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CASE REPORT

TOXICOLOGY

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Investigation of Postmortem Absorption and Redistribution After the Application of a Fentanyl Patch

ABSTRACT: Fentanyl deaths have increased with availability of transdermal patches. Interpretation of postmortem fentanyl levels may be complicated by postmortem redistribution and absorption of fentanyl from a patch. We applied an unused 100-μg/h fentanyl patch onto the lower abdomen of a decedent with no premortem fentanyl exposure. Ocular fluid, blood, and urine were collected prior to placement, and the decedent was refrigerated for 23 h. Prior to the autopsy, urine, subcutaneous tissue under the patch, and samples from the same anatomic sites were obtained. We observed no fentanyl in any postpatch placement samples (LOD: 0.1 ng/mL for blood and vitreous fluid, 1.0 ng/mL urine, 2.0 ng/g for tissues). Although we observed no postmortem absorption of fentanyl, this was only a single case; therefore, we recommend that patches be removed after receipt of a cadaver before initiation of an autopsy, with the location of removed patch documented.

KEYWORDS: forensic science, fentanyl transdermal patch, postmortem redistribution, postmortem absorption, liquid chromatography/mass spectrometry, stratum corneum

Fentanyl is an analgesic and anesthetic agent that is about 100 times more potent than morphine and has been used since 1963. When the drug was first made available, deaths due to fentanyl overdose were infrequent. The drug was largely administered in the hospital by trained medical professionals, and patients were monitored. On August 7, 1990, a transdermal fentanyl patch (Duragesic®) was approved to Janssen Pharmaceuticals by the U.S. Food and Drug Administration (FDA) (1). Patches are available in 25, 50, and 100 μg/h dosages. The availability of the patch enabled patients more freedom of dosing themselves. While the patch enabled a constant and more convenient delivery of drug for patients with chronic pain, it also accelerated the availability and abuse of the drug, resulting in a dramatic rate of fentanyl abuse. Using data from the Drug Abuse Warning Network (DAWN), the number of emergency department cases where fentanyl was mentioned increased 50-fold from 1994 to 2002 to nearly 1600 cases (2). During this same time interval, the number of prescriptions increased by only sevenfold (2). From 2002 to 2009, the number of fentanyl mentions redoubled to over 3500 mentions according to DAWN (3). In an attempt to reduce the incidence of toxicity and abuse, the FDA issued a series of warnings aimed at the public, directed to adults and children (4–6).

Interpreting results of fentanyl use as a cause of death from postmortem examinations is a challenge due to the postmortem redistribution phenomenon. The ratio of postmortem blood collected centrally (C) to that collected peripherally (P) has been used as a measure of redistribution, under the assumption that the central blood is more subjected to changes (7). While there are some cases where the C:P ratio is >1.0, suggesting that there is postmortem redistribution to the central tissues, there are also deaths that are associated with the C:P ratio at or around 1.0, indicating that the redistribution had not or was less likely to have occurred (8).

If postmortem blood and tissue concentrations are to be used in the investigation of fentanyl deaths, it would be helpful to determine whether there is postmortem release of fentanyl after death from patches left on the decedent. In the context of gastrointestinal absorption of drugs, Pelissier-Alicot et al. (9) suggested that filtration could occur for small water-soluble molecules through damaged pores if there is a concentration gradient. These authors further opined that weak organic bases (e.g., fentanyl) cross lipid membranes by active transport. This requires energy such as ATP, which is not available shortly after death. Palmer suggested that the specific transdermal transport of fentanyl across the skin is highly unlikely due to the absence of circulation needed to drive release (10). Also, if the patch is located in a peripheral area of the body, for example, lower leg or arm, systemic transdermal absorption postmortem is unlikely. Nevertheless, there are no studies that have addressed this issue. We examined postmortem fentanyl concentrations in a variety of fluids and tissues after the application and incubation of a trans-

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dermal patch on the skin of a decedent that was naïve to fentanyl use prior to death.

Materials and Methods

Case Report and Study Protocol

The decedent was a 50-year-old Caucasian man with no documented medical history other than lower extremity varicose veins. He was on no prescribed medication but did abuse cigarettes, marijuana, and ethanol. He had not seen a physician for *c.* 15 years prior to his death. According to his wife, the decedent had a blood pressure of 280/160 mmHg taken at a local pharmacy shortly before his death. On the day of his death, the decedent complained of dizziness and pain in his right arm. He offered no specific complaints of chest pain, left arm pain, or jaw pain. No difficulty in breathing was noted. His wife witnessed him become unresponsive while resting in a chair. Emergency medical services responded to the scene and documented an initial rhythm of asystole. Attempts at cardioversion were unsuccessful.

