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CASE REPORT

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A Fatal Drug Interaction Between Oxycodone and Clonazepam*

ABSTRACT: A case is presented of a fatal drug interaction caused by ingestion of oxycodone (Oxycontin®) and clonazepam (Klonapin®). Oxycodone is an opium alkaloid used in long-term pain management therapy. Clonazepam is a benzodiazepine used for the treatment of seizures and panic disorders. The Drug Abuse Warning Network (DAWN) has reported an increase of 108% in the last two years of emergency department episodes related to Oxycontin®. Six billion prescriptions were written for Oxycontin® in the year 2000, an 18-fold increase from four years previous (1). Oxycontin has recently gained enormous notoriety at the local and national levels; however, there are very few previously documented cases of lethal drug interactions between oxycodone and clonazepam. Synergistic effects between these two drugs are postulated to arise from different agonistic mechanisms producing similar physiological changes. It is also theorized that clonazepam may inhibit the metabolism of oxycodone. A 38-year-old white female was found dead in Jefferson County, Tennessee in March of 2001. The deceased had physical evidence of previous drug abuse and positive serological findings of hepatitis B and C. Prescription pill bottles filled under the name of the deceased, as well as another name, were found with the body. Serum, urine and gastric contents from the deceased were screened for numerous drugs and metabolites using a combination of thin layer chromatography and immunoassay techniques (EMIT and FPIA). Analysis of biological specimens from the deceased revealed the presence of: benzodiazepines, opiates (oxycodone), and trazodone metabolites in the serum; cannabinoids, benzodiazepines, opiates (oxycodone), trazodone, trazodone metabolites, nicotine, and nicotine metabolite in the urine; and benzodiazepines, opiates (oxycodone), nicotine, and nicotine metabolite in the gastric contents. Quantitative analyses for clonazepam was performed by high performance liquid chromatography (HPLC) and revealed a plasma concentration of 1.41 μg/mL. Plasma oxycodone and urine 11-nor-carboxy-delta-9-tetrahydrocannabinol concentrations were determined by gas chromatography/mass spectrometry and revealed concentrations of 0.60 μg/mL and 27.9 ng/mL, respectively. The deceased had pathologies consistent with severe central nervous system (CNS) and respiratory depression produced by high concentrations of clonazepam and oxycodone including collapsed lungs, aspirated mucus, and heart failure. The pathologies were sufficient to cause death, which was officially attributed to a drug overdose; however, the manner of death was unknown.

KEYWORDS: forensic science, oxycodone, clonazepam, lethal drug interaction

Oxycodone HCl (Oxycontin®), 14-hydroxydihydrocodeinone, is a thebaine derived opium alkaloid that is indicated for the treatment of moderate to severe pain. Oxycontin® is an extended release formula of oxycodone HCl that is used in pain management programs that extend over long periods of time and is applicable to the case reported herein. Oxycodone binds predominately to the μ-opiate receptor located throughout the central nervous system (2). The μ-opiate receptor agonism results in anxiolysis, euphoria, feelings of relaxation, respiratory depression, constipation, miosis, and cough suppression, as well as analgesia. Respiratory depression to the level of cessation is typically the primary cause of death in narcotic overdoses. Oxycodone pharmacokinetic data are given in Table 1.

Clonazepam (Klonapin®), 5-(2-chlorophenyl)-1,3 dihydro-7-nitro-2H-1,4-benzodiazepin-2-one, is a short-acting benzodiazepine that is indicated for treatment of panic and seizure disorders. The specific pharmacodynamic mechanism of action is unknown, although it is believed to enhance the activity of gamma aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous system (3). Toxic plasma concentrations of clonazepam are known to produce cyclic comas in which the patient repeatedly gains and loses consciousness (4). Clonazepam pharmacokinetic data are given in Table 2.

