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Distribution of Δ^9-Tetrahydrocannabinol and 11-Nor-9-Carboxy-Δ^9-Tetrahydrocannabinol Acid in Postmortem Biological Fluids and Tissues From Pilots Fatally Injured in Aviation Accidents

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16. Abstract

Despite a long history of research on the pharmacology of Δ^9-tetrahydrocannabinol (THC), the primary active cannabinoid in marijuana, little is known of its distribution in postmortem fluids and tissues. This study presents postmortem fluid and tissue data for THC and its major metabolite, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH), from 55 pilots involved in fatal aviation accidents from 2005 – 2012.

Utilizing immunoassay screening followed by confirmation using gas chromatography/mass spectrometry, blood, urine, liver, brain, lung, heart, kidney, and muscle were analyzed, as needed, for each of the 55 cases.

Particular attention was paid to lung as this tissue is exposed to cannabinoid-rich marijuana smoke. Mean THC concentrations in blood, liver, lung, and kidney were 12.5 ng/mL, 52.8 ng/g, 766.0 ng/g, and 27.1 ng/g, respectively. Mean THCCOOH concentrations in those same specimens were 34.1 ng/mL, 322.4 ng/g, 38.0 ng/g, and 138.5 ng/g, respectively. Limited data were available for heart tissue (2 cases), muscle (2), and brain (1). Heart THC concentrations were 184.4 and 759.3 ng/g. The corresponding heart THCCOOH measured 11.0 and 95.9 ng/g, respectively. Muscle concentrations for THC were 16.6 and 2.5 ng/g; corresponding THCCOOH, "positive" and 1.4 ng/g. The only brain tested in this study showed 0.0 ng/g THC and 2.9 ng/g THCCOOH, low concentrations that correlated with low values in other specimens from this case.

This research emphasizes the need for cannabinoid testing in postmortem forensic toxicology laboratories. In addition, it demonstrates the usefulness of a number of tissues, most notably lung, for these analyses.

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CONTENTS

Distribution of Δ^9-Tetrahydrocannabinol and 11-Nor-9-Carboxy-Δ^9-Tetrahydrocannabinol in Postmortem Biological Fluids and Tissues From Pilots Fatally Injured in Aviation Accidents

INTRODUCTION ---------- 1
- Materials and Methods ---------- 2
- Chemicals and Reagents ---------- 2
- Pilot Demographics ---------- 2
- Specimen Collection ---------- 2
- Cannabinoid Screening ---------- 2
- Confirmation and Quantitation of Cannabinoids ---------- 2

RESULTS ---------- 3
- Blood ---------- 3
- Urine ---------- 3
- Tissues ---------- 4

DISCUSSION ---------- 4
CONCLUSIONS ---------- 7
REFERENCES ---------- 7

Distribution of Δ^9-Tetrahydrocannabinol and 11-Nor-9-Carboxy-Δ^9-Tetrahydrocannabinol in Postmortem Biological Fluids and Tissues From Pilots Fatally Injured in Aviation Accidents

INTRODUCTION

The National Institute on Drug Abuse (NIDA) found that 18.1 million people were classified as current marijuana (*Cannabis sativa*) users in the United States and 5.4 million people aged 12 and older used marijuana on a daily or almost daily basis in the prior 12 months.[1] A significant number of Americans (4.2 million) were clinically diagnosed as being dependent on, or abusers of, marijuana in 2011. Marijuana contributed to 455,668 emergency department visits with the highest use among 21-24 yr olds.[2] As evidenced by a recent report from the European Union, cannabis use is common in many parts of the world.[3]

As a result of such widespread marijuana use and its well-documented effects on behavior and psychomotor performance, workplace drug testing and postmortem forensic toxicology laboratories are frequently called upon to analyze biological specimens for evidence of marijuana ingestion. Forensic toxicologists then are asked to opine on the effects of marijuana and any correlations that might be drawn between those effects and cannabinoid concentrations in biological specimens.

The relationship between concentrations of the primary psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (THC), and its effects on behavior and psychomotor performance has been the focus of decades of research.[4] THC has a broad spectrum of central nervous system (CNS) and physiological actions. In addition to the well-studied euphoria, other dose-related effects of THC on the CNS include deficits in time perception, concentration and information processing, as well as impaired learning and memory. These effects can last up to 28 days after abstinence from the drug.[5] Effects on performance skills associated with driving have demonstrated detriments in such areas as vision, perceptual motor skills, decision making, and reaction time.[4,6]

Negative effects on complex skills associated with flying an airplane have also been demonstrated. Using an ATC-510 flight simulator, research authored by Meacham et al. and Janowsky et al. showed that smoking marijuana increased major and minor flying errors, altitude and heading deviations, and radio navigation errors.[7-9] Leirer et al. found performance decrements in pilots were based upon the additive effects of marijuana, age, and task difficulty.[10] Another study by the same group demonstrated a significant carry-over effect on pilot performance up to 24 h post administration of a moderate dose of marijuana.[11] An important finding of this study was that all but one of the pilots studied were not aware they were impaired.