The decedent sustained a witnessed cardiac event and was pronounced dead at his residence at 8:19 pm. After the scene investigation performed by members of the medical examiner's office, the body was transported to the District 21 Medical Examiner's Office, Fort Myers FL and logged into the facility at 11:06 p.m. (Day 1). The body was placed in the incoming cooler 2.8 h after having been pronounced dead. The body had no external or internal evidence of decomposition. The decedent was a 76-inch, 244 pound (body mass index 29.7 kg/m^2) white male, who appeared to be the stated age of 50 years. There were no surgical scars identified. Rigor mortis and nonblanching lividity were present at the time of autopsy. To determine eligibility for the study, postmortem toxicology was performed on Day 2. Results were negative for ethanol, acetone, methanol, and isopropanol by headspace gas chromatography, and negative for amphetamines, barbiturates, benzodiazepines, buprenorphine, cannabinoids, cocaine metabolite, fentanyl, methadone, opiates, salicylates, and tricyclic antidepressants. Testing was conducted at the Wuesthoff Reference Laboratory (Viera, FL) using immunoassays from Neogen Corp. (Lexington, KY). Gas chromatography–mass spectrometry and liquid chromatography tandem MS were positive for nicotine metabolite, caffeine, and caffeine metabolite from the vitreous fluid and left femoral blood samples. Having been deemed eligible, family members of the decedent were given details of the study and asked for their permission to participate. No review for human subjects research was deemed necessary as the subject was deceased. Once the authorization/release was signed, the decedent was removed from the cooler and prepatch postmortem samples were obtained on the same day at 9:15 a.m. Ocular fluid (red top) and subclavian blood, superior vena cava blood (confirmed at the time of autopsy), and femoral blood were drawn and submitted in gray top tubes. The actual anatomic sites were marked with an "x" for future reference. These samples presumably devoid of fentanyl were used as the background or baseline. A new 100 ug/h Duragesic transdermal patch was used. As per the instructions from the package insert, a single patch was placed on the decedent's right lower/lateral abdomen. The decedent was then placed back into the refrigerator for 23 h.

The autopsy was conducted on Day 3. Postfentanyl patch placement and pre-autopsy samples were obtained from the same sites as sampled on the previous day. The underlying skin/subcutaneous tissues directly underneath the patch were dissected and submitted for analysis. During the autopsy, urine, and samples of brain, lungs, liver, and kidneys were obtained and frozen. All samples were forwarded, in dry ice, overnight to NMS Labs (Willow Grove, PA) for analysis.

The decedent was noted to be overweight (BMI 29.7) and without evidence of acute blunt, penetrating or sharp force injury. The lower extremities had no evidence of pretibial edema. His face and upper thorax were plethoric, commonly visualized in individuals who die of cardiac issues. Internally, the pericardial sac was invested by a large amount of bright yellow, dense adipose tissue. A pericardial effusion was not noted. The heart was enlarged (560 g) and was associated with an increase in subepicardial adipose tissue. The left main coronary artery, the left anterior descending coronary artery, the left circumflex coronary artery, and the right main coronary artery had severe to critical atherosclerotic disease with near luminal occlusion. Both atria were dilated, and the left ventricle had concentric hypertrophy. The right ventricle was dilated. The myocardium had no gross evidence of recent or remote infarctions. Microscopically, the heart muscle had myocyte hypertrophy and foci of interstitial fibrosis but no evidence of acute ischemia. The lungs were heavy, wet, and congested. Microscopically, the lungs had emphysematous changes. Both kidneys were surrounded by dense, bright yellow adipose tissue. The kidneys had granular subcapsular surfaces (consistent with nephrosclerosis and hypertensive and arteriosclerotic cardiovascular disease). The cause of death was certified as "hypertensive and arteriosclerotic cardiovascular disease", and the manner of death was ruled "natural."

Fentanyl Analysis

Tissue samples were prepared by homogenizing one part tissue with three parts deionized water. Liquid–liquid extraction was used to clean up the samples and concentrate any analytes for analysis. Briefly, 25 uL of deuterated internal standard mixture (0.04 ng/uL d5-fentanyl and 0.02 ng/uL d5-norfentanyl) was added to 0.5 mL of sample (diluted tissue homogenate or blood). Samples were made basic with 5 uL ammonium hydroxide and 4.0 mL of n-butyl chloride: acetonitrile (4:1) was added. Samples were mixed by rotation for 20 min and then centrifuged. The organic layer was transferred to a clean labeled test tube, and the sample was evaporated to dryness at $55 \pm 5°C$ under nitrogen. The sample was reconstituted in 200 uL in a 80:20 mixture of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in methanol). After vortexing, the reconstituted sample was transferred to an autosampler vial for analysis. Fentanyl analysis was performed by liquid chromatography–tandem mass spectrometry. The chromatographic and mass spectrometric conditions used for this study are shown in Table 1.

