Phenazepam is a benzodiazepine derivative that has been in clinical use in Russia since 1978 and is not available by prescription in the United States; however, it is attainable through various internet websites, sold either as tablets or as a reference-grade crystalline powder. Presented here is the case of a 42-year-old Caucasian male who died as the result of combined phenazepam, morphine, codeine, and thebaine intoxication. A vial of white powder labeled “Phenazepam, Purity 99%, CAS No. 51753-57-2, Research Sample”, a short straw, and several poppy seed pods were found on the scene. Investigation revealed that the decedent had a history of ordering medications over the internet and that he had consumed poppy seed tea prior to his death. Phenazepam, morphine, codeine, and thebaine were present in the blood at 386, 116, 85, and 72 ng/mL, respectively.

Introduction

The internet provides virtually unlimited access to medical information and a forum for discussion of numerous health-related topics to over one billion people globally (1). Unfortunately, it also facilitates the dissemination of potentially hazardous substances by providing an alternative source of illicit and prescription drugs (1,2). Furthermore, not only can drugs be obtained through the internet, but also instructions, advice, and first-person accounts of drug abuse practices (1,3).

Phenazepam (fenazepam) [7-bromo-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one] is a benzodiazepine derivative which was first synthesized in 1975 through the joint efforts of pharmacologists from the Academy of Medical Sciences of the USSR and chemists from I.I. Mechnikov Odessa National University in Ukraine (4,5). Its clinical use has been widespread in the former Soviet Union since 1978, where it is one of the most extensively prescribed benzodiazepines (5–7). Structurally, phenazepam is similar to clonazepam (Figure 1).

Like other benzodiazepines, phenazepam is a therapeutically useful anxiolytic with anticonvulsive and hypnotic activity. Some report that it is comparable to lorazepam in regard to its dose and therapeutic action (8), but others report that it has a higher median lethal dose (LD$_{50}$) in rats than benzodiazepines such as chlordiazepoxide and diazepam (5).

In the United States, phenazepam is not currently approved for prescription use and is not listed under the Controlled Substances Act. Accordingly, distribution of the drug remains uncontrolled. Phenazepam is, however, making its way into the United States (9,10) and infiltrating illicit drug markets in Sweden and Finland (8,11,12). Many internet websites provide potential global access to this drug with little difficulty, and available preparations include tablets and reference-grade crystalline powders.

As a fairly comprehensive information resource, the internet provides a great deal of information concerning the use of poppy seed tea (PST) to individuals hoping to achieve opiate-induced euphoria or control pain. Additional interest in PST is sparked by the claim by some users that the consumption of PST mitigates the severity of withdrawal symptoms from opiates (13). Several websites advertise the sale of seed pods, and

Figure 1. Structure of phenazepam.
seeds can be purchased in bulk from supermarkets or health food stores at a relatively low cost. Detailed instructions for preparation of PST, as well as anonymous forums for discussion of its use are easily found following a web search for “poppy seed tea.” A widely used method for the preparation of PST involves washing or soaking generous quantities of dried seeds in water containing lemon juice. The resultant liquid is then consumed. With this method, significant amounts of morphine, codeine, and other opiate alkaloids are extracted from the residual coating and capsular debris of the seeds (13).

Poppy seeds harvested in different years or from different regions of the world display a wide range of concentrations of morphine (2–251 mg/kg seeds), codeine (0.4–57 mg/kg seeds) (14), and compounds such as thebaine, papaverine, and noscapine (narcotine). Accordingly, teas prepared from different varieties of seeds will also exhibit variations in the concentrations of such compounds. PST prepared from “recipes” provided by PST users has been shown to contain morphine in concentrations ranging from 10 to 105 mg/kg seeds and codeine concentrations ranging from 3 to 11 mg/kg seeds (13).

It has been demonstrated that approximately 50% of the morphine and codeine in poppy seeds can be extracted by soaking seeds in water for 5 min with constant agitation (15). Users of PST report that the onset of effects usually occurs within 15 min of ingestion and that these effects can be felt for up to 24 h, depending on the amount of tea consumed (13).

Here, a fatality involving concurrent use of phenazepam and PST is presented.