Scant research has been done to assess cannabinoid concentrations in postmortem specimens. Most work on postmortem samples has been performed on fluids.[12-15] Lin and Lin reported on urine, blood, vitreous humor, and bile concentrations of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) in traffic fatalities.[16] An animal model for THC distribution in the pig found that postmortem changes in the concentrations of THC do occur and that postmortem blood THC values from multiple sites did not correlate with perimortem concentrations.[17]

A lack of postmortem data has resulted in a dearth of knowledge regarding the postmortem distribution of cannabinoids in humans primarily because cannabinoid testing is not routine in many postmortem toxicology laboratories due to the long-held belief that marijuana does not directly cause death. This assertion has recently been challenged, however, with evidence that cannabis induced increases in heart rate, and alterations in blood pressure may result in significant cardiovascular toxicity.[18,19]

The high lipid solubility and rapid distribution of THC into the tissues, coupled with extensive metabolism by the liver, present significant challenges to interpreting cannabinoid concentrations. Moreover, when attempting to correlate analytical data, the postmortem forensic toxicologists have additional problems. While blood from a peripheral site is considered the fluid specimen of choice for evaluating marijuana's contribution to a postmortem case, this specimen is not always available for analysis because of trauma, decomposition, exsanguination, or embalming. It is imperative, therefore, that the forensic toxicology community understands how cannabinoids are distributed in postmortem fluids and tissues to assign interpretive value, if possible, to these alternate specimens.

In 2010, the Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) reported increasing concentrations of THC and THCCOOH in postmortem blood and urine from aviation accidents between 1997 and 2006.[20] This publication coincided with other research showing that cannabis potency has increased.[21] Two articles in 2011 specifically investigated postmortem cannabinoid distribution. Holland et al. examined the postmortem redistribution of THC, its equipotent metabolite, 11-hydroxy-THC (11-OH-THC), and the inactive metabolite, THCCOOH, in 19 medical examiner cases.[22] Their findings included only a slight degree of postmortem redistribution (central:peripheral concentration ratio < 2.0) and a rising trend of redistribution with increasing postmortem interval. Lemos and Ingle examined blood and urine cannabinoid concentrations in 30 postmortem cases.[23] Interestingly, they found mean central/peripheral blood concentration ratios of 0.62, 1.07, and 0.99 for THC, THCCOOH, and 11-OH-THC, respectively. Their findings suggested only a modest degree of postmortem redistribution despite THC having characteristics associated with significant redistribution such as a high volume of distribution (10 L/kg). These authors

also concluded that cannabinoid testing should be routine in all postmortem investigations.

Through its mission to perform toxicological investigations on pilots fatally injured in aviation accidents, the Bioaeronautical Sciences Research Laboratory at CAMI has a unique opportunity to study postmortem fluid and tissue concentrations of THC and its metabolites in pilots.[24] These data can then be used to characterize the patterns of marijuana use in the aviation industry and its effect on pilot performance.

In the current publication, we describe the results of a study undertaken at CAMI to further characterize the postmortem fluid and tissue distribution of THC and THCCOOH in aviation accident pilot fatalities. The principle route of administration for marijuana is smoking.[4] After inhalation, the lung is exposed to cannabinoid-laden smoke. THC and numerous other cannabinoids are absorbed and deposited. Therefore, a particular focus of this research was an evaluation of lung tissue as a potentially acceptable choice of biological specimen for cannabinoid analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Methanolic, deuterated and non-deuterated THC and THCCOOH (1.0 mg/mL) were obtained from Cerilliant Corporation (Round Rock, TX). All solvents were HPLC grade or higher, unless otherwise indicated, and were obtained from Pierce Chemical Company (Rockford, IL) and Fisher Scientific (Pittsburgh, PA). All aqueous solutions were prepared using double deionized water, which was obtained using a Purelab® Ultra water system (Siemens Corp., Syracuse, NY). The derivatization reagents, boron trifluoride (BF_3) and trifluroacetic anhydride (TFAA), were obtained from Pierce Chemical Company. The pH of all solutions was measured using a Corning model 430 pH meter (Corning Life Sciences, Acton, MA) connected to a Corning 3-in-1 model pH electrode.

Pilot Demographics

Between 2005 and 2012, our laboratory received biological samples from 2121 fatal aviation accidents, 2045 of which were from pilots operating the aircraft. Cannabinoids were detected in 55 pilots (2.7% of the pilot fatalities). There were 54 male pilots and 1 female pilot. Their mean age was 44.8 ± 11.6 yr (median, 46 yr). The majority of the fatal accidents in which cannabinoids were detected in the pilots occurred in Florida (7) and California (7).

Specimen Collection

Details of the sample submission procedure at CAMI were published previously.[25] Specimens were submitted via commercial courier in collection kits designed specifically by CAMI. Each case was assigned a unique accessioning number and the samples were stored frozen (–20°C) until time of analysis.

