Reduced Interference by Phenothiazines in Amphetamine Drug of Abuse Immunoassays

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- **Context.**—Emergency department physicians frequently request urine drug screens, but many are unaware of their limitations, including the potential for false-positive results. Promethazine, a phenothiazine derivative, is used for the treatment of allergies, agitation, nausea, and vomiting. Many patients taking promethazine are subject to urine drug screens and any potential interferences are important to recognize.

- **Design.**—During an 11-month period, all patients presenting to the Massachusetts General Hospital emergency department who had a finding of promethazine in their serum drug screen, and who also had a urine drug screen performed, were selected for inclusion in the study. The urine drug screen results (n = 22 patients/samples) were then studied.

- **Objective.**—To determine if promethazine use can cause false-positive urine amphetamine results in widely used drug of abuse immunoassays.

- **Results.**—Thirty-six percent of patients taking promethazine had false-positive test results for urine amphetamines using the EMIT II Plus Monoclonal Amphetamine/Methamphetamine Immunoassay. Sixty-four percent of patients showed cross-reactivity greater than 20% higher than the blank calibrator rate. In a separate, related study, no promethazine-induced false-positive results were seen with the EMIT II Plus, Triage, and Testcard 9 amphetamine assays, or the Triage methamphetamine assay. Reduced chlorpromazine interference was also seen with these other assays.

- **Conclusions.**—False-positive urine amphetamine results can be obtained in patients taking promethazine. Promethazine metabolite(s), and not the parent compound, are the likely cause of these urine false-positive results obtained with EMIT II Plus Monoclonal Amphetamine/Methamphetamine Immunoassay. Immunoassays from different manufacturers can have very different “interference” profiles, which the pathologist and laboratory scientist must understand and relay to clinicians.

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important for clinical laboratories to report any new observations.

Data from our toxicology laboratory reveal that many patients taking promethazine also have ‘amphetamine’ detected in their urine, even though the patient denies taking any form of amphetamine. We investigate the validity of these amphetamine results in an attempt to determine any interference of promethazine in the EMIT-MAM assay. Finally, we report results of 6 different test systems on urine samples from patients ingesting promethazine or chlorpromazine.

MATERIALS AND METHODS

We identified all patients presenting to the emergency department during an 11-month period who had promethazine detected in their serum toxicology screen and who also had a urine toxicology screen performed. If promethazine was detected in a patient’s serum, we then used the laboratory information system to retrospectively identify those patients who also had an EMIT-MAM urine toxicology screen performed at the same time as their serum analysis. All patients with promethazine detected in their serum and who also had an EMIT-MAM urine toxicology screen performed were included in the study (n = 22). Results of the EMIT-MAM drug screen were obtained from the laboratory information system and used in the data analysis.

Blood and urine samples obtained from patients in the emergency department are sent to the laboratory via a pneumatic tube transportation system and processed immediately on receipt by the laboratory. On completion of analysis (usually 1–3 hours), all urine and serum samples are stored frozen at –20°C.

Toxicology analysis for all serum and select urine specimens (as described later in an adjunct study) was performed using liquid chromatography with photodiode array detection (LC-PDA) by the detection limits for phenothiazines, amphetamines, and amphetamine-like compounds using LC-PDA are as follows: amphetamine (20 µg/L), chlorpromazine (20 µg/L), methamphetamine (35 µg/L), MDMA (70 µg/L), ephedrine (60 µg/L), phentermine (20 µg/L), promazine (20 µg/L), and pseudoephedrine (70 µg/L).

Urine toxicology screens were performed on a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, Ind) using the EMIT-MAM assay. If the enzyme rate observed using a patient’s urine sample exceeds the rate generated using the 1000 µg/L d-methamphetamine calibrator, the sample is considered presumptive positive for amphetamines. The assay also has a limit of detection of 1000 µg/L for d-amphetamine.

In an adjunct study, we selected consecutive patients from the emergency department who were found to have either promethazine (n = 3) or chlorpromazine (n = 6) in their serum and a positive result from an EMIT-MAM urine amphetamine screen. Their serum and urine samples were stored at –20°C for further toxicology analysis. The LC-PDA analysis of the urine (promethazine) or the LC-PDA analysis of serum (chlorpromazine) was performed to determine whether the patients had taken amphetamines. None of the samples had amphetamines detected. All 9 samples producing false-positive urine amphetamine results were also analyzed using 5 other immunoassay platforms, in addition to the EMIT-MAM assay previously described (Table 1).

