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Brief reports

Vortioxetine use may cause false positive immunoassay results for urine methadone

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ABSTRACT

Background: Urine immunoassays are frequently employed for methadone screening because they are relatively inexpensive and widely available. However, immunoassays are notoriously prone to false positives. We report that the use of vortioxetine (Trintellix® in the USA and Canada, Brintellix® worldwide) could cause false positives in the Roche KIMS Methadone II Urine immunoassay (MDN2).
Methods: We performed a spiking study using a parent drug vortioxetine concentration of 7500 ng/ml.
Results: Urine specimens from seven patients on typical vortioxetine doses tested positive for methadone in the Roche assay but negative for methadone in a confirmatory (GC/MS) assay and two other immunoassay platforms. Because of the pharmacokinetics of vortioxetine and the high cross-reactivity of a metabolite in the MDN2 assay, routine use of the drug could cause false positives even without detectible parent drug in the urine.
Conclusions: Vortioxetine is commonly prescribed for mood disorders, which have high prevalence in patients treated for opioid addiction. For that reason, it is important that clinicians are aware of this interference.

1. Introduction

The last decade has seen a sustained epidemic of opioid use and abuse and a concomitant increase in the demand for urine drug testing (UDT). UDT is used to verify that a patient is adherent with prescribed medications and abstaining from illicit drug use. The CDC recommends urine testing before prescribing opiates and at least annually to "assess for prescribed medications as well as other controlled prescription drugs and illicit drugs" [1]. Between 2011 and 2017, the College of American Pathologists (CAP) saw a 55% increase in the volume of proficiency testing for methadone immunoassays and a 588% increase for fentanyl immunoassays [2].

As indirect methods, immunoassays are prone to false positives by design. Clinicians are increasingly aware of these imperfections, as reviewed elsewhere [3,4]. Confirmatory testing by mass spectrometry after chromatography (LC/MS or GC/MS) for a specific drug or its metabolites is the most specific and sensitive test for the presence of that substance. However, routine use of confirmatory MS methods is currently limited by cost and availability.

At our institution, we use the Roche Kinetic Interaction of Microparticles in Solution (KIMS) Methadone II Urine immunoassay (hereafter MDN2) to screen for the presence of methadone in urine. At the request of the ordering provider, the sample can be sent to a reference lab for confirmation by GC/MS. In our institutional experience, few of the positive screens are sent for confirmatory testing, suggesting that clinicians are relying on the MDN2 result as the primary laboratory evidence for methadone use.

2. Methods

2.1. Analytic standard

Vortioxetine: HBr was from Astatech, Inc. A working methanolic stock (1 mg/ml) was prepared gravimetrically and used to make diluted standard solutions via dilution with deionized water.

2.2. Setting and patients

All patient samples were submitted to the Massachusetts General Hospital (MGH) Core Laboratory for drug of abuse testing as ordered by the patient's treating clinician. The Core Laboratory processes samples from inpatients and outpatients at MGH. This work was performed as part of a clinical quality assurance program and thus specific institutional review board approval was not required.

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2.3. Methadone immunoassays

The primary immunoassay used in this report was the Roche MDN2 which is based on the kinetic interaction of microparticles in solution (KIMS) technique. Samples with immunoreactivity less than the 300 ng/ml calibrator are reported as negative. Samples with immunoreactivity equal to or greater than the 300 ng/ml calibrator are reported as positive. We used the Roche Cobas 500 series analyzers for all Roche MDN2 immunoassay testing.

The Multigent methadone assay on the Architect Analyzer (Abbott Laboratories) is a competitive homogenous enzyme immunoassay. The enzyme labeled drug competes with drug from the urine for a fixed number of specific antibody binding sites. In the absence of drug in a given sample, the specific antibody binds to the drug labeled with glucose-6-phosphate dehydrogenase (GP6PDH) and the enzyme activity is inhibited. The qualitative cutoff is 300 ng/ml. This assay was performed at the Boston Medical Center Clinical Laboratory.