Clonazepam enhances the effect of opioids when used in combination, and it can act as a substitute when heroin is not available (5). Clonazepam's efficacy may be lower than that of heroin with respect to euphoria; however, its effects can partially satisfy an addict's craving for heroin in addition to relieving some of the symptoms of withdrawal (i.e., cravings and nervousness). A recent investigation of 151 fatalities has revealed that benzodiazepines were present in over 40% of cases in which the cause of death was a heroin overdose (6). The synergistic and potentially fatal effects of

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TABLE 1—*Oxycontin® pharmacokinetics**

Formulation	10, 20, 40, 80, 160 mg tablets
Dosage	Symmetric, every 12 h, dosing with appropriate formulation†
Therapeutic plasma concentrations	0.01–0.16 μg/mL
Toxic plasma concentrations	0.2–5.0 μg/mL
Lethal plasma concentrations	4.3–14 μg/mL (0.4–2.7 μg/mL when combined with other CNS depressants)
Peak plasma concentrations	0.0106–0.0985 μg/mL†,‡, 2–3 h after oral dose. Oxycontin® provides oxycodone delivery for over 12 h
Maximum daily dose	20–23^0 mg/day†
Duration of action	approx. 12 h
Bioavailability	60–87%
Excretion	Primarily via the kidneys 19% free oxycodone 50% conjugated oxycodone <14% conjugated oxymorphone
Plasma binding	45%
Volume of distribution	1.8–3.7 L/kg
Half life	4–6 h
Clearance	0.8 L/min for adults

* From Refs 2, 13–15.
† Dependeing on formulation given or opioid tolerance.
‡ 160 mg tablet data not given.

TABLE 2—*Clonazepam pharmacokinetics.**

Formulation	0.5, 1, 2 mg tablets
Dosage	Initially, 0.25–0.5 mg every 8 h may be increased 0.5 mg every 3 days to control seizures
Therapeutic plasma concentrations	0.007–0.12 μg/mL
Toxic plasma concentration	0.069 μg/mL
Lethal plasma concentrations	None reported
Peak plasma concentrations	0.007–0.024 μg/mL, 1–4 h after 2 mg oral dose
Maximum daily dose	Varies depending on seizure proliferation
Duration of action	Approx. 12 h
Bioavailability	90%
Excretion	Biliary primarily as 7-amino-clonazepam
Plasma binding	86%
Volume of distribution	1.5–4.4 L/kg
Half life	19–60 h
Clearance	1.55 mL/min/kg

* From Refs 3, 13–15.

combining clonazepam and oxycodone are postulated to arise from their different agonistic mechanisms to produce similar physiological effects. Oxycodone and clonazepam are preferentially biotransformed by different isoforms of cytochrome P-450 (CYP450) for their primary metabolism (2D6 and 3A4, respectively); however, the cross reactivity of CYP450-3A4 may allow for the metabolism of oxycodone, especially in high therapeutic and toxic concentrations (7). The data suggest that oxycodone's metabolism may be hindered by clonazepam. Hepatic pathologies and/or CYP450-3A4 inhibitors (e.g., cannabinoids, cimetidine, grapefruit juice, etc.) will further exacerbate the increase in plasma concentration of clonazepam and oxycodone (8).

Case History

A 38-year-old white female was found dead in Jefferson County, Tennessee. The specific details as to the exact location and position of the body were unavailable. Medications found at the scene included: trazodone (Deryrel®), hydroxyzine (Atarax®), naproxen sodium (Naprosyn®), aspirin/acetaminophen/caffeine (Excedrin®), hydrocodone/acetaminophen (Lortab®), Oxycodone HCl (Oxycontin®) and sulindac (Clinoril®). An empty pill bottle for oxycodone/acetaminophen (Percocet®) was found in addition to a pill bottle for hydrocodone/acetaminophen (Lortab®), which only contained a few coins. An empty unidentifiable pill container and cap was also found. We were unable to determine whether or not the deceased had followed the prescribed regimen for dosing of her medications based on the dates on the pill bottles and remaining pills. The sulindac prescription was nearly a year old and contained more tablets than prescribed. The narcotic pill bottles did not contain any of the indicated medications. Prescription drug abuse was suspected due to some of the medication being filled under a different name and at various pharmacies.

