Prescription Compliance or Illicit Designer Drug Abuse?

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CASE DESCRIPTION

A 62-year-old Caucasian woman underwent evaluation for possible liver transplantation. She had end-stage cirrhosis secondary to hepatitis C and alcohol abuse. Among the tests ordered were urine screens for various drugs of abuse, including amphetamines, for which the patient’s urine sample was positive. Given the patient’s history of alcohol abuse and possible other drug abuse, her physicians questioned her in detail about recent use of any illicit substances. The patient stated emphatically that she had used no recreational drugs for the past 15 years. The physicians wondered what could be the cause of a false-positive result and relayed to the laboratory that the patient was on several medications. The laboratory arranged to have the initial sample analyzed for amphetamines by GC-MS. The result was reported as negative for amphetamine, methamphetamine, 3,4-methylenedioxy-methamphetamine (MDMA),3 and 3,4-methylenedioxyamphetamine (MDA). In addition, the laboratory contacted the manufacturer of the screening immunoassay used [Amphetamines II, a kinetic interaction of microparticles in solution (KIMS) assay; Roche Diagnostics] and was informed that the medications that the patient had been prescribed, namely trazodone and bupropion, had been tested and did not exhibit any cross-reactivity.

DISCUSSION

Amphetamines are a group of compounds that include amphetamine, methamphetamine, MDMA, and a large number of “designer drugs.” Widespread abuse has been reported worldwide and has increased over the last decade (1). In the clinical laboratory, the detection of amphetamine abuse has become increasingly important because of the potential for adverse drug–drug interactions involving existing prescription pharmaceuticals. Many antidepressants and antipsychotics function by affecting concentrations of dopamine, norepinephrine, and/or serotonin. Likewise, amphetamines increase concentrations of these biogenic amines by blocking their uptake by plasma membrane and/or vesicular transporters. Thus, in addition to their well-documented habit-forming properties and toxicities (2), amphetamines are of concern to clinicians because of their potentially toxic or fatal drug–drug interactions with similarly acting prescription drugs.

Screening immunoassays designed to detect amphetamines have had particularly high rates of false positives because of the structural similarities between amphetamines and drugs such as nasal decongestants (pseudoephedrine) and weight-loss supplements (synephrine). Consequently, manufacturers test such structurally related compounds to demonstrate the analytical specificity of their assays. Knowing that they do can lead to a false sense of security, because most toxicology testing in clinical laboratories is performed with urine samples, and many drugs are excreted in the urine as metabolites. Therefore, testing the parent compound is not sufficient to prove that a given drug will not interfere with an immunoassay.

That is certainly the case with trazodone, in which <1% of the parent drug is excreted in the urine (3). Trazodone is a triazolopyridine derivative widely prescribed as an antidepressant drug. Pharmacologically, trazodone acts as a serotonin antagonist and reuptake

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1 Nonstandard abbreviations: MDMA, 3,4-methylenedioxy-methamphetamine; MDA, 3,4-methylenedioxyamphetamine; KIMS, kinetic interaction of microparticles in solution (assay); mCPP, m-chlorophenylpiperazine; 5-HT, 5-hydroxytryptamine.
inhibitor (4). Interestingly, trazodone’s primary metabolite is \textit{m}-chlorophenylpiperazine (mCPP), which is itself an abused “designer drug” and thought to be responsible for some of the effects attributed to trazodone. It is a potent serotonin agonist, exhibiting affinities for the 5-hydroxytryptamine (5-HT) receptors (5-HT1, 5-HT1, 5-HT1A, 5-HT2, and 5-HT2C) (5). mCPP has been reported as a drug of abuse for more than a decade and is used either by itself or in combination with other illicit drugs (6).

