Distribution of Butalbital in Postmortem Tissues and Fluids from Non-Overdose Cases*

Russell J. Lewis¹, Robert D. Johnson, Travis L. Southern, and Dennis V. Canfield
Civil Aerospace Medical Institute, Federal Aviation Administration, Toxicology and Accident Research Laboratory, AAM-610, P.O. Box 25082, Oklahoma City, Oklahoma 73125

Abstract
During the investigation of fatal aviation accidents, postmortem samples from the pilots/co-pilots are submitted to the Federal Aviation Administration's (FAA) Civil Aerospace Medical Institute (CAMI) for toxicological analysis. Although therapeutic levels for most drugs are typically reported in the scientific literature for blood and plasma, blood specimens are received in only approximately 70% of our cases. Therefore, it is imperative for an accident investigator and forensic toxicologist to be able to estimate drug concentrations in an aviation accident victim's blood from available tissue drug concentrations. This is exemplified by a recent aviation fatality in which butalbital was identified in the muscle tissue of a pilot. In this case, no blood was available for analysis, but investigators needed to know the approximate butalbital concentration expected in the victim's blood. Certain side effects of butalbital, such as drowsiness, sedation, dizziness, and a feeling of intoxication, could affect pilot performance and become a significant factor in an aviation accident. Thus, our laboratory determined the distribution of butalbital in various postmortem tissues and fluids. The distribution coefficients for butalbital, expressed as specimen/blood ratios, were found to be as follows: 0.66 ± 0.09 (muscle, n = 4), 0.98 ± 0.09 (kidney, n = 4), 0.87 ± 0.06 (lung, n = 4), 0.75 ± 0.03 (spleen, n = 4), 0.96 ± 0.07 (brain, n = 3), 2.22 ± 0.04 (liver, n = 4), and 0.91 ± 0.17 (heart, n = 2). The results obtained from our limited number of cases suggest that muscle, kidney, lung, spleen, brain, liver, and heart could be used, in a cautious and conservative fashion, to estimate butalbital blood concentrations.

Introduction
The Federal Aviation Administration's (FAA) Civil Aerospace Medical Institute (CAMI) is responsible under Department of Transportation (DOT) orders to "conduct toxicologic analysis on specimens from...aircraft accident fatalities" and "investigate...general aviation and air carrier accidents and search for biomedical and clinical causes of the accidents, including evidence of...chemical (use)." Therefore, following an aviation accident, samples are collected at autopsy and sent to CAMI's Toxicology and Accident Research Laboratory where toxicological analysis is conducted on various postmortem fluids and tissues.

Because of the violent collisions, explosions, and fires often associated with aircraft accidents, crew members' bodies may be fragmented, incinerated, disintegrated, or scattered over large areas of rough terrain or bodies of water. In many cases, the search for remains results in only small fragments of tissues being submitted for toxicological analysis. In fact, the FAA's Toxicology and Accident Research Laboratory receives blood specimens in only 70% of fatal aviation accidents. Therapeutic levels of a drug are typically reported in the scientific literature for blood or plasma only. Because blood is not available in all cases, it may be necessary for accident investigators and forensic toxicologists to estimate drug concentrations in an accident victim's blood from an available tissue's drug concentration. This is exemplified by a recent aviation fatality where butalbital was identified in the muscle tissue of a pilot. In this case, no blood specimen was available for analysis, but investigators wanted to know the approximate butalbital concentration expected in the victim's blood.

Butalbital, a short-acting barbiturate found in combination with other drugs such as acetaminophen, aspirin, codeine, and caffeine, is commonly prescribed for the treatment of tension headaches (1,2). There are specific side effects, however, that could affect a pilot's performance and become a significant contributory factor in an aviation accident. The most serious of these side effects includes drowsiness, sedation, dizziness, and a feeling of intoxication (1,2). Additional side effects of barbiturate exposure include a withdrawal syndrome that is characterized by psychosis, personality changes, or seizures and a rebound syndrome that is characterized by chronic and/or daily pain or headaches (3,4).