As can be seen in Table 1, not all fluids and tissues were analyzed for every case. As with most postmortem forensic toxicology laboratories, not all specimens are routinely analyzed. In addition, due to the severity of injuries from aviation accidents, not all tissue and fluid samples are available for analysis. Between 2005 and 2012, forty-three cases had blood available for cannabinoid testing, the majority of which (39) were drawn from the heart or thoracic cavity. Two samples were obtained from the subclavian vein (#17 and #22) and one from the femoral vein (#23). One blood specimen was labeled "peripheral" (#51). All blood samples tested in this study were collected in gray top Vacutainer® blood collection tubes containing potassium oxalate and sodium fluoride.

Cannabinoid Screening

Blood was initially analyzed using enzyme-linked immunosorbant assay following the method specification prescribed by the manufacturer (Immunalysis Corp, Pomona, CA). Tissues were homogenized with extraction buffer (4×tissue weight) before analysis by the manufacturer's method. Cutoff THCCOOH concentrations for the cannabinoid assay were 15 ng/mL for blood and 40 ng/g for other tissues.

Urine specimens were screened by fluorescence polarization immunoassay following the manufacturer's method specifications on the AxSYM™ system (Abbott Labs, North Chicago, IL). The cutoff concentration of THCCOOH in urine was 25 ng/mL.

In addition to open controls and calibrators, the CAMI laboratory also includes blind controls in each batch of samples as an added check of analytical quality.

Confirmation and Quantitation of Cannabinoids

Confirmation and quantitation of THC and THCCOOH in blood and tissues were accomplished by solid phase extraction (SPE) and negative-ion chemical ionization (NCI) gas chromatography/mass spectrometry (GC/MS) utilizing modified, previously published procedures that were validated for use at CAMI.[26] Urines were extracted in the same manner, but the extracts were analyzed by GC/MS using electron impact ionization (EI).

Prior to extraction, tissues were homogenized in aqueous 1% sodium fluoride (1:2) and 3.0 gram of the homogenate was analyzed. Deuterated THCCOOH (50 ng d_3-THCCOOH) was added as internal standard to 3.0 mL blood or 3.0 g tissue homogenate in glass, screw-capped test tubes. Cold (–4°C) acetonitrile (9.0 mL) was added to each specimen. The tubes were shaken vigorously for 3.0 min and centrifuged at 2,000 rpm for 5 min. The supernatant was transferred to a clean, glass tube. The samples were evaporated to approximately 2.0 mL in a 40°C water bath under a stream of nitrogen.

Urine samples were subjected to alkaline hydrolysis prior to extraction. As with the blood and tissue samples, 50 ng d_3-THCCOOH was added as the internal standard to 3.0 mL urine. This was followed by the addition of 300 µL of 10 M potassium hydroxide and placement of the samples in a heating block at 70°C for 15 min. The samples were allowed to cool and the pH adjusted to 6.0 by the addition of 165 µL of glacial acetic acid. The pH was further adjusted to 6.0 with glacial acetic acid, if necessary.

Following column activation with the sequential addition of 2.0 mL methanol and 2.0 mL of 0.1 M acetate buffer (pH 7.0), 5.0 mL of 0.1 M acetate buffer and 250 µL of methanol were added to each sample, calibrator, and control tube. The tubes were vortexed for approximately 10 s and poured over their respective Bond Elut Certify II SPE columns (Agilent Technologies, Santa Clara, CA). A Cerex ® positive pressure manifold (Varian Corporation, Palo Alto, CA) was used to facilitate elution (2.5 – 5.0 psi). The columns were rinsed with 1.0 mL 0.1 M acetate buffer (pH 7.0) and allowed to dry at 25 psi for 5 min.

THC and THCCOOH were eluted in 2 fractions. THC was eluted in the neutral fraction into glass round-bottom tubes with 3.0 mL of a hexane:ethyl acetate (95:5) solvent mixture. The THC eluates were carefully transferred into glass, conical, screw-capped tubes such that no water accompanied the organic phase into the second tube. The SPE columns were then rinsed with 4.0 mL of a methanol:water (1:1) solvent mixture and dried at 25 psi for 5 min. THCCOOH was eluted in an acidic fraction from the SPE columns, into new glass, round bottom test tubes with 4.0 mL 1% acetic acid in a hexane:ethyl acetate (75:25) mixture. The THCCOOH eluates were carefully transferred into glass, conical, screw capped tubes such that no water contaminated the organic phase in the second tube. Both the THC and the THCOOH eluates were dried to residue in a nitrogen evaporator set at 40ºC (N-Evap, Organomation Associates, Berlin, CA).

The THCCOOH residues were treated with 200 µL methanolic BF_3, vortexed, and placed in a heating block at 70ºC for 20 min. The extracts were allowed to come to ambient temperature, and 2.0 mL deionized water and 5.0 mL hexane were added. The tubes were shaken vigorously for 3.0 min and centrifuged at 2000 rpm for 5 min. The upper organic layer was transferred to the THC eluate tubes, and the lower aqueous layer was discarded. All tubes were evaporated to dryness in the nitrogen evaporator set at 40ºC.

