Clinical Chemistry 64:8
1143–1147 (2018)

Clinical Case Study

A Comatose Patient with a Bluish Tongue

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CASE

A 26-year-old Caucasian man collapsed in the morning after having drunk several glasses of beer the night before. The paramedics found him comatose with a blood pressure of 140/70 mmHg, a regular pulse of 60/min, a respiratory rate of 14/min, and a Glasgow Coma Scale of 5 (E1M3V1). The patient did not respond to verbal stimuli and appeared to have a bluish tongue. There were no focal neurologic signs, and his pupils were reactive to light and were not miotic. Blood analysis showed no abnormalities except for an alcohol concentration of 200 mg/dL. Urine analysis for benzodiazepines and opioids was negative, as well as blood analysis for paracetamol. In the absence of neurologic abnormalities, intracerebral pathology or epilepsy did not seem likely. He was admitted to the Intensive Care Unit for close neurological observation and supportive measures.

To rule out an intoxication other than alcohol, relatives searched his house for medicines and drugs. An empty bottle of Rivotril® (clonazepam, liquid, 25 mg) was found that belonged to his girlfriend. No other substances were found aside from alcohol. Additional history from his relatives and friends did not reveal any other clues.

DISCUSSION

Because clonazepam was the only substance suspected and because of the bluish tongue (Rivotril is a blue liquid), clonazepam intoxication was considered likely despite the negative benzodiazepine screening result. Flumazenil (Anexate®) 0.5 mg was administered as an antidote. His Glasgow Coma Scale immediately improved to 15, which strongly suggested a benzodiazepine intoxication, in this case most likely due to clonazepam. A blood sample from admission, analyzed by LC-MS/MS, proved to be positive for clonazepam with a concentration of 79 ng/mL (therapeutic range, 30–60 ng/mL).

QUESTIONS TO CONSIDER

1. What are some of the limitations of urine benzodiazepine screening assays?
2. What additional testing should be performed on this patient?
3. What antidote could potentially be used in this patient?

The patient recovered without any permanent damage and was discharged after 2 days.

Benzodiazepines are one of the most commonly used drugs in self-intoxication. In large doses they can cause coma, respiratory depression, and death (especially when used with coingestants such as tricyclic antidepressants, serotonin reuptake inhibitors, monoamine oxidase inhibitors, or alcohol). The incidence of intoxication with benzodiazepines varies between 9% and 84% in different countries *(1)*. In 2016, 4133 benzodiazepine intoxications were reported in the Netherlands, of which 176 involved clonazepam *(2)*. These are reported clinical intoxications; however, the true incidence is probably higher.

Clonazepam is a benzodiazepine that is primarily known for its anticonvulsant property *(3)*. The therapeutically active clonazepam is metabolized to 7-aminoclonazepam (7-AC; Fig. 1), and the metabolites are excreted in urine with <1% as unchanged drug *(4)*. If benzodiazepine intoxication is suspected, a urine toxicology screening can be helpful to establish the diagnosis and treat the patient appropriately. However, there are some pitfalls regarding these screening methods.

Benzodiazepine screening assays are semiquantitative urine immunoassays calibrated with a specific benzodiazepine (e.g., diazepam, lormetazepam). Our hospital uses the BENZ Flex® reagent cartridge with the Dimension Vista® 1500 from Siemens; the antibodies are calibrated with lormetazepam standards. The literature indicates cross-reactivities between 17% and 108% for clonazepam and <10% for its main metabolite in urine (7-AC), regardless of the immunoassay used *(5)*. A likely explanation for the false-negative screening result in our patient is the poor cross-reactivity for 7-AC of our benzodiazepine assay, i.e., approximately 4%.

In addition, the urine sample from our patient was slightly dilute, with a urine creatinine value of 45 mg/dL

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Received June 16, 2017; accepted November 1, 2017.
DOI: 10.1373/clinchem.2017.278200

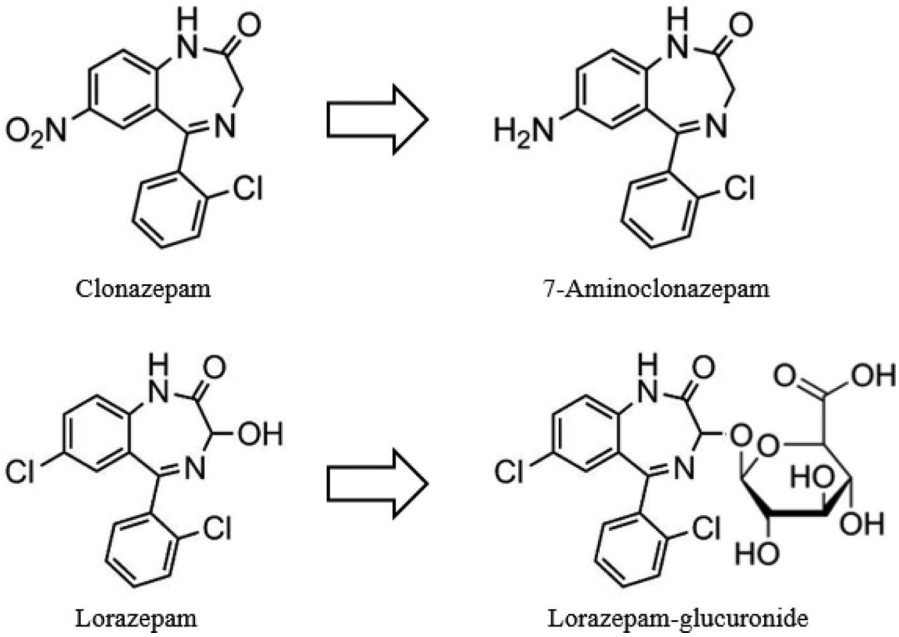

Fig. 1. Molecular structures of clonazepam and lorazepam and their metabolites, 7-aminoclonazapem and lorazepam-glucuronide.

and a specific gravity of 1.002. Although the definition of a dilute urine sample is a creatinine value of ≤20 mg/dL and specific gravity of ≤1.003 *(6)*, and the sample from our patient therefore did not fully meet these criteria, the degree of dilution of a urine sample is always important to consider, as it can contribute to a false-negative drug screening test *(6)*. The dilute nature of the urine in our patient was probably caused by initial fluid resuscitation in the emergency room.