A limited amount of butalbital distribution data, predominantly from drug-overdose fatalities, has been reported (5,6). Utilizing drug-overdose data in estimating blood values in therapeutic cases is of limited value because of potential abnormal

---

* This research is part of an Office of Aviation Medicine Research Report.
1 Author to whom correspondence should be addressed.
Drug pharmacokinetics and/or postmortem redistribution, which is evident in the wide range of distribution coefficients reported (7). Because scientific information concerning the distribution of butalbital at therapeutic levels is not available, our laboratory set out to determine its distribution in various postmortem tissues and fluids. A search of the Toxicology and Accident Research Laboratory database identified only two fatal aviation cases that reported positive for butalbital in blood and also had a full complement of biological tissues and fluids, including muscle, liver, kidney, lung, spleen, brain, heart, urine, and bile, available for analysis. This report details the quantitative and distribution of butalbital in postmortem samples and identifies specimens that may be suitable for estimating blood concentrations of butalbital.

Materials and Methods

Chemicals and reagents

Butalbital and butalbital-d₅ standards were purchased from Cerilliant (Cerilliant Corp., Round Rock, TX) at 1.00 mg/mL concentrations in methanol. The derivatization reagent, Meth-Elute, was obtained from Pierce Chemical Co. (Rockford, IL). Distilled-deionized water was prepared using a MilliPore Milli-Q benchtop purification device (Millipore, Continental Water Systems, El Paso, TX) and was used for all reagent preparations. All other necessary chemicals and reagents were obtained from commercial sources in high purity and used with no further purification.

Gas chromatographic–mass spectrometric conditions

All analyses were performed using a benchtop gas chromatograph–mass spectrometer (GC–MS), which consisted of a Hewlett-Packard (HP) 6890 series GC connected to an HP 5973 quadrupole MS operating with a transferline temperature of 280°C and a source temperature of 250°C. The MS was auto-tuned on a daily basis using perfluorotributyl-amine. The electron multiplier voltage was set at the autotune voltage with no offset. All chromatographic separations were achieved using an HP-ULTRA 1 cross-linked 100% methyl slyxane capillary column (12-m × 0.2-mm i.d., 0.33-μm film thickness). Helium was used as the carrier gas with a flow rate of 1.0 mL/min. An HP 6890 autosampler was used to inject 1 μL of sample extract into the GC. The GC was equipped with a split/splitless injection port operated in the splitless mode with a purge time of 1 min, then ramped at 40°C/min to a final temperature of 290°C, which was held for 1 min, yielding a total run time of 7.83 min. Butalbital had a retention time of 3.5 min. Standard solutions of butalbital and butalbital-d₅ were separately analyzed using the full scan mode from 50 to 500 amu in order to select unique quantitation and qualifier ions. The ions chosen were m/z 196, 181, and 138 for butalbital, and m/z 201, 200, and 184 for butalbital-d₅. Once appropriate ions were selected, the MS was operated in selected ion monitoring mode (SIM) with a dwell time of 40 ms.

Butalbital concentrations were determined using an internal standard calibration procedure. The calibration curve was prepared by plotting the linear regression of the analyte/internal standard response factor versus the analyte concentration. Response factors were determined for each specimen, and the various analyte concentrations were then obtained from the calibration curve.

Preparation of standards

The calibration curve was prepared by diluting a butalbital standard in whole blood yielding concentrations ranging from 25 to 800 ng/mL. Controls were prepared in whole blood at 80, 160, and 320 ng/mL using separate drug standards and were used to validate the calibration curve. The internal standard solution was prepared by diluting a standard of butalbital-d₅ with water to yield a final concentration of 400 ng/mL.

Sample handling

The positive butalbital cases identified by the Toxicology and Accident Research Laboratory database spanned a 2-year period. All samples were analyzed at one time to avoid interassay variations. Blood butalbital values determined in this study were compared to those previously determined to ensure no deterioration occurred. In all cases, blood was stored at –20°C in tubes containing 1% (w/v) sodium fluoride/potassium oxalate until analysis. All other specimen were stored without preservation at –20°C until analysis.