Case History

A 42-year-old Caucasian male was found unresponsive in bed by his wife when she returned home from nursing school. She called 911, and EMS responded to the scene where he was pronounced dead. The woman reported that her husband was an alcoholic, had a history of drug abuse, and visited his doctor five months earlier for treatment of insomnia and asthma. She also stated that he had been unemployed for an extended period of time and spent his days at home accessing the internet, through which he had previously purchased medications. A search of the decedent’s bedroom yielded a short straw, several poppy seed pods (Figure 2), a plastic measuring cup containing several lemon slices, and a small glass vial labeled “Phenazepam, Purity 99%, CAS No. 51753-57-2, Research Sample”. The vial contained a small amount of white powder (Figure 3). When later questioned about the seed pods, the decedent’s wife indicated that they were “opium poppy seed pods” that her husband used to make tea.

A complete autopsy was performed, revealing marked obesity (body mass index = 37), cardiomegaly (660 g), and pulmonary findings consistent with bronchial asthma. Marked pulmonary edema, diffuse visceral congestion, and moderate cerebral edema were also documented. Samples submitted to the toxicology laboratory included blood from the subclavian vein, urine, gastric contents, liver, and vitreous humor. Powder from the glass vial was submitted at a later date by law enforcement.

Materials and Methods

Subclavian blood was analyzed for volatiles using direct injection and gas chromatography–flame-ionization detection (GC–FID) with t-butanol employed as the internal standard. Preparation of the sample involved the addition of 2 mL t-butanol to 1 mL subclavian blood and vortex mixing prior to manual injection of approximately 0.5 µL onto an HP 6890 series GC with an FID. Subclavian blood (precipitated with acetone and reconstituted in 0.1 M, pH 6.5 phosphate buffer) and urine were screened for a panel of drugs by enzyme immunoassay on a MGC 240 analyzer using DRI® reagent kits purchased from the manufacturer (Microgenics, Fremont, CA). An alkaline drug screen on urine was performed using Toxi-Lab® Toxi-Tubes® A (Varian, Palo Alto, CA) and GC–mass spectrometry (MS). A minute amount of powder from the glass vial was dissolved in methanol and analyzed by GC–MS.
Mass spectral data from analysis of the powder was compared with that of a phenazepam standard (Lipomed, Cambridge, MA).

Next, benzodiazepine and opiate confirmations were performed. Negative serum was purchased from Sigma Aldrich (St. Louis, MO). Codeine, codeine-d₆, morphine, and morphine-d₆ were obtained from Cerilliant (Round Rock, TX). Methanol and formic acid were purchased from EMD Chemicals (Gibbstown, NJ), and acetonitrile was from JT Baker (Phillipsburg, NJ). Deionized water was prepared using a Milli-Q system (Millipore, Bedford, MA).

Benzodiazepine calibrators consisted of negative serum fortified with phenazepam, diazepam, nordiazepam, clonazepam, 7-aminoclonazepam, temazepam, oxazepam, alprazolam, lorazepam, midsazolam, demoxepam, chlordiazepoxide, and norchloridiazepoxide at concentrations of 5, 10, 25, 50, 100, 250, 500, and 1000 ng/mL. Controls at concentrations of 20, 200, and 650 ng/mL were prepared independently. Blank serum was extracted and included in the analysis. Sample preparation was performed using a modification of a method described by Karinen et al. (16). Two-hundred microliters of calibrators, controls, and samples were added to 2.0-mL polypropylene microcentrifuge tubes, and 10 ng diazepam-d₅ was added as internal standard to each tube. Protein precipitation was achieved through the addition of 1.0 mL cold acetonitrile/methanol (85:15). Each tube was vortex mixed briefly and centrifuged at 32,000 × g at 2°C for 15 min. Supernatants were transferred to 12 × 75-mm glass culture tubes and dried at 45°C under a stream of nitrogen. Residues were reconstituted in 150 µL initial mobile phase (0.1% formic acid/acetonitrile with 0.1% formic acid, 72:28) and placed into microcentrifuge tubes, centrifuged a second time, and transferred to autosampler vials for analysis.

Opiate calibrators consisted of negative serum spiked with morphine, codeine, oxycodone, oxymorphone, hydrocodone, dihydrocodeine, hydromorphone, and 6-acetylmorphine at concentrations of 2.5, 5, 10, 20, 50, 100, 500, and 1000 ng/mL. Controls at concentrations of 7.5, 200, and 650 ng/mL were prepared and included in the analysis, along with a blank serum. Sample preparation was performed as described, with 10 ng morphine-d₆ and codeine-d₆ added to each tube as internal standards for morphine and codeine. Again, residues were reconstituted in 150 µL initial mobile phase (0.1% formic acid/acetonitrile with 0.1% formic acid, 99:1) and placed into microcentrifuge tubes for a second centrifugation prior to analysis.