Materials and Methods

Specimen Collection

Blood was collected from three locations in the body by venipuncture as follows: four 15 mL tubes from the right femoral vein, two 15 mL tubes from the left femoral vein, two 15 mL tubes from the right atrium, and stored in sterile plain Vacutainer® tubes or Vacutainer® tubes containing potassium oxalate and sodium fluoride. Vitreous humor was collected by optic puncture and stored in a sterile plain 15 mL Vacutainer® tube. Urine was collected by bladder puncture and stored in a plastic container. Gastric contents were stored in a plastic container. Serum and plasma were separated by centrifugation (TJ-6, Beckman, Arlington Heights, IL) at 3000 rpm for 10 min. Biological fluids were stored at 2–4°C until analysis.

Analytical Methods

Biological specimens (serum, gastric contents, and urine) were screened for various acidic, basic and neutral drugs and metabolites including: narcotics, over-the-counter analgesics, barbiturates, benzodiazepines, cannabinoids, cocaine, phencyclidine, phenothiazines, sympathomimetic amines, and tricylic antidepressants by a combination of thin layer chromatography, specific colorimetric, and enzyme immunoassay procedures.

The quantitation of clonazepam in plasma involved the alkalinization of the specimens and calibrators to a pH of 9.2 with concentrated NH_4OH, and the addition of 10 μL of 0.5 mg/mL of prazepam (Sigma, St. Louis, MO) as an internal standard to a specimen volume of 1.0 mL. A one point standard curve, which has previously demonstrated linearity up to a concentration of 5.0 μg/mL clonazepam, was prepared as follows: a 1.0 mg/mL methanolic stock standard of clonazepam was prepared by adding 10.0 mg of clonazepam powder (Sigma) to a 10 mL volumetric flask and diluting to the mark with methanol. A 5.0 μg/mL working standard solution was prepared by adding a 50 μL aliquot of the clonazepam methanolic stock standard solution to a 10 mL volumetric flask and diluting to the mark with blank serum (adult bovine, Sigma). The 5.0 μg/mL working standard solution was diluted 1:1 with blank serum to prepare the calibrator solution, 2.5 μg/mL of clonazepam. A quality control specimen was also prepared by diluting the 5.0 μg/mL working standard solution 1:4 with blank serum, yielding a

1.0 μg/mL clonazepam solution. All specimens were then extracted with 5.0 mL of butyl chloride on a tube rocker (American®, Miami, FL) for 20 min (9). The organic phase was separated from the aqueous phase and evaporated overnight to dryness at room temperature. The residue was then reconstituted with 50 μL of the mobile phase solution (acetonitrile/0.015 M pH 3.3 phosphate buffer, 40:60, ACS grade, Fisher Scientific, Fair Lawn, NJ). All solvents used were HPLC grade (Fisher Scientific). The extract was chromatographed on a Waters (Milford, MA) liquid chromatography analyzer with a 4μ C_{18} Radial-Pak Cartridge with a Lambda Max 480 UV detector at 254 nm and a Hewlett Packard (Palo Alto, CA) 3390A integrating recorder. The mobile phase was pumped at a flow rate of 2.0 mL/min at room temperature. The clonazepam standard eluted at 1.86 min (0.2097 relative retention time to the internal standard, prazepam). Concentrations were determined by interpolation of peak area ratios of clonazepam to prazepam.

The quantitation of oxycodone in plasma involved the alkalinization of the specimens and calibrators to a pH of 9.5 with concentrated NH_4OH, and the addition of 10 μL of 0.10 mg/mL of d^3-codeine (Cerilliant Corp., Round Rock, TX) as an internal standard to a specimen volume of 2.0 mL. A two point standard curve, which has previously demonstrated linearity up to a concentration of 1.0 μg/mL oxycodone, was prepared as follows: a 1.0 mg/mL methanolic stock standard of oxycodone was prepared by adding 11.2 mg of oxycodone · HCl (Sigma) to a 10 mL volumetric flask and diluting to the mark with methanol. A 10 μL aliquot of the oxycodone methanolic stock standard solution was placed in a 10 mL volumetric flask and diluted to the mark with blank serum to yield a 1.0 μg/mL oxycodone working solution. Volumetric serial dilutions of the working solution yielded 0.5 and 0.25 μg/mL oxycodone solutions. The standard curves comprised the 0.25 and the 1.0 μg/mL oxycodone concentrations, while the 0.5 μg/mL solution was prepared as a quality control specimen. All specimens were then extracted with 10.0 mL of butyl chloride, back extracted into hydrochloric acid and finally partitioned into chloroform then evaporated to dryness under nitrogen (9). All solvents used were HPLC grade (Fisher Scientific). Residue was reconstituted in 50 μL of methanol and chromatographed on a 5890 Series II gas chromatograph with a 5972 Mass Selective Ion Detector (Hewlett Packard, Palo Alto, CA) using a 0.25 mm × 30.0 m DB-5 capillary column. Chromatographic conditions were: injector 275°C; helium carrier at 1.0 mL/min; column 115°C for 0.5 min then 20°C/min to 280°C then held for 5.0 min; transfer line 280°C. Mass spectrometer detector conditions were set using Standard Spectra Autotune. The target ion was 315.3 with a retention time of 10.3 min (0.9514 relative retention time to the internal standard, d^3-codeine, target ion 302.3). Concentration was interpreted by linear curve fit of oxycodone: d^3-codeine peak area ratios.

