CASE REPORT

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Detection of Alprazolam in Three Cases of Methadone/ Benzodiazepine Overdose

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ABSTRACT: Benzodiazepine abuse is common among clients at methadone maintenance clinics. Diazepam and lorazepam are readily detected by immunological screening methods and confirmed by GC/MS. Alprazolam has been relatively difficult to confirm. We recently reported a modification of an existing serum HPLC procedure which allows us to analyze whole blood. We report here three cases of fatal drug overdose caused by co-ingestion of methadone and alprazolam. In all three cases, alprazolam was detected by HPLC and could not be identified by alkaline extraction GC/MS. Postmortem blood concentrations of methadone were at the lower range or below the concentrations previously identified in methadone overdose fatalities, suggesting an increased risk from co-ingestion of methadone and alprazolam.

KEYWORDS: forensic science, forensic toxicology, methadone, alprazolam, benzodiazepines, overdose

Although benzodiazepines as a class are felt to have a relatively low abuse potential in the general population, they are frequently abused by opiate dependent individuals (1). Several reports indicate that use of diazepam, lorazepam, and alprazolam among methadone patients is more likely to be abusive than therapeutic (2,3). These benzodiazepines are reported to augment the euphoric effects of methadone (2,3). Although detection of diazepam and lorazepam is relatively straightforward, screening and analysis of alprazolam has presented a challenge to forensic toxicologists. We recently reported modification of an existing HPLC procedure that permits analysis of whole blood with a sensitivity of 18 ng/mL and linearity from 18 to 200 ng/mL (4). We here describe three cases of fatal drug overdose involving co-ingestion of methadone and alprazolam. In each case, EMIT suggested the presence of benzodiazepines, GC/MS analysis of whole blood failed to confirm the EMIT results, but HPLC allowed detection and quantification of whole blood alprazolam.

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Laboratory Procedures

Screening: Postmortem urine samples were screened on the Syva EMIT Plus Analyzer using EMIT reagents and calibrators according to the manufacturer, Syva, Palo Alto, CA. Volatile analysis was performed with a head space procedure (5), using *n*-propanol as the internal standard. The column was a 6 foot Porapak-S at a temperature of 180°C. Instrumentation was Shimadzu GC-I 4A, Kyoto, Japan.

Alkaline drug screens were performed on blood, liver, and gastric contents following extraction as described (6). A Hewlett-Packard 5971 GC/MS System (Palo Alto, CA) equipped with a 12.5 m by 0.2 mm HP-1 methyl silicone column was used. The column temperature was programmed from 70 to 280°C at 20°C/min. Helium carrier gas flow was 1 mL/min. A total ion scan was performed.

HPLC analysis was performed on a Shimadzu LC-6a (Kyoto, Japan) system using reagents for benzodiazepines as supplied by Bio-Rad, Hercules, CA (7). A UV detector was used at 242 nm and 0.01 AUFS. Extraction of blood, liver homogenate, and gastric contents was carried out as described using a solid phase extraction column, reagents, and internal standard supplied by Bio-Rad (Hercules, CA).

Case Histories

Case 1 was a 20-year-old female with a history of benzodiazepine and hydrocodone abuse found dead in her apartment. Postmortem urine screens were positive for benzodiazepines and opiates. Alkaline drug screen on whole blood by GC/MS detected only methadone, 0.14 mg/L. Blood and liver alprazolam concentrations by HPLC were 30 μg/L and 230 μg/kg. No other drugs were detected. Death was certified as an accidental methadone and alprazolam overdose.

Case 2 was a 33-year-old male with a history of drug abuse who had repeatedly threatened suicide in the past. He was found dead several hours after being observed ingesting methadone and alprazolam. Postmortem urine screens were positive for benzodiazepines and cocaine metabolites. Alkaline drug screen on whole blood by GC/MS detected methadone, 0.33 mg/L. Blood and liver alprazolam concentrations by HPLC were 23 μg/L and 120 μg/kg, respectively. A low concentration of cocaine (0.01 mg/L) was present in whole blood. Death was certified as an accidental methadone and alprazolam overdose.