The combined neutral and acidic fraction residues were treated with 300 µL chloroform and 300 µL of TFAA. The tubes were vortexed, capped, and incubated in a heating block at 70ºC for 20 min. The samples were evaporated to dryness, reconstituted with 150 µL hexane, and transferred to amber glass autosampler vials in preparation for instrumental analysis.

The blood, urine, and tissue extracts were analyzed by GC/MS. A 6890 gas chromatograph, a 5973 mass selective detector, and a 7673 autosampler were used in the analysis (Agilent Technologies, Santa Clara, CA). The GC/MS was equipped with a 100% methylsiloxane capillary column (0.2 mm I.D. × 12 m length × 0.33 µm film thickness). The carrier gas was ultra-high purity helium. The injector temperature was 250ºC; the transfer line was maintained at 300ºC. The initial oven temperature of 70ºC was ramped to 290ºC at 30ºC/min and held at 290ºC for 2.67 min.

For bloods and tissues, the MS was operated in NCI mode using methane as the reagent gas. Selected ion monitoring was used for identification and quantitation of THC and THCCOOH and their deuterated analogues. NCI typically produces little fragmentation, thus a single ion was monitored for each analyte (THC, 410 m/z; THC-d_3, 413 m/z; THCCOOH, 454 m/z; THCCOOH-d_3, 457 m/z).

Urine extracts were analyzed using the same GC temperature program but with the MS operating in EI mode. Selected ion monitoring was used to identify and quantitate of THC, THCCOOH, and deuterated internal standards (THCCOOH, 439, 454, 395 m/z; THCCOOH-d_3, 442, 457, 398 m/z).

Identification of the analytes was based upon retention time of the respective ions. Quantitation of samples and controls (blind and open) was based upon peak area ratios (analyte/internal standard) compared to a standard curve. The limit of detection and limit of quantitation for THC and THCCOOH by NCI were 1.0 ng/mL in blood and 1.0 ng/g in tissues. For THCCOOH in urine by EI, the limits of detection and quantitation were 2.5 ng/mL.

RESULTS

Results from the analysis of THC and THCCOOH in fatally injured pilots from aviation accidents are found Table 1. This study was initiated to evaluate the distribution of THC and THCCOOH in postmortem samples with a particular interest in investigating the usefulness of lung tissue as an alternative matrix for the detection of cannabinoids. Therefore, the bulk of the data is composed of blood and lung concentrations of THC and THCCOOH, along with urine concentrations of THCCOOH. Urinary THC is not currently analyzed at CAMI. Liver, brain, heart, kidney, and muscle were analyzed for THC and THCCOOH as the cases warranted.

All 55 pilots who tested positive for cannabinoids by one of the screening procedures described above were thus carried forward for confirmation and quantitation.

Blood

Of the 55 cases that screened positive for cannabinoids, 42 had blood samples available for confirmation and quantitation for THC and THCCOOH. Of the 42 blood samples that underwent confirmatory testing, quantitative data was obtained for THC and THCCOOH in 40 cases. One blood (#50) was attempted but found to be unsuitable for THC analysis due to the possible presence of interfering substances. Another sample (#4), while meeting criteria for a positive identification for both THC and THCCOOH, was reported only as "positive."

Blood THC concentrations ranged from 0 to 69.2 ng/mL (n=41). Mean and median blood THC concentrations were 12.5 (±18.1) and 5.0 ng/mL, respectively. THCCOOH concentrations in blood (n=41; 1 case reported "positive") ranged from 0 to 200.5 ng/mL with a mean of 34.1 (±51.3) ng/mL. The median THCCOOH concentration in blood was 14.8 ng/mL.

Urine

The parent compound, THC, is not analyzed in urine at CAMI, thus, only THCCOOH concentrations are reported in this study. Urine THCCOOH concentrations (n=30) ranged

from 2.7 ng/mL to 2549.4 ng/mL. The mean and median THCCOOH concentrations in urine were 315.4 (± 510.9) ng/mL and 98.4 ng/mL, respectively.

Tissues

Lung. We were particularly interested in investigating lung tissue as a possible alternative matrix for identifying and quantitating cannabinoids. Since the primary route of administration for marijuana is inhalation, a reasonable hypothesis was that cannabinoids, particularly THC, would accumulate in the lungs. As a result, considerably more lung samples were tested than other tissues (Table 1).