The quantitation of 11-nor-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) in urine specimens involved the hydrolysis of glucuronide conjugates by the addition of 200 μL of 10N NaOH (ACS grade, Fisher Scientific) and heating to 60°C for 20 min after which 20 μL of 10 μg/mL d^3-11-nor-carboxy-delta-9-tetrahydrocannabinol (d^3-THC-COOH) was added as an internal standard. Specimens were then adjusted to pH 3.5 by the addition of glacial acetic acid (ACS grade, Fisher Scientific). The THC-COOH was extracted from the specimens using solid phase extraction cartridges (UCT, Bristol, PA) which were conditioned with: HPLC grade methanol (Fisher Scientific), 18 Mohm deionized water, then 100 mM phosphate buffer (Sigma). The samples were applied to the cartridge and subsequently washed with: 18 Mohm deionized water, 100 mM acetic acid (Fisher Scientific), then HPLC grade hexane (Burdick and Jackson, Muskegon, MI). The drugs bound to the column were eluted with a 50/50 (v/v) solution of hexane/ethyl acetate (Burdick and Jackson, Fisher Scientific). The eluate was evaporated to dryness at room temperature under a vacuum. The residue was derivatized with BSTFA with 1% TCMS (Sigma) overnight. The derivatized sample was analyzed using the above mentioned GC/MS instrument with the following conditions: injector 250°C; helium carrier at 1.0 mL/min; column 175°C for 0.5 min then 30°C/min to 300°C then held for 2.5 min; transfer line 300°C. Mass spectrometer detector conditions were set using Standard Spectra Autotune. The target ion was 371.2 with a retention time of 6.24 min (1.000 relative retention time to the internal standard ion of 374.2). Concentration was interpreted by linear curve fit of THC-COOH: d^3-THC-COOH peak area ratios.

Results

Toxicological Findings

Analytical results are given in Table 3. The serum drug screen was negative for ethanol and positive for benzodiazepines, trazodone and opiates (oxycodone). The gastric drug screen yielded positive results for benzodiazepines, nicotine and metabolites, and opiates. The urine drug screen was negative for ethanol and yielded positive results for benzodiazepines, nicotine and metabolites, opiates, trazodone and metabolites, and cannabinoids. Quantitative analysis of the femoral plasma revealed an oxycodone concentration of 0.60 μg/mL, a clonazepam concentration of 1.41 μg/mL, and metabolites of trazodone (no parent drug was found). Quantitative analysis of the urine revealed a THC-COOH concentration of 27.9 ng/mL.

Pathological Findings

The body was that of a well-developed, well-nourished, 52.2 kg adult white female. The deceased was a right below-the-elbow amputee and had various well healed scars present in sporadic areas over the body. The deceased had partial collapse of the lungs due to inadequate breathing, aspiration of mucus, pulmonary edema, and heart failure. The lungs also contained polarizable plant material, small hemorrhages, bacteria, and crystallized materials suggesting possible previous IV drug use; however, no recent