The EMIT II Plus amphetamine assay (EMIT-A†) was performed on a Hitachi 911 analyzer. Similar to the EMIT-MAM, the EMIT-A uses a 1000 µg/L d-methamphetamine calibrator. Urine sample results with enzyme rates above this cutoff are considered positive. The Triage TOX Drug Screen (Biosite Incorporated, San Diego, Calif) and TesTcard 9 (Varian, Inc, Palo Alto, Calif) point-of-care drugs of abuse immunoassays for amphetamine and methamphetamine were performed according to the manufacturers’ instructions. The Triage amphetamine and methamphetamine assays use calibrator cutoff concentrations of 1000 µg/L. In the TesTcard 9 system, 300 µg/L of d-amphetamine and 500 µg/L of d-methamphetamine are used as calibrator cutoff concentrations for the amphetamine and methamphetamine assays, respectively.

RESULTS

We retrospectively reviewed the results of all patients who presented to the Massachusetts General Hospital emergency department during an 11-month period who had promethazine detected in their serum. From these patients, we found 22 who also had a urine toxicity screen performed; these 22 patients were included in this study (Table 2). Using LC-PDA, no amphetamine or amphetamine-related compounds were detected in the serum of any of the patients. Eight (36 %) of the 22 patients taking promethazine had positive test results for urine amphetamine (Table 2 [results in bold]). Results greater than 100% of the cutoff calibrator were considered positive. In addition, 4 (29 %) of the 14 negative results were more than 50% higher than the amphetamine-free blank calibrator used in the assay (Table 2 [results in italic]), suggesting some effect of promethazine on assay results. Sixty-four percent of patients taking promethazine showed some effect of promethazine on assay results. Sixty (29 %) of the 14 negative results were more than 50% higher than the amphetamine-free blank calibrator used in the assay.

In another experiment, we studied paired urine and serum samples from 9 patients in the emergency department using 5 different amphetamine immunoassay plat-
Promethazine is used in a variety of clinical situations and its potential cross-reactivity in drugs of abuse assays is clinically relevant. Promethazine use appears to be increasing according to findings at this institution. Promethazine was detected in 23 (1.8%) of 1290 serum toxicology samples obtained from patients presenting to the emergency department during a recent 47-day period. A 20-µg/L limit of detection for promethazine using LC-PDA allows detection of the drug in serum samples from patients taking typical therapeutic doses. As a consequence, our study population resembles a typical one from an emergency department, not a group of patients with known toxic serum promethazine concentrations. More than one third of our patient population with a positive finding for serum promethazine tested positive for amphetamine in urine using EMIT-MAM. In a previous study, 18 inmates received 50 mg of promethazine every day and their urine was subsequently screened by an earlier-generation EMIT-MAM assay for drugs of abuse.

### Table 3. Results of Paired Serum/Urine Samples Having Both Positive Serum Toxicology Screens for Promethazine or Chlorpromazine, and a False-Positive Urine Enzyme Multiplied Immunoassay Technique II Plus Monoclonal Amphetamine/Methamphetamine Assay (EMIT-MAM)*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Serum LC-PDA Findings</th>
<th>Urine LC-PDA Findings</th>
<th>EMIT-MAM†</th>
<th>TRIAGE</th>
<th>TestCard 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EMIT-A†</td>
<td>A</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Promethazine</td>
<td>Negative for amphetamines</td>
<td>Positive (146)</td>
<td>Negative (&lt;5)</td>
<td>N N N</td>
</tr>
<tr>
<td>2</td>
<td>Promethazine</td>
<td>Negative for amphetamines</td>
<td>Positive (133)</td>
<td>Negative (&lt;5)</td>
<td>N N N P</td>
</tr>
<tr>
<td>3</td>
<td>Promethazine</td>
<td>Negative for amphetamines</td>
<td>Positive (161)</td>
<td>Negative (&lt;5)</td>
<td>N N N</td>
</tr>
<tr>
<td>4</td>
<td>Chlorpromazine</td>
<td>Not tested</td>
<td>Positive (179)</td>
<td>Negative (&lt;5)</td>
<td>N N N</td>
</tr>
<tr>
<td>5</td>
<td>Chlorpromazine</td>
<td>Not tested</td>
<td>Positive (267)</td>
<td>Negative (22)</td>
<td>N N N P</td>
</tr>
<tr>
<td>6</td>
<td>Chlorpromazine</td>
<