All chromatography was performed using a Waters Acquity ultra-performance liquid chromatograph (UPLC®) with separation achieved on an Acquity UPLC BEH C₁₈ 2.1 × 100-mm (1.7 μm) column (Waters, Milford, MA). For each analysis, column temperature was maintained at 40°C. All chromatographic runs were performed using linear gradients where solvent A was 0.1% formic acid and solvent B consisted of acetonitrile with 0.1% formic acid. For phenazepam, the initial conditions of solvent A/solvent B (72:28) were maintained for 0.1 min. Solvent A was then decreased to 58% over 2.2 min and maintained until 2.7 min. It was then reduced to 50% at 3 min and to 5% at 3.5 min, where it was held until 4.5 min. The system was returned to initial conditions and held for 1 min prior to the next injection. For morphine and codeine, solvent A was decreased from 99% to 0% over 5.0 min. At 5.5 min, initial starting conditions were restored and maintained for 1 min until the subsequent injection. Average retention times (in minutes) observed under these chromatographic conditions were 3.22 for phenazepam, 3.34 for diazepam-d₅ and codeine-d₆ were added to each tube as internal standards for morphine and codeine. Again, residues were reconstituted in 150 µL initial mobile phase (0.1% formic acid/acetonitrile with 0.1% formic acid, 72:28) and placed into microcentrifuge tubes for a second centrifugation prior to analysis.

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MS–MS analysis was carried out using a Waters TQ Detector with ionization in electrospray positive mode. Target compounds were analyzed using multiple reaction monitoring (MRM) with argon as the collision gas. Instrument parameters for phenazepam, morphine, and codeine are given in Table I, and details of the MRM transitions and conditions are shown in Table II. One quantification transition and two target transitions were monitored for drugs of interest, whereas only a quantification transition and a single target transition were...
monitored for deuterated internal standards. The LOQ for benzodiazepine and opiate analysis was set to that of the lowest calibrator, 5 ng/mL for benzodiazepines and 2.5 ng/mL for opiates. Both assays were found to be linear over the range of calibrators used, with $r^2 = 0.99$ for each drug of interest. Within-run precision was calculated at concentrations of 20, 200, and 650 ng/mL for phenazepam and concentrations of 7.5, 200, and 650 ng/mL for morphine and codeine ($n = 3$ for each concentration). For phenazepam, coefficients of variation ($\%CV$) ranged from 0.68% to 7.5%; for morphine, $\%CV$ ranged from 0.77% to 11.1%; and for codeine, $\%CV$ ranged from 2.67% to 6.24%. To be acceptable, all ion ratios had to be within ±20% of the average ion ratios for the calibrators. Additionally, concentrations of positive controls had to be within ±20% of their expected concentrations. Prior to analysis of case samples, blood controls were prepared, extracted, and quantified using serum curves. For each drug, these controls met the acceptability parameters noted above; therefore, serum curves were used for analysis. Matrix effects were evaluated using post-column infusion of drugs of interest with simultaneous injection of blank extracted matrix (blood and serum) under desired chromatographic conditions. This assessment did not reveal any notable ion suppression or enhancement.

Results

No ethyl alcohol or other volatile compounds were detected in subclavian blood by GC–FID. Both subclavian blood and urine screened positive for benzodiazepines and opiates by enzyme immunoassay. Preliminary data collected by our laboratory using spiked urine suggested that phenazepam demonstrates significant cross-reactivity for the Microgenics DRI Benzodiazepine Assay. The following compounds were detected in the urine alkaline drug screen by GC–MS through matches with NIST, AAFS, and in-house spectral libraries:

Figure 4. MRM chromatograms of extracted blank (A), 200 ng/mL control (B), and case (C) for phenazepam and diazepam-d$_5$.

Figure 5. MRM chromatograms of extracted blank (A), 200 ng/mL control (B), and case (C) for morphine and morphine-d$_6$. 

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nicotine, cotinine, hydrocodone, codeine, and thebaine. Free phenazepam was found in the blood at a concentration of 386 ng/mL. Unconjugated morphine and codeine were quantified in the blood at 116 and 85 ng/mL, respectively, by LC–MS–MS. MRM chromatograms of extracted blanks, 200 ng/mL controls, and case specimens for phenazepam, morphine, and codeine are shown in Figures 4–6. Thebaine was quantified in the blood by GC–MS at 72 ng/mL (NMS Labs, Willow Grove, PA). Heroin use was excluded based on investigative findings and the absence of 6-acetylmorphine and acetylmorphine in the blood and urine (17,18). The powder in the vial was confirmed to be phenazepam with no other drugs detected; however, further study of purity of the phenazepam preparation was not performed.