TABLE 3—*Analytical results.*

Specimen	Result
Drug Screen	
Blood	Benzodiazepines, trazodone, opiates (oxycodone)
Urine	Benzodiazepines, cannabinoids, nicotine and metabolites, trazodone and metabolites, opiates (oxycodone)
Gastric	Benzodiazepines, nicotine and metabolites, opiates (oxycodone)
Plasma Drug Quantitation	
Oxycodone	0.60 μg/mL
Clonazepam	1.41 μg/mL
Trazodone	None detected (metabolites only)
Urine Drug Quantitation	
11-nor-carboxy delta-9-THC	27.9 ng/mL

* Three 40 mg Oxycontin® tablets were found in the gastric contents.

venipunctures were apparent. Serological analysis yielded positive results for hepatitis B virus (HBV) and hepatitis C virus (HCV). These results were consistent with microscopic analysis of the liver which revealed acute passive congestion with marked chronic triaditis and fine steatosis with degeneration and regeneration of heptocytes, suggesting active hepatitis C and resolved hepatitis B.

Discussion

An acute overdose with oxycodone can be manifested by respiratory depression, somnolence progressing to stupor or coma, skeletal muscle flaccidity, cold and clammy skin, constricted pupils, bradycardia, hypotension, and death (2). Review of case reports has indicated that the risk of fatal overdose is further increased when Oxycontin® is abused concurrently with alcohol or other CNS depressants, including other opioids (2,10). Oxycodone use, addiction and death occurs across a spectrum of people ranging from older patients who initially were prescribed the drug to young recreational drug users (11). Overdoses of clonazepam by itself produce symptoms similar to other CNS depressants including: somnolence, confusion, coma, and diminished reflexes (3). Death from benzodiazpines are rare when taken alone (12). Based on the minimum volume of distribution (V_d) for oxycodone and assuming maximal bioavailability (f), an estimated dose was calculated from the plasma concentration of oxycodone (C_p) as Dose = ($V_d \times C_p$)/f. In order to achieve a plasma concentration of oxycodone of 0.60 μg/mL, the deceased may have either ingested an acute overdose of at least 65 mg of oxycodone in the course of a therapeutic oxycodone regimen, and/or had the normal metabolism attenuated by concomitant administration of clonazepam. An estimation of the dose of clonazepam needed to achieve the plasma the plasma concentration measured was calculated using the minimum volume of distribution, and maximum bioavailability for clonzepam. The deceased may have either ingested an acute overdose of at least 123 mg of clonazepam, and/or the drug's metabolism was severely attenuated during a normal therapeutic regimen due to concomitant administration of oxycodone.

The subject's oxycodone plasma concentration was considered to be toxic (0.2–5.0 μg/mL, six times the maximum therapeutic concentration) (13–15). Lethal concentrations have been reported in the range of 4.3–14 μg/mL, and 0.4-0.7 μg/mL when combined with at least one other CNS depressant (15). An acute clonazepam overdose of 14–32 mg taken orally produced a plasma concentration of 0.069 μg/mL (15). The therapeutic plasma concentration of clonazepam ranges from 0.007 to 0.12 μg/mL, less than one-tenth the level present in the deceased (13,15). The reported concentrations reflect the concentrations present at the time of death and are not falsely elevated due to postmortem redistribution as a femoral specimen was used for analysis.

Conclusion

Other than the aforementioned references, there are almost no published records of the interaction between oxycodone and clonazepam. The case presented herein suggest that clonazepam's metabolism preferentially occupied the activity of CYP450-3A4; therefore the metabolism of oxycodone may have been competitively inhibited. The cannabinoids and trazodone present in the urine indicates previous use; however, the absence of parent compounds in the plasma does not allow one to correlate cannabinoid and trazodone concentrations with an effect, synergistic or otherwise. We can only postulate that within a few days before the subject's death, the activity of CYP450-3A4 was further inhibited by these two substances (16). A further reduction in metabolism of clonazepam and oxycodone was attributed to compromised hepatic functions by active hepatitis C. The combination of drugs found in the deceased with decreased hepatic function manifested elevated blood concentrations of clonazepam and oxycodone. This in turn exerted a synergistic effect of severe CNS and respiratory depression. Based on the pathological and laboratory findings, death resulted from anoxic conditions produced by inadequate respiration. The cause of death was determined to be a drug overdose, the manner of death was unable to be determined.

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