Sample preparation and extraction procedure

Tissues were homogenized with a Brinkmann Tissue Homogenizer (Brinkmann Instruments, Westbury, NY) following a 1:1 dilution with water. Three-milliliter aliquots of specimen fluids, calibrators, and controls and 2-g samples of tissue homogenates were transferred to individual 15-mL screwtop vials. To each specimen, calibrator, and control, 1 mL of the internal standard solution (400 ng/mL) was added. The samples were vortex mixed and allowed to stand for 10 min. The cellular debris and proteins were precipitated and removed from the samples by adding 9 mL of cold acetonitrile, mixing on a rotary extractor for 15 min, and finally centrifuging at 820 × g for 5 min. The supernatant was transferred to 15-mL culture tubes and evaporated in a water bath at 40°C under a stream of dry nitrogen to a volume of less than 1 mL. To this, 3 mL of 0.1M sodium acetate buffer (pH 7.0) was added. The extracts were transferred to solid-phase extraction (SPE) columns that were pre-conditioned with 5 mL methanol followed by 2 mL sodium acetate buffer (pH 7.0). The SPE columns used were Bond Elute Certify II™ procured from Varian (Harbor City, CA). Care was taken not to dry the column prior to sample addition. A column flow rate of 1–2 mL/min was maintained in each step using a Varian 24 port positive pressure manifold with a nitrogen pressure of approximately 3 psi. Once the samples had passed through the columns, the columns were washed with 1 mL 0.1M sodium acetate buffer (pH 7.0), dried with 25 psi nitrogen for 5 min, and then washed with 2 mL hexane/ethyl acetate (95:5). Butalbital was eluted from the columns with 4 mL hexane/ethyl acetate (75:25). Eluents were evaporated to dryness in a water bath at 40°C under a stream of dry nitrogen. The ex-
tricts were reconstituted in 75 μL MethElute, transferred to GC autosampler vials, and analyzed once.

Results and Discussion

The mass spectrum of butalbital and butalbital-d5 provided numerous ions. From these ions m/z 196 was selected as the quantitation ion for butalbital and m/z 201 was selected as the quantitation ion for butalbital-d5. Ions m/z 181 and 138 were used as qualifier ions for butalbital, and m/z 200 and 184 were chosen as qualifier ions for butalbital-d5. A calibration curve (correlation coefficient = 0.998) of six points was constructed from 25 to 800 ng/mL and was employed for butalbital quantitation for all specimens. With appropriate specimen dilutions, all results fell within the calibration curve. We found the LOQ and LOD to be 25 and 3 ng/mL, respectively. The extraction procedure provided a clean extract in a relatively short period of time.

Therapeutic levels of butalbital in plasma range from 1 to 10 μg/mL (8). The blood concentrations found in the four cases examined ranged from 0.221 to 11 μg/mL. With butalbital having a blood/plasma ratio of 1.0 (6), blood concentrations in these cases ranged from slightly above therapeutic to slightly below it. The distribution and concentration of butalbital found in the various cases are presented in Table I. The wide range of butalbital blood concentrations found suggest that the victims took the drug at differing times prior to the occurrence of the accident and/or they took different dosages of the drug.

The distribution coefficients for butalbital, expressed as specimen/blood ratios, are summarized in Table II. As can be seen in the table, the distribution coefficients for muscle, kidney, lung, spleen, brain, liver, and heart all have a coefficient of variation (CV) of less than 20%. The relatively small CVs associated with these distribution coefficients suggest that, in these cases, butalbital was in the post-absorption phase. Although butalbital has been reported to undergo significant postmortem redistribution at lethal levels (7), the small CVs associated with the distribution coefficients presented here suggest that postmortem redistribution was not a major factor in these cases.

The distribution of butalbital in urine and bile was initially investigated. These experiments were discontinued because of large variations in the initial distributions obtained. These findings were not unexpected, however, because of the excretory nature of these fluids. Therefore, urine and bile were excluded from this study.

There are no widely accepted criteria for what constituted an acceptable distribution coefficient; however, we believe it is feasible and reliable to use a tissue or fluid distribution coefficient to estimate a blood concentration in cases where blood is not available if the distribution coefficient has a CV of less than 20%. Therefore, the results obtained from our limited number of cases suggest that butalbital concentration in muscle, kidney, lung, spleen, brain, liver, and heart could be used to estimate blood values ranging from slightly below to slightly above therapeutic levels. Although, admittedly, a study involving a greater number of samples from a larger pool of cases needs to be completed in order to more definitively verify these results, the findings from this study indicate the possible utility of various postmortem tissues in approximating butalbital blood concentrations at therapeutic levels.

Conclusions

Ideally, all toxicological analyses would be conducted using blood samples. Because of the nature of forensic toxicology, however, the laboratory must rely upon the specimens available for analysis. There is an obvious need for the ability to estimate drug concentrations in blood from values determined in various other tissues or fluids. In this study, our laboratory established the distribution of butalbital at levels ranging from slightly below to slightly above therapeutic in various postmortem specimens. The results obtained from the limited number of cases examined suggest that the tissues discussed herein could be used, in a cautious and conservative fashion, to estimate therapeutic levels of butalbital in blood.

References


Manuscript received July 15, 2002;
revision received October 8, 2002.