The Medtox Methadone assay on the MEDTOXScan instrument (Medtox Diagnostics) is a one step, competitive, membrane-based immunochromatographic test. The result is reported as positive or negative with a cutoff of 200 ng/ml. This assay was performed at the Nantucket Cottage Hospital Clinical Laboratory.

2.4. Vortioxetine testing

Vortioxetine was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) by Quest Diagnostics. This method had a detection limit of 10 ng/ml. This is a quantitative assay.

2.5. Confirmatory urine methadone testing

The patient urine samples that tested positive for methadone by the MDN2 assay were sent for confirmatory testing (Mayo Medical Laboratories). This assay had a limit of detection of 100 ng/ml for methadone and the methadone metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP).

2.6. Urine sample inclusion criteria

It was necessary to determine if the concentrations of vortioxetine that were shown to cause a false positive interference in the methadone assay in the spiking studies were clinically applicable. To this end, we selected all positive urine methadone samples received by the laboratory over a two-month period in which the donor did not have a methadone prescription but did have one for vortioxetine. This determination was made by review of the electronic medical record.

3. Results

Fig. 1 shows a graph of a spiking experiment, in which vortioxetine was added to deionized water to test for immunoreactivity in the MDN2 assay. Any signal above zero is reported as positive. It takes approximately 7500 ng/ml of vortioxetine in the immunoassay to generate a positive result, thus demonstrating cross-reactivity of 4% for vortioxetine in the MDN2 assay.

Table 1 shows the results of 7 patient urine samples that gave positive methadone immunoassay results and whose medical records indicated they were prescribed vortioxetine but not methadone. Four of the 7 patient urine samples had detectable vortioxetine as measured by LC-MS/MS. However, based on the spiking experiments that showed a concentration of 7500 ng/ml was required to cause a false positive on the MDN2, the concentrations of vortioxetine found in the patient urine samples (ranging from < 10–55 ng/ml) were not high enough. Fig. 2 shows the structures of methadone, vortioxetine and Lu AA34443, the major vortioxetine metabolite. Based on the similarities in the structures, it was hypothesized that methadone immunoassays from different manufacturers may also be vulnerable to this interference from vortioxetine. Where available, aliquots of the 7 patient urine samples were testing using two other methadone immunoassays and they all tested negative (Table 1).

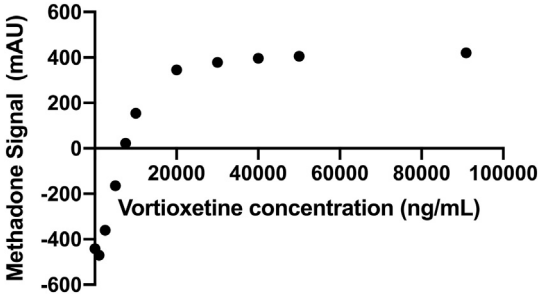

Fig. 1. Concentration of vortioxetine in urine (X-axis in ng/ml) vs. raw signal (Y-axis in mAU) in the KIMS Methadone II immunoassay (MDN2). Each dot represents the mean of duplicate measurements. Measurements of 0 or greater are resulted as "positive."

4. Discussion

Urine immunoassays are widely used to monitor drug use. However, because the assays do not measure the drugs of abuse or their metabolites directly, immunoassays are vulnerable to interferences. Some substances interfere directly with the signal from the antibody, such as excess biotin in a sample interfering with assays that rely on biotinylated antibodies [5,6]. Other substances can interfere if their structures are so similar to the target analyte that they cross-react with the antibodies used in the assay [4]. The cross-reactivity becomes clinically significant if it impacts the test interpretation of drug testing in a substantial patient population. Our data demonstrate that vortioxetine use can cause false positives in the Roche MDN2 immunoassay. Further, samples can test positive in the MDN2 assay even with very small amounts of the parent drug present in the sample.