**RESOLUTION**

The positive immunoassay result for amphetamines combined with the history of polysubstance abuse caused concerns that the patient was abusing illicit drugs and therefore would not be a good candidate for liver transplantation. The GC-MS confirmation test, however, failed to detect amphetamine, methamphetamine, MDA, or MDMA in the patient’s urine. Appropriately, the laboratory contacted the physicians and updated its report to reflect the fact that the initial immunoassay result was a false positive. In an effort to identify the source of the positive result in the immunoassay screening assay, the laboratory sent the patient’s sample to San Francisco General Hospital, which had in place a screen for “designer drugs” that used an LC-TOF-MS method. Recently, high–mass accuracy mass spectrometers that use TOF technology have become extremely powerful screening tools in clinical laboratories (7). Large numbers of drugs can easily be screened against preestablished libraries, with compounds identified by their retention times and a calculated molecular formula. The molecular formula is generated from the exact mass of the compound and the isotope-abundance ratios of each of the elements (7). Analysis of the patient’s sample with a method that screens for 40 compounds of the amphetamine type, including the most widely reported “designer drugs,” identified mCPP at a concentration of approximately 9000 ng/mL.

When this result was reported back to the clinical laboratory at Beth Israel Deaconess Medical Center, a new question arose: Was the patient taking a designer amphetamine rather than the amphetamines included in the standard GC-MS screen? Because the laboratory had been assured that the KIMS assay did not detect trazodone, they wondered whether trazodone might interfere with the mass spectrometry method. A review of the compounds’ chemical structures suggested that an isobaric interference caused by the parent trazodone molecular ion was unlikely, but interrogation of trazodone’s chemical structure revealed an mCPP moiety (Fig. 1, shaded). A literature search confirmed that mCPP is an active metabolite of trazodone (8).

To determine whether mCPP cross-reacted with the KIMS amphetamine immunoassay and therefore was responsible for the positive result in the amphetamine screen, we spiked mCPP into a drug-free urine sample over a range of physiologically relevant concentrations (Table 1A). A positive result was obtained for all samples with mCPP concentrations \(\geq 5000\) ng/mL. Of note is that the patient’s sample contained approximately 9000 ng/mL mCPP. From these data, we concluded that the trazodone metabolite mCPP was the cause of the positive amphetamine result for this patient. We also spiked the metabolite mCPP into 3 other

**Fig. 1. Structures of several amphetamine-type stimulants (amphetamine, pseudoephedrine, MDMA), trazodone, and mCPP (a trazodone metabolite).**
widely used amphetamine immunoassays and found that the Microgenics CEDIA®/H23041 Amphetamines/Ecstasy assay gave a positive result of 20,000 ng/mL, but neither the Emit®/H23041 II Plus Amphetamines assay (Beckman Coulter) nor the AxSYM II Amphetamine/Methamphetamine II assay (Abbott Diagnostics) showed any reactivity to concentrations as high as 100,000 ng/mL (Table 2B).

As this manuscript was in preparation, a separate report that mCPP cross-reacts with the KIMS immunoassay was published (9); however, that study used an assay specific for mCPP rather than the more general TOF mass spectrometry assay we have described. More importantly, their study focused exclusively on the Roche KIMS assay and did not include data for other widely used immunoassays. We recommend that laboratories be aware of the limitations of their specific amphetamine assays. Laboratories that use the Emit II and AxSYM assays should not expect to have false positives from patients prescribed trazodone, whereas laboratories that use CEDIA or Roche KIMS assays can expect false-positive results. Additionally, it is important to point out that arylpiperazine motifs, such as mCPP, are common in many current pharmaceuticals, including aripiprazole, nefazodone, perospirone, and ziprasidone (10). Thus, it is possible that dealkylation of these drugs could also cause false-positive amphetamine results, but this supposition remains to be shown directly. In any case, any positive result in a screening immunoassay must be considered presumptive until it is confirmed with another method based on a different principle. Depending on the downstream implications of the results, we recommend that discrepancies be investigated further by reviewing the patient’s history and obtaining a list of medications, including all prescribed, over-the-counter, and herbal products. Careful evaluation of the current literature will reveal any published reports of substances that interfere with the immunoassay. Next, we recommend contacting the manufacturer of the immunoassay to determine whether the patient’s medications or their metabolites have been directly tested for cross-reactivity. Finally, additional testing, such as comprehensive drug screens or other types of targeted screens (i.e., designer drugs and so forth) could be used to discover the identity of the interfering compound.

Table 1. KIMS assays for mCPP (A) and comparison of mCPP immunoassays (B).