THC and THCCOOH were present in the lung in considerable quantities. THC was confirmed present in 40 lung specimens, 39 quantitatively (1 specimen was reported as confirmed positive without quantitative results). The mean THC concentration (n=39) was found to be 766.0 (± 1204.2) ng/g. The range of THC concentrations was 6.4 to 6379.5 ng/g, with a median concentration of 220.0 ng/g. The mean THCCOOH concentration in lung (n=37; 3 were confirmed positive only) was 38.0 (± 59.4) ng/g. The range of THCCOOH concentrations was 0.0 to 313.0 ng/g, with a median of 18.7 ng/g. Based upon the mean values, the THC concentration in the lung was 20 times higher than that of THCCOOH.

Liver. Under the analytical conditions used for this study, the cannabinoids proved to be more difficult to extract and identify from liver. THC was successfully analyzed in only 14 (41.6%) of 24 attempted analyses. The remaining 10 samples were unsuitable for analysis. The mean THC concentration (n = 14) for the successful tests was 52.8 (± 72.4) ng/g. The range of concentrations was 0.0 to 237.4 ng/g, with the median being 28.0 ng/g.

The analysis of THCCOOH in liver was relatively more successful. All 24 cases in which THCCOOH analysis was attempted met acceptance criteria. One of the cases (#34) was reported confirmed positive with no quantitative value because the concentration was above the limit of detection but below the lowest calibrator. The mean (n = 23) THCCOOH concentration found in liver was 322.4 (± 632.9) ng/g. Concentrations ranged from 8.0 to 2515.3 ng/g, with a median of 96.9 ng/g. THCCOOH in liver was present in considerably higher concentrations than that of THC.

Kidney. Confirmation and quantitation of THC and THCCOOH were performed on 13 of the 55 cases that screened positive for cannabinoids. Both compounds were detectable in kidney. The mean THC concentration was 27.1 (± 36.3) ng/g, while the mean THCCOOH concentration was 138.5 (± 134.8) ng/g. THC ranged from 0.0 to 122.0 ng/g (median, 15.3 ng/g), and THCCOOH ranged from 2.8 to 392.1 ng/g (median, 88.7 ng/g). THCCOOH was also found to be present in higher amounts than the parent compound.

Other tissues. Very limited data were obtained for muscle, brain, and heart, but these specimen types were included for completeness. No statistical analysis was performed on these specimens.

Muscle was analyzed for THC and THCCOOH in only 2 cases. In case#30, THC was found in muscle at 16.6 ng/g, with THCCOOH being confirmed positive but below the lowest calibrator. Case #37 also tested positive for THC and THCCOOH in muscle at 2.5 ng/g and 1.4 ng/g, respectively.

THC was not detected in the brain of case #37, the only brain analysis performed from 2005 to 2012. This correlates with the very low concentration of THC found in the muscle (2.5 ng/g) from the same case. THCCOOH was found in the brain of case #37 at 2.9 ng/g, also correlating with the low muscle concentration of 1.4 ng/g.

Heart tissue was analyzed for THC and THCCOOH in 2 cases (#8 and #44). Both compounds were easily detected in this specimen type. THC and THCCOOH heart concentrations found in case #8 were 184.4 ng/g and 11.0 ng/g, respectively. THC and THCCOOH heart concentrations found in case #44 were 759.3 ng/g and 95.9 ng/g, respectively. THC in heart was present in relatively higher concentrations than its carboxylic acid metabolite, consistent with concentrations found in the lung tissue.

DISCUSSION

Little systematic research has been done to determine the postmortem distribution of cannabinoids in humans. CAMI analyzes cannabinoids in postmortem samples collected from fatally injured pilots as part of routine toxicological investigations into aviation accidents. As a result, this study was performed to evaluate the prevalence of marijuana in pilot fatalities from aviation accidents. In addition, we specifically determined the detectability and distribution of THC and THCCOOH in postmortem fluids and tissues.

Cannabinoids were confirmed in fluids and/or tissues from 55 (2.7%) of 2045 deceased pilots submitted to CAMI for toxicological evaluation from 2005 to 2012. By comparison, the Office of the Chief Medical Examiner of San Francisco, California reported that, of 1,338 postmortem toxicology cases from July 1, 2010 to June 30, 2011, cannabinoids were confirmed in 30 (2.2%) accidental deaths.[27] The Florida Department of Law Enforcement reported a 4.3% positive rate in postmortem cases in 2012.[28] Positive rates for cannabinoids in automobile drivers in 3 recent roadside studies were 7.7% in Spain, 8.5% in California (USA), and 9.8% in Australia.[29-31]

The mean age (44.8 ± 11.6 yr) and median age (46 yr) of the 55 pilots in the present study are consistent with data from the previously published Office of Aerospace Medicine report showing a median age of 45 for civil aviation pilots.[32] Interestingly, the mean and median pilot ages found in the present study are higher than previously published reports for cannabinoid-positive automobile drivers and autopsy cases. For example, Jones et al. reported a mean age of 33 yr (range 15 – 66 yr) for their study investigating the frequency of THC in Driving Under the Influence of Drugs (DUID) cases in Sweden.[33] Mean and median ages were 38.2 and 35 yr, respectively, in a study of postmortem cannabinoid concentrations from 30 autopsy cases.[23]