Case 3 was a 37-year-old male with a history of drug abuse who was enrolled in a methadone maintenance program. Postmortem urine screens were positive for benzodiazepines and propoxyphene. Alkaline drug screen on whole blood by GC/MS detected methadone (0.70 mg/L), propoxyphene (0.13 mg/L), norpropoxyphene (0.47 mg/L), and carisoprodol (2.3 mg/L). Blood and liver alprazolam concentrations by HPLC were 26 μg/L and 170 μg/kg, respectively. Death was certified as an accidental multiple drug overdose.

Results and Discussion

Table 1 is a summary of the drug concentrations detected in the three cases. The blood concentrations of methadone detected in these cases are in or below the lower range of those previously reported in fatal overdose cases (0.4 to 1.8 mg/L) (8). The concentrations of alprazolam were also substantially below those reported in five suicidal overdoses, (122 to 390 μg/L, mean 230) (9). Although little direct information is available concerning pharmacologic interactions between benzodiazepines and methadone (9), several lines of indirect evidence suggest they may have synergistic interactions. First, the combination of anesthetic doses of the opiate, fentanyl, with low doses of diazepam induced substantial decreases in mean arterial pressure and systemic vascular resistance in patients undergoing coronary artery bypass surgery (10). Second, low doses of naloxone inhibit the induction of unconsciousness by diazepam (11), suggesting that endorphins or opioid receptors are involved in the response to benzodiazepines. Third, it has been found that subanalgesic doses of alfentanil potentiate midazolam-induced unconsciousness (12). Finally, individuals who abuse methadone and benzodiazepines report that benzodiazepines "boost the high" associated with methadone ingestion (2,3). In addition, some users report that they deliberately ingest amounts of methadone and alprazolam which almost induce unconsciousness in an attempt to produce a "near death" experience [Huggins, N., personal communication]. Our cases may be the result of misjudgement of the appropriate dose to produce this effect.

It is noteworthy that in each case an EMIT screen was positive for benzodiazepines, but that an alkaline drug screen on whole blood by GC/MS failed to detect alprazolam, which was ultimately confirmed on solid phase extraction and HPLC. The solid phase extraction HPLC alprazolam method has been available in our laboratory only since November/94. We identified two cases in the prior year in which low concentrations of methadone, (0.07 and 0.18 mg/L) were detected in whole blood, and EMIT screen was positive for benzodiazepines, but none were detected by alkaline extraction GC/MS. Although plausible anatomic causes of death were identified in both cases, the circumstances of death were compatible with drug overdose, and it is possible that HPLC would have detected alprazolam and the deaths been classified as overdoses. It has been reported that methadone patients are aware of the difficulty of detecting alprazolam and may be selecting this particular benzodiazepine in order to evade detection (13).

In summary, these findings demonstrate the usefulness of our recently described solid-phase extraction HPLC method for alprazolam detection. It is possible that deaths among methadone users will be misclassified as natural and that abuse of alprazolam by methadone patients will go undetected if a sensitive confirmation method for alprazolam is not used. Finally, it appears that the combination of relatively low doses of methadone and alprazolam may present greater risks than previously appreciated.

TABLE 1—*Drug concentrations in three overdose cases.*

	Methadone*		Alprazolam†		Other Drugs*	
	Blood	Liver	Blood	Liver	Blood	
Case 1	0.14	NA	30	NA		
Case 2	0.33	4.5	23	120	Cocaine	0.01
Case 3	0.70	9.8	26	170	Propoxyphene	0.13
					Norpropoxyphene	0.93
					Carisoprodol	2.3

*Methadone and "other drug" concentrations: Blood mg/L, liver mg/kg.
†Alprazolam concentrations: Blood μg/L, liver μg/kg.
NA: Not analyzed.

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