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<tr>
<th>A. Roche KIMS Assay</th>
<th>Signal</th>
<th>Result</th>
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<tbody>
<tr>
<td>mCPP concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100,000 ng/mL</td>
<td>168</td>
<td>+</td>
</tr>
<tr>
<td>20,000 ng/mL</td>
<td>105</td>
<td>+</td>
</tr>
<tr>
<td>10,000 ng/mL</td>
<td>84</td>
<td>+</td>
</tr>
<tr>
<td>5000 ng/mL</td>
<td>31</td>
<td>+</td>
</tr>
<tr>
<td>1818 ng/mL</td>
<td>-61</td>
<td>-</td>
</tr>
<tr>
<td>d-Amphetamine concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1250 ng/mL (positive control)</td>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td>750 ng/mL (negative control)</td>
<td>-85</td>
<td>-</td>
</tr>
<tr>
<td>0 ng/mL</td>
<td>-218</td>
<td>-</td>
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<table>
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<th>B. Result with mCPP spike of:</th>
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<tr>
<td>Immunoassay</td>
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<tr>
<td>KIMS</td>
</tr>
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<td>CEDIA</td>
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<td>Emit II</td>
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<td>AxSYM</td>
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a (A), The KIMS assay is calibrated such that a signal of 0 corresponds to 1000 ng/mL d-amphetamine. Samples with signals ≥0 are reported as positive. Two QC samples run in parallel demonstrated that the assay and instrument were running properly (positive control, 1250 ng/mL d-amphetamine; negative control, 750 ng/mL), generating signals of 40 and -85, respectively. Drug-free urine generated a signal of -218. Although yielding a technically negative result, even at a concentration of 1818 ng/mL, mCPP generated a signal higher than the 750-ng/mL d-amphetamine control. (B), Results from 4 different immunoassays of a drug-free urine sample spiked with mCPP at 5000, 20,000, and 100,000 ng/mL: Roche KIMS Amphetamines II assay, Microgenics CEDIA Amphetamines/Ecstasy assay, Abbott Diagnostics AxSYM Amphetamine/Methamphetamine II assay, and Beckman Coulter Emit II Plus Amphetamines assay. Positive results (+) and negative results (-) are at the indicated concentrations.
Clinical Case Study

POINTS TO REMEMBER

- Although generally accurate, positive immunoassay results must be considered presumptive until confirmed by mass spectrometry.
- A false-positive result can be caused by the apparent drug or by one of its metabolites. Many manufacturer-based interference studies evaluate only the parent compound and therefore do not rule out the potential of a given drug to produce a false positive.
- We recommend that laboratory scientists facing discrepant drug-screening results review the clinical situation and prescribed medications, consider sending the sample for confirmation, contact the immunoassay manufacturer for additional data not available in the package insert, stay engaged with the medical team, and consider additional testing to determine the source of the false positive.
- Interference profiles differ dramatically, both quantitatively and qualitatively, among assays from different manufacturers. It is important to understand the limitations and interferences of your specific immunoassay.
- A negative result in a drug screen does not mean complete absence of a drug. One needs to know what cutoff threshold is used by each laboratory for each drug or drug class.
- It is important to confirm results with the same sample that generated the initial positive result. A new sample may not contain the same interfering drugs in the same concentrations.

Commentary

Michael Ganetsky*

I dislike the urine drug of abuse screen (UDOA). As an emergency physician and medical toxicologist, I find that the UDOA creates unnecessary work and grief without affecting patient management, because clinicians confuse it for a diagnostic test.

The UDOA is routinely ordered for emergency department patients with psychiatric and neurologic complaints to answer the following question: Is this patient’s presentation caused by an intoxication? The UDOA is the wrong test in this setting. The UDOA looks for metabolites (instead of the parent compounds that cause the intoxication), thus increasing the window of detection, a goal for workplace drug testing. Additionally, the UDOA typically has a low negative predictive value because it cannot test for all substances that are potentially intoxicating. UDOA results hardly ever affect patient management, a limitation that was recognized more than 35 years ago (1), but our practice of routinely ordering this screen has not changed.

In my medical toxicology practice, my colleagues frequently ask me to interpret the results of a positive UDOA result. It is typically for a punitive purpose, such as to deny a patient opiates or to prevent listing the patient for transplantation, such as in the case Petrie et al. describe. Because these immunoassays frequently have false positives

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References


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