80:20 mixture of methylene chloride/isopropanol, which was prepared fresh daily. Eluents were evaporated to dryness in a TurboVap® Concentration Workstation at 40°C (Caliper Life Sciences; Hopkinton, MA) under a stream of dry nitrogen. The procedure used is a standard analytical procedure of our laboratory for multi-opiate analysis, requiring derivatization. Therefore, even though oxycodone may be analyzed without derivatization, the derivatization step was performed so that analytical parameters could be directly compared to those that had been previously obtained. The derivatization was accomplished by adding 50 µL of pyridine, followed by 50 µL of propionic anhydride to each specimen tube. The samples were capped tightly, vortexed, and incubated at 40°C for 60 min. The tubes were allowed to cool to room temperature, and the contents were evaporated to dryness under a stream of dry nitrogen in a TurboVap® set at 40°C. Once dry, the contents of each tube were reconstituted in 50 µL of ethyl acetate and transferred to GC/MS vials for analysis.

RESULTS AND DISCUSSION

The procedure used for the analysis of oxycodone was reproducible and sensitive. Oxycodone and oxycodone-d_3 chromatographic peaks suffered no interference from endogenous/exogenous matrix components. The mass spectra of oxycodone and oxycodone-d_3 provided numerous high mass ions. It should be noted that an isotope ion was used as one of the qualifier ions for both oxycodone and oxycodone-d_3. This approach was necessary due to cross-contribution between the analyte and oxycodone-d_3 when other ions were evaluated. The linear dynamic range (LDR), limit of detection (LOD), and limit of quantitation (LOQ) for oxycodone were determined while utilizing bovine whole blood as the matrix. The LDR were determined to be 3.13 – 800 ng/mL. Correlation coefficients for the calibration curves used were greater than 0.997 when a weighting factor of 1/X was employed. The LOD was defined as the lowest analyte concentration detectable that met the above-discussed identification criteria. The LOQ was defined as the lowest analyte concentration detectable that not only met all identification criteria, as discussed above, but also had an experimentally determined concentration within ± 20% of its prepared value. The LOD for oxycodone was determined to be 1.56 ng/mL. The LOQ for this compound was determined to be 3.13 ng/mL.

Carryover was not found to be a problem on the GC/MS; however, it was initially investigated and subsequently monitored by the use of ethyl acetate blank injections. The injection of an ethyl acetate blank following the 800 ng/mL calibrator showed no carryover contamination. Subsequently, ethyl acetate blanks were utilized between each postmortem specimen throughout the sample sequence to verify that no sample-to-sample contamination had occurred.

Whole blood oxycodone concentrations found in the four cases examined ranged from 0.027 to 0.742 μg/mL and were within 10% of the value originally determined. This finding verified that no deterioration in oxycodone blood concentration had occurred during storage. Therapeutic concentrations of oxycodone in whole blood range from 0.010 to 0.100 μg/mL.[17] Toxic levels of oxycodone have been reported to be 0.200 - 5.000 μg/mL and vary greatly.[17] Blood concentrations in these cases could generally be above therapeutic and neared possibly toxic levels. The concentration of oxycodone in each postmortem specimen from these four cases is given in Table 1. With a relatively large Vd (277 ± 186 L/kg),[4] oxycodone was expected to be found at high concentrations in the tissues analyzed, which was consistent with our findings. The mean concentrations (μg/mL, μg/g) of oxycodone found were: blood 0.408 (0.027-0.742, n=4); urine 7.36 (2.20 and 12.5, n=2); vitreous humor 0.083 (0.048 and 0.118, n=2); liver 1.46 (0.103-3.35, n=4); lung 0.477 (0.047-1.35, n=4), kidney 0.991 (0.045-3.12, n=4), spleen 1.10 (0.115-2.43, n=4); muscle 0.165 (0.017-0.400, n=4); brain 0.437 (0.032-1.36, n=4); and heart 0.900 (0.038-3.19, n=4). The distribution coefficients for oxycodone, expressed as specimen type/blood ratio ± SD, are summarized in Table 2. The oxycodone

Table 1. Oxycodone concentrations obtained from 4 pilot fatalities.*

Case	Blood	Urine	VH†	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart
1	0.317	12.5	—‡	3.35	1.35	0.312	2.43	0.400	1.36	3.19
2	0.741	—	—	2.12	0.315	0.638	1.58	0.157	0.220	0.273
3	0.027	—	0.048	0.103	0.047	0.045	0.115	0.017	0.033	0.038
4	0.137	2.20	0.119	0.403	0.195	0.155	0.272	0.085	0.136	0.099

* All concentrations shown in units of g/mL or g/g
† Vitreous Humor
‡ Specimen type not available for analysis

Table 2. Postmortem tissue distribution coefficients for oxycodone.

	Urine/ Blood	VH*/ Blood	Liver/ Blood	Lung/ Blood	Kidney/ Blood	Spleen/ Blood	Muscle/ Blood	Brain/ Blood	Heart/ Blood
n	2	2	4	4	4	4	4	4	4
Mean	27.8	1.32	5.04	1.96	1.16	4.00	0.68	1.69	3.14
s.d.†	11.7	0.45	3.22	1.41	0.30	2.30	0.38	1.53	4.02
CV‡	42	34	64	72	26	57	55	90	128

* Vitreous Humor
† Standard deviation
‡ Coefficient of variation

distribution coefficients for these specimen types had coefficient of variation (CV) values between 26 and 128%.

Although blood drug concentrations may aid in determining impairment and/or cause or manner of death, the availability of blood samples from aviation accident fatalities may not be always an option. This limitation is primarily due to the destructive nature of aviation accidents on the human body. In fact, the FAA laboratory receives blood from only approximately 70% of the fatal cases, and the majority of the blood samples are collected from the chest cavity. Such blood samples can not only readily experience postmortem redistribution, but they can easily be contaminated with drug(s) from ruptured organs. Therefore, caution must be exercised when using drug values obtained from chest cavity blood.

Based upon the present study, an attempt was made to establish distribution coefficients for oxycodone between the various tissue/fluids and blood. Those coefficients might be useful to potentially estimate a blood concentration in cases where blood is not available. Though the CV values varied considerably (Table 2), the results obtained from the limited number of cases suggest that oxycodone blood concentrations cannot be estimated from any of the tissue types examined.The large range in distribution values within a given specimen/blood ratio could be due to the postmortem redistribution of the drug with respect to the different postmortem intervals, the blood collection sites, and/or presence of other drugs.[18] It might also be affected by whether the victim was a chronic or an acute user of oxycodone. It is well established that the postmortem redistribution of drugs is more pronounced with basic drugs as they have large apparent volumes of distribution. For oxycodone, the Vd is 277 ± 187 l/kg.[4,12,13] In spite of these limitations and variations, it is still important to gain knowledge of drug concentrations in tissues as these analytical values may help in differentiating therapeutic use of the drugs from their overdose.[19] Additionally, such information will contribute to the limited tissue distribution data available in the literature for this drug. However, one has to be careful when drawing final conclusions from this data because the blood collection sites and postmortem intervals were often not known or not established.

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