Other benzodiazepines can exhibit low cross-reactivities for a more general reason related to metabolism. Several benzodiazepines are excreted in urine as a glucuronide metabolite *(7)*, and many immunoassays will fail to detect these metabolites. However, by treating the sample with β-glucuronidase, the glucuronidated benzodiazepine metabolite is converted to the free form before analysis, and the cross-reactivity improves substantially. For example, the cross-reactivity for lorazepam-glucuronide [the metabolized fraction of lorazepam (Fig. 1)], varies between 1% and 2% in frequently used immunoassays. After addition of β-glucuronidase, the cross-reactivity increases to almost 50% *(7)*. We do not use β-glucuronidase treatment of urine in our hospital.

Because clonazepam is frequently used in drug-facilitated sexual assaults, several articles have reported the detection of urinary metabolites after a single drug dose. Cheze et al. *(8)* investigated urine samples from healthy volunteers after a single dose of 2 mg clonazepam, and observed a peak urine 7-AC concentration of 55.0 ng/mL (measured by LC-MS/MS) 24 h after ingestion. This is in accordance with the long half-life of clonazepam (30–40 h) *(4)*. Negrusz et al. *(9)* also investigated the elimination of 7-AC in healthy subjects, finding peak urine concentrations <50 ng/mL at 6 h and <184 ng/mL at 24 h after ingestion of 3 mg clonazepam. The 7-AC peak concentrations in urine measured by chromatographic methods found in these studies were low compared to cutoff values used for positive testing with most commercially available benzodiazepine screening assays. For instance, our assay (Dimension Vista 1500 analyzer) uses a cutoff concentration of 200 ng/mL, calibrated with lormetazepam. As a consequence, these tests would probably yield false-negative results, regardless of the extent of cross-reactivity for 7-AC if comparable thresholds were used. We do not know the exact time of intoxication in our patient, but we estimate that the time between ingestion and presentation at the emergency department was between 6 and 12 h. Even though he ingested far more clonazepam than the subjects in the studies cited above, the relatively short time span between ingestion and urine analysis is probably another explanation for our negative screening result.

It is clear that benzodiazepine screening assays have several limitations, and negative screening results should be interpreted with caution. On the other hand, physicians should be aware that a positive screening result

could mask other potentially threatening or treatable conditions. If a patient is known to use benzodiazepines and presents in a comatose state, a positive screening result does not exclude other conditions. Therefore, a good medical history, thorough physical examination and additional (laboratory) tests should always be performed to rule out other problems, such as a cerebrovascular accident, epilepsy, or metabolic disorder.

The serum clonazepam concentration of 79 ng/mL (therapeutic range, 30–60 ng/mL) found in our patient was above the upper limit of the therapeutic range and, due to clinical circumstances, considered toxic. To our knowledge, the patient was naïve to benzodiazepines, which may have contributed to the comatose effect of this concentration of clonazepam. Furthermore, an alcohol concentration in blood of 200 mg/dL was found. Because alcohol can aggravate the effect of benzodiazepines *(1)*, this coingestion could have contributed to the clinical presentation.

In our patient, flumazenil was administered as a diagnostic tool. Benzodiazepines have a seizure-protective effect, and flumazenil should therefore not be used in patients who present with seizures, or if ingestion of proconvulsive drugs (such as tricyclic antidepressants) is known or suspected. A recent review showed that the use of flumazenil in a population admitted at the emergency department with (suspected) benzodiazepine intoxication was associated with an increased risk of adverse events compared to placebo *(10)*. However, it remains difficult to distinguish an adverse effect of flumazenil administration from the effect of the intoxication itself. Moreover, the benefit of flumazenil, for example to prevent endotracheal intubation, could outweigh the possible risks. The use of flumazenil should therefore be considered as a therapeutic or diagnostic tool. Known or suspected cointoxication with tricyclic antidepressants is an absolute contraindication for the use of flumazenil *(10)*. This patient did not have a seizure at presentation and was not suspected to have coingested proconvulsive drugs. He was continuously monitored on admission and no adverse events were observed.

Despite the fact that many of these pitfalls of urine benzodiazepine screening assays have been reported, we noted that many of our clinicians lack this awareness. To prevent misdiagnoses and over- or undertreatment of patients with a benzodiazepine intoxication, laboratories should play an educational role. In our hospital, we educate clinicians to appreciate the risk of false-negative or false-positive screening results and more importantly to rely on their clinical judgment.

POINTS TO REMEMBER

- A negative urine screening result does not rule out a benzodiazepine intoxication.
- Benzodiazepine screening assays are typically semiquantitative urine immunoassays calibrated with a specific benzodiazepine. Many immunoassays exhibit poor cross-reactivity with urine metabolites of the parent drug.
- The clinical presentation is key in establishing the diagnosis of benzodiazepine intoxication, and a confirmatory analysis (in urine or blood) with chromatographic methods could be performed to definitively rule out a benzodiazepine intoxication when screening results are questioned.
- Flumazenil can be administered as an antidote or diagnostic tool in benzodiazepine intoxications. It should not be used in patients who present with seizures or in patients with suspected ingestion of proconvulsive drugs (such as tricyclic antidepressants).

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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