Forensic toxicologists are asked to correlate blood concentrations of cannabinoids with the well-known behavioral effects of marijuana. In the current study, blood concentrations of THC and THCCOOH varied widely from 0 to 69.2 ng/mL for THC and 0 to 200.5 ng/mL THCCOOH (Table 1). The mean concentration of THC in blood from this study was 12.5 ng/mL. This represents an approximately 1.7-fold increase in mean THC concentrations over the mean concentration (7.2 ng/mL) found in a previous CAMI study examining cannabinoid data in fatal aviation accidents from 2002 to 2006.[20] The mean concentration of THCCOOH, 34.1 ng/mL, represents a 1.5-fold increase over the previous CAMI study. The increase in mean blood concentration of THC and THCCOOH in the current study may reflect the continuing trend showing an increase in THC potency seen in confiscated cannabis preparations by the NIDA Potency Monitoring Program.[21, 34]

A difficulty in interpreting blood concentrations in aviation accidents is that the vast majority of blood samples submitted to CAMI are obtained from the thoracic cavity, rather than any particular organ or blood vessel such as the heart or femoral vein. The reason for this is that extensive damage to the body results from an aviation accident, making the body cavity the only source of blood available for analysis. The interpretive value of cavity blood, therefore, is quite low as it is subject to contamination from multiple sources, including diffusion from the liver, gastric contents, and urine from the bladder. Therefore, interpretation is limited to proving exposure to marijuana.

Four samples were identified as heart blood (#32, #49, #50, #55). There were 2 samples from the subclavian vein (#17, #22), 1 sample from the femoral vein (#23), and 1 sample labeled "peripheral" (#51). However, it should be emphasized that even blood from intact organs, such as heart, subclavian vein, and, to some degree, femoral vein, may be subject to the well-known phenomenon of postmortem redistribution, the diffusion of drugs along concentration gradients from highly concentrated tissues to lower concentrated blood.[35] Recent research comparing heart blood and peripheral blood cannabinoid concentrations suggests that cannabinoids exhibit only a modest degree of postmortem redistribution despite having chemical characteristics suggesting a propensity for a greater effect.[22, 23]

Urine was only analyzed for THCCOOH. The mean and median concentrations were 315.4 ± 510.9 ng/mL and 98.4 ng/mL, respectively. The range of concentrations was 2.7 to 2549.4 ng/mL. These data almost mirror urinary THCCOOH concentrations found in a study of traffic fatality cases (mean, 314 ng/mL; median, 415 ng/mL; range, 44 – 2330 ng/mL).[16] Urine samples were subjected to alkaline hydrolysis with 10 M potassium hydroxide and heat at 70°C. Therefore, there was no data from these cases on the percentage of free versus conjugated THCCOOH for this study. In addition, we do not routinely analyze for THC in urine. This would require enzymatic hydrolysis with β-glucuronidase to cleave the ether bonded glucuronide.[36]

While there are a few reports in the scientific literature examining postmortem fluid concentrations of cannabinoids (blood, urine, vitreous, bile), little data exists regarding postmortem tissue cannabinoid concentrations.[13, 16] Table 1 shows tissue data for THC and THCCOOH found in the 55 cases submitted from 2005 to 2012. Most of the tissue testing was performed on lung, liver, and kidney. Heart, muscle, and brain, however, were also included in this dataset (Table 1) for completeness. But the very limited number of analyses for these specimens prevented any statistical evaluation.

Given that marijuana is usually consumed by smoking, the inhaled cannabinoids are introduced into the body through the pulmonary tract via the mouth, through the trachea, and into the lungs. Users generally inhale deeply and hold the smoke in their lungs as long as possible in order to maximize absorption of the pharmacologically active compound, THC. Consequently, it seems reasonable to hypothesize that THC would be present in significant concentrations in postmortem lung tissue.

As can be seen in Table 1, lung tissue from 40 of 55 fatally injured pilots was analyzed for THC and THCCOOH. THC and THCCOOH were easily detectable in lung tissue using the described methodology. As hypothesized, THC was found in noteworthy concentrations, albeit highly variable, ranging from 6.4 to 6379.5 ng/g and a median of 220.0 ng/g. The mean of 766.0 ± 1204.2 ng/g was over 14 fold higher than the next highest mean tissue concentration of 52.8 ng/g found in liver. These data support the hypothesis that THC can be detected in lung in high concentrations, likely the result of marijuana inhalation and the extensive storage capacity due to the large surface area of the lungs, with roughly 300 million alveoli. It would be expected, though as yet not studied, that the concentration pattern would be different if marijuana was consumed orally.