Discussion

The number of deaths resulting from the use of opioid compounds in combination with benzodiazepines has displayed an alarming increase in recent years (19). When taken in excess, opioids can often elicit significant respiratory depression, and when used in conjunction with benzodiazepines, enhancement of opioid-mediated central nervous system depression may be observed (20,21). In this case, assessing the relative contributions to fatal toxicity of phenazepam and the poppy seed derived compounds, as well as the extent of their combined effects, was especially challenging. Another factor to be considered was the assessment of the decedent’s natural disease and associated respiratory insufficiency, which may have left the decedent more vulnerable to fatal respiratory depression in the presence of these compounds. Interpretation of toxicity produced by opioid compounds is dependent on several factors, particularly the extent to which the user has developed tolerance to the compounds. Studies indicate that morphine and codeine at the concentrations found in the presented case are capable of producing toxicity in those who lack tolerance to opioid medications (22). Although the decedent was reported to abuse drugs, the available information did not specifically point to a history of ongoing opioid abuse, and as such, it is not clear whether any level of opioid tolerance was present. Had the decedent been using opioid drugs or PST for a period of time, one would expect him to have developed some degree of pharmacodynamic tolerance to the compounds detected, and the concentrations observed may not have been lethal. Although reliable information pertaining to the interpretation of blood thebaine concentrations is limited, the detection of thebaine in the urine and blood corroborates the decedent’s reported consumption of PST (23,24).

Phenazepam intended for oral administration is supplied in 0.5–2-mg tablets, with the normal adult dose being 0.5 mg 2–3 times daily for the treatment of anxiety (6). Kankaanpää et al. (25) reported a pharmacologically relevant concentration of phenazepam to be 300 ng/mL, although an effective therapeutic concentration range of 30–70 ng/mL for treatment of neurosis has also been reported (8). Peak plasma concentrations from 8 to 15 ng/mL following a 2-mg oral dose have been described (8), and single oral doses of the drug at 3 and 5 mg produced average blood concentrations of 24 and 38 ng/mL, respectively (6). Furthermore, blood phenazepam levels in 20 subjects arrested for impaired driving ranged from 18 to 400 ng/mL (8).

In this case, the possibility that phenazepam alone contributed to severe cardio-pulmonary distress is likely, given the suggested potency of the drug (8). However, information pertaining to phenazepam in the scientific literature is limited and somewhat contradictory; therefore, a reliable estimation of its degree of toxicity is difficult. Mrozkowska et al. (12) report a non-fatal case in which a 26-year-old male ordered phenazepam over the internet and reported ingesting a significant quantity of the drug (400–600 mg). This resulted in an extremely high blood concentration (1.2 µg/g, or approximately 1200 ng/mL) seven days post-ingestion, though it should be noted that this study did not identify whether this concentration referred to free or total phenazepam. The concentration of

![Figure 6. MRM chromatograms of extracted blank (A), 200 ng/mL control (B), and case (C) for codeine and codeine-d₆.](https://academic.oup.com/jat/article-abstract/34/8/527/887422/figure6.png)
phenazepam in the present case report suggests the consumption of an amount of phenazepam significantly in excess of that which would be seen in a clinical setting and some level of impairment and/or toxicity would be expected. On the other hand, based on the non-fatal outcome highlighted in the case presented by Mrozkowska et al. (12), ascribing fatal toxicity to a phenazepam blood concentration of 386 ng/mL is unwarranted.

Although it could not be ascertained whether the decedent had previously used phenazepam, it would not be surprising if death ensued after his first use of the drug. It seems likely that the decedent may not have had full knowledge or appreciation of the purity of the drug he purchased via the internet. Any prior personal experience or familiarity he had regarding insufflation of a given amount of crushed tablets containing pharmacologically inert ingredients and his naivety about the potency of the “99% pure” drug purchased through the internet could have led to the inadvertent exposure to a significant and unintentionally large quantity of the drug.

Conclusions

To summarize, it was the opinion of the certifying pathologist that the cause of death was combined morphine, codeine, thebaine, and phenazepam intoxication in the setting of recreational abuse of PST and non-prescribed phenazepam with marked obesity acting as a contributory factor to death. This case is unique in that 1. phenazepam and PST are rarely observed in postmortem investigations; 2. it is the only reported fatality involving both phenazepam and PST; and 3. the phenazepam and the instructions for use and preparation of PST were apparently obtained by the user from the internet. Furthermore, the fatal outcome in this case report underscores the potentially negative role played by the internet in facilitating access to illicit/uncontrolled substances and hazardous drug abuse practices.

References