Results and Discussion

Heart blood, femoral blood, subclavian blood, and vitreous fluid collected before and after patch application and urine, liver, brain, kidney, and lung tissue collected after patch application were analyzed. Fentanyl was not detected in any sample given with the reporting limits of 0.1 ng/mL for blood and vitreous fluid, 1.0 ng/mL for urine specimens, and 2.0 ng/g for all tissues.

TABLE 1—*LC-MS/MS conditions used for the fentanyl assay.*

Injection volume	5 μL	
HPLC column	Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm)	
LC instrumentation	Waters Acquity Ultra Performance LC	
Elution	Gradient	
MS instrumentation	Micromass Quattro Micro tandem mass spectrometer	
Source ionization	Atmospheric pressure ionization	
Acquisition parameters		
Voltage for capillary	3500 V	
Cone	14 V	
Extractor	1.0 V	
Lens	0.0 V	
Source temperature	120°C	
Desolvation temperatures	400°C	
Cone gas flow rate	Off	
Desolvation gas flow rate	450 L/h	
MRM Transition ions (*m/z*)		
D5-Fentanyl	342.2 → 188.2 (quant)	342.2 → 105.2 (qualifier)
Fentanyl	337.2 → 188.2 (quant)	337.2 → 105.2 (qualifier)
D5-Norfentanyl	238.2 → 84.1 (quant)	238.2 → 182.1 (qualifier)
Norfentanyl	233.2 → 84.1 (quant)	233.2 → 55.1 (qualifier)

The interpretation of postmortem fentanyl continues to be challenging, and the results of this study do nothing to resolve the issue of redistribution. There have been other approaches of estimating postmortem fentanyl concentrations such as urine, testing for metabolites, and liver as a matrix (10). Of these approaches, testing liver appears to have the most promise. Anderson and Muto suggested liver fentanyl values exceeding 69 μg/kg was consistent with overdose deaths while values <31 μg/kg as therapeutic use (8). More recently, Palamalai et al. (11) reported similar cutoffs of 56 and 23 μg/kg, respectively for liver fentanyl concentrations. These investigators demonstrated that the ratio of liver to peripheral blood decreased as a function of increasing interval between death and the autopsy, consistent with more postmortem redistribution of the blood to a greater extent than the liver. The slope of a plot of liver fentanyl concentrations versus the death-to-autopsy interval was somewhat flat.

Our results suggest that postmortem absorption of fentanyl from a transdermal patch does not occur under the conditions of this study. The skin remains largely intact 24 h after death and acts as a barrier in the absence of blood flow despite the presence of a substantial drug concentration gradient. It may be possible that some absorption can occur with prolonged decedent storage beyond 1 day, or at storage temperatures above 2–8°C. For this case, we mimic typical postmortem conditions, where a decedent is delivered to the morgue within 24 h after death and immediately refrigerated until an autopsy is conducted. This experimental condition does not mimic the condition whereby a body is discovered a day or more after death and is left at ambient temperature. If a body is left at elevated ambient temperatures, transdermal absorption of drugs could occur from a patch with the degradation of skin integrity. We did select an unused patch with the highest fentanyl concentration available on the market to test the "worse-case" scenario.

Roy and Flynn studied the *in vitro* permeability of decedent skin to fentanyl (12). They demonstrated short diffusion lag times of <30 min utilizing heat-isolated epidermis and dermatomed cadaver skin sections. The permeability was greatly enhanced when the stratum corneum was removed by tape stripping and the pH raised from 4 to 8. This suggested that the stratum corneum was a principle barrier to percutaneous absorption of fentanyl. Fentanyl, as a weak base, will be present in a "free base" form in an alkaline environment, which will render it into a nonionized state and thus able to more readily penetrate biological membranes. The difference in permeability from this *in vitro* model, and our intact decedent model, may be explained by the use of warm temperatures (37°C) and use of buffer solutions in a diffusion cell model to approximate an *in vitro* system of a living human. The colder temperatures, intact stratum corneum, lower surface and tissue pH, and lack of a circulating diffusion compartment may collectively explain the lack of cutaneous permeability of fentanyl from a transdermal patch in an intact decedent.

The interpretation of postmortem fentanyl concentrations may also be clarified should there be any situations whereby a fentanyl patch is placed onto a decedent after death. Our study refutes the argument that a fentanyl patch remaining on a decedent has the potential for sample contamination and/or regional diffusion of drug to falsely elevate a postmortem level of fentanyl even if sampling is in close approximation to the site of the patch. However, given that we were not able to test prolonged refrigerated times or room temperature, decedents who have fentanyl patches, we recommend that patches be removed as soon after a decedent is received and logged into the facility from the autopsy suite, unless the autopsy is scheduled to be performed immediately. Photographs of the bodies would need to be taken before any therapeutic/medical devices such as transdermal patches are removed to document their location.

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