THCCOOH was also detected in lung tissue, but in far lower concentrations (20 times less) than THC. The mean concentration of THCCOOH in lung was 38.0 ± 59.4 ng/g and the median concentration was 18.7 ng/g. For this study, these THCCOOH values were comparable to those found in postmortem blood but were the lowest mean and median concentrations of the tissues tested (Table 1). The presence of THCCOOH is not surprising since it is the major THC metabolite detected in humans. The low concentration may be due to the hydrophilic nature of THCCOOH resulting in reduced deposition in the tissues. However, drug metabolizing enzymes, including cytochrome P450 enzymes, are known to be present in extrahepatic organs, including lung.[37,38] The reduced THCCOOH concentration in lung may also be the result of low metabolic enzyme activity in human lung compared to other tissues.[39]

It is clear from the current study that lung tissue is suitable for detecting THC and THCCOOH in autopsy cases under the analytical conditions utilized for this project. The usefulness of lung concentrations of THC and THCCOOH for determining behavior or impairment of an individual remains elusive. Information on when a pilot may have smoked or ingested marijuana prior to an aviation accident is rarely, if ever, available for CAMI investigators. Thus, more research is necessary to evaluate any possibility of correlation between postmortem lung concentration and the behavioral effects of THC.

Table 1. Specimen type and analytical data for cannabinoids in 55 pilots involved in fatal aviation accidents.

Case no.	Collection Site (blood)	THC							THCCOOH							
		Blood	Liver	Brain	Lung	Heart	Kidney	Muscle	Blood	Urine	Liver	Brain	Lung	Heart	Kidney	Muscle
1	cavity	7.5	23.0						121.9		162.6					
2	NA						22.5			543.2					392.1	
3	cavity	4.0	0.0						CP		10.6					
4	cavity	CP							4.3	79.2						
5	cavity	4.8			115.0				29.8	1113.5			39.4			
6	cavity	5.1			83.9				12.2	101.5			7.5			
7	cavity	23.9			1365.9				37.3				38.8			
8	cavity		NS		2863.7	184.4				308.2	123.5		18.7	11.0		
9	cavity	1.8			219.2				7.8	8.3			10.5			
10	cavity	20.3			1524.9				178.6	545.2			101.8			
11	cavity	2.8			133.1				27.0	456.8			29.6			
12	cavity	7.0			438.0				28.4				22.0			
13	cavity	64.9	43.4		1220.7				168.1		2515.3		313.0			
14	cavity	2.2	80.6		170.2				14.8		96.9		18.0			
15	cavity	39.7							128.0	2549.4						
16	cavity	0.0							3.7	36.9						
17	subclavian	7.8							16.7							
18	NA		0.0				10.4			29.7	52.1				88.7	
19	cavity	0.0							2.7	2.7						
20	cavity	3.1	0.0		95.0				4.8		250.4		6.3			
21	cavity	69.2	NS		1556.9				200.5	349.0	1207.5		91.8			
22	subclavian	51.1			1302.8				32.3	451.4			44.8			
23	femoral	3.3			351.0				8.6	554.5			13.8			
24	cavity	47.9			1508.1				40.2				36.2			
25	cavity	7.5			538.7				29.1				20.1			
26	cavity	0.0							1.2	18.6						
27	cavity	3.2	NS		135.0				10.1	140.9	111.3		11.5			
28	cavity	6.6			232.2		0.0		13.4	69.0			10.8		70.3	
29	cavity	5.9			179.9		15.3		11.7	116.0			10.3		129.1	
30	NA		NS				42.1	16.6			22.3				53.8	CP
31	cavity	0.0			21.2				5.6	56.6			CP			
32	heart	34.2	51.3		415.5				97.4	334.6	135.0		129.3			
33	cavity	0.0			12.6		0.0		0.0	11.9			0.0		2.8	
34	NA		0.0		CP						CP		CP		CP	
35	cavity	3.7			673.5		47.0		25.5				44.6		371.1	
36	NA		NS		220.0		72.6				104.7		29.5		176.0	
37	NA			0.0				2.5		30.4		2.9				1.4
38	cavity	0.0			6.4				1.6	23.8			1.2			
39	cavity	16.9	237.4		2901.0				15.6	348.2	37.2		10.2			
40	NA		NS		17.8		0.0				52.2		0.0		26.7	
41	NA		NS		1976.0		122.0				249.4		37.2		208.1	
42	cavity	3.5			24.1				1.6	23.0			0.0			
43	cavity	8.2	33.0		1363.5				27.2		1751.1		34.7			
44	NA				6379.5	759.3							168.6	95.9		
45	cavity	0.0	NS		1080.9				0.0	12.5	9.4		CP			
46	cavity	2.1	107.7		19.8				2.4		15.7		0.0			
47	NA		NS				0.0				8.0				6.4	
48	cavity	16.6			275.6		20.5		24.2	987.9			32.6		261.8	
49	heart	0.0			183.1				6.8				5.8			
50	heart	NS	NS		46.0				11.1	95.2	87.3		9.4			
51	"peripheral"	7.2							17.4							
52	NA		0.0		13.8						44.9		8.7			
53	cavity	2.7							5.4	65.0						
54	NA		0.0		16.5		0.0				26.0		3.4		14.2	
55	heart	13.9	162.8		192.1				23.9		341.7		45.4			
Min		**0.0**	**0.0**		**6.4**		**0.0**		**0.0**	**2.7**	**8.0**		**0.0**		**2.8**	
Median		**5.0**	**28.0**		**220.0**		**15.3**		**14.8**	**98.4**	**96.9**		**18.7**		**88.7**	
Max		**69.2**	**237.4**		**6379.5**		**122.0**		**200.5**	**2549.4**	**2515.3**		**313.0**		**392.1**	
Mean		**12.5**	**52.8**		**766.0**		**27.1**		**34.1**	**315.4**	**322.4**		**38.0**		**138.5**	
SD		**18.1**	**72.4**		**1204.2**		**36.3**		**51.3**	**510.9**	**632.9**		**59.4**		**134.8**	