Fig. 2 shows the structural similarity between methadone, vortioxetine, and the major metabolite Lu AA34443. If the vortioxetine metabolite Lu AA34443 is present in greater concentration than the parent drug in urine and also shows cross-reactivity with the MDN2 immunoassay, the false positive results in all seven samples are easily explained. The metabolite Lu AA34443 is not commercially available, nor is there a commercially available assay to measure Lu AA34443 in urine. We are therefore reliant on data from the vortioxetine manufacturer (Takeda) regarding Lu AA34443 concentrations in the urine of patients taking vortioxetine.

Fifty-nine percent of a vortioxetine dose is eventually eliminated in the urine but less 0.01% as the parent drug [7]. The patent drug is often undetectable in pharmacokinetic studies. Instead, the major metabolite in the urine is Lu AA34443, which is present at least 81× the concentration of vortioxetine itself [8]. Taking the average of our urine parent drug concentrations, 17 ng/ml, a conservative estimate of the Lu AA34443 urine concentration would be at least 1377 ng/ml (17 ng/ml x 81). Moreover, the drug manufacturer also provides an estimate of the mean amount of Lu AA34443 in the urine of a human taking 20 mg per day of vortioxetine: 14,000 ng/ml [9].

While we were conducting this study, Roche revised its package insert cross-reactivity table for the MDN2 assay to include vortioxetine, with a similar percent cross-reactivity claim (4%) to that reported here [10]. The insert also lists the metabolite, Lu AA34443, as a cross-reacting substance at the level of 14%. Therefore, it takes slightly > 2000 ng/ml Lu AA34443 to create a false positive in the immunoassay (300 ng/ml/0.14). Our conservative estimate above (1377 ng/ml) makes false positives plausible while the manufacturer's estimate is > 6 × the amount required to cause a false positive. In addition, there are any number of other metabolites with unknown cross-

Table 1
Results of methadone and vortioxetine testing on 7 patient samples by immunoassay and mass spectrometric methods. The prescribed dose is listed in the second column. IA, Immunoassay; mAU, milli-Absorbance Units; LC-MS/MS, Liquid Chromatography/Tandem Mass Spectrometry; GC–MS, Gas chromatography/Mass Spectrometry; QNS, quantity not sufficient for testing.

Sample	Vortioxetine dose (mg/day)	Vortioxetine LC-MS/MS (ng/ml)	Methadone GC–MS	Methadone roache IA (mAU)	Methadone abbott IA (mAU)	Methadone medtox IA
1	5	< 10	negative	40 (positive)	0 (negative)	negative
2	5	16	negative	358 (positive)	-9 (negative)	QNS
3	5	11	negative	217 (positive)	61 (negative)	negative
4	20	23	negative	106 (positive)	11 (negative)	QNS
5	10	QNS	negative	240 (positive)	96 (negative)	negative
6	20	< 10	negative	132 (positive)	37 (negative)	negative
7	10	55	negative	91 (positive)	−4 (negative)	negative

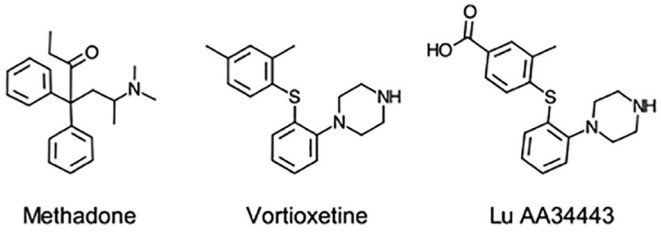

Fig. 2. Structures of methadone, vortioxetine, and the vortioxetine metabolite Lu AA34443.

reactivities that also might contribute to a (false) MDN2 immunoassay signal. In practice, this means a urine sample of a person taking vortioxetine but not methadone can test positive for methadone and negative for vortioxetine.

Vortioxetine was approved for use in the U. S. in 2013 and is now widely prescribed [11]. The incidence of depression in patients being treated for opiate use is 5–10 times higher than that of the general population [12]. It is not an uncommon occurrence for a patient in a methadone maintenance program to be taking vortioxetine. Physicians rely on laboratory guidance and available scientific literature to interpret results of urine drug tests. It is important for physician providers to be aware of this significant interference.

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