CP = Confirmed positive only

NA = Not available

NS = Sample not suitable for analysis.

THC was also detected in liver (Table 1). Mean and median THC concentrations were 52.8 ± 72.4 ng/g and 28.0 ng/g, respectively. The analysis of THC was more problematic, as evidenced by the 10 cases (14 of 24 total cases) in which the liver was determined to be unsuitable for analysis. For all 10 cases, THC analysis was attempted (they have corresponding THCCOOH concentrations), but unknown interfering substances inhibited adequate recovery of THC or interfered with the chromatography of the selected ions for THC or the deuterated internal standard.

THCCOOH in liver was confirmed in all 24 cases analyzed. The highest mean tissue concentration was found in liver for this study, at 322.4 ± 632.9 ng/g and a median of 96.9 ng/g. One case was reported as "positive" without quantitation. The liver is the primary metabolic organ for THC and, thus, would be expected to contain a significant THCCOOH concentration. The concentrations were highly variable between cases.

Kidney proved to be a useful specimen for detecting THC and THCCOOH. Both cannabinoids extracted well from this tissue. THCCOOH was detected in higher concentrations than THC. Mean and median concentrations of THCCOOH were 138.5 ± 134.8 ng/g and 88.7 ng/g, respectively. Mean and median THC concentrations were 27.1 ± 36.3 ng/g and 15.3 ng/g, respectively. The higher THCCOOH, compared to THC, may reflect the deposition of THCCOOH from the bloodstream as the metabolite is being excreted. The metabolic enzymes found in kidney may also affect THCCOOH production.[38]

THC and THCCOOH were detected in heart tissue in the 2 cases that were tested (#8 and #44). It is interesting to note that the THC concentrations in both cases (184.4 ng/g and 759.3 ng/g) were significantly higher than the corresponding THCCOOH metabolite (11.0 ng/g and 95.9 ng/g). This large concentration difference may be due to simple diffusion from the lungs, which are in close proximity to the heart. It may also be the result of the normal circulation path of the blood of the marijuana user. Once it returns from the body, blood is pumped by the right ventricle of the heart into the lungs via the pulmonary artery. While in the lungs, oxygen and inhaled THC are deposited in the blood. The newly oxygenated, and THC-rich blood returns to the heart via the pulmonary veins, flowing into the left atrium and left ventricle. The heart tissue, therefore, is exposed to high concentrations of THC from the lungs.

Muscle tissue was analyzed in 2 cases (#30 and #37). THC and THCCOOH were detected in both cases, suggesting that muscle is a viable alternative for cannabinoid testing. Brain was tested for THC and THCCOOH in only one case (#37). Results for other tissues from this case, including lung, were low. It was not surprising, therefore, that brain was negative for THC and only 2.9 ng/g THCCOOH was present. More cannabinoid data are needed for these tissues before any estimation can be made on their interpretive value.

CONCLUSIONS

This study adds to the growing body of data describing the postmortem distribution of THC and THCCOOH. To our knowledge, it is the first research project focusing on pilots involved in fatal aviation accidents. In addition, this is the first study to provide a comprehensive evaluation of the postmortem distribution of THC and THCCOOH in multiple specimen types, combining blood and urine concentrations with data from liver, lung, kidney, muscle, heart, and brain.

Mean blood THC concentrations in this study from 2005 to 2012 show an increase over that from a previous study from CAMI spanning 1997 to 2006.[20] As suggested by the authors of the first publication, increasing blood concentrations in deceased pilots from this study seem to correlate with increasing marijuana potency.

A variety of tissue types are useful for detecting marijuana use in postmortem cases. THC is present in high concentrations in lung due to the popular inhalational route of administration. THCCOOH is also detectable in lung, albeit in much lower concentrations than THC. From the 2 cases tested, heart tissue appears to be another site where THC and THCCOOH are easily detected. More research is necessary before it is known whether or not tissues will provide any interpretive help with time of ingestion or psychomotor impairment. A weakness of this study is the lack of data from the brain, the primary site of action for the behavioral and performance effects of cannabinoids. Further research will focus on the brain to assist forensic toxicologists with the interpretation of the cannabinoid concentrations found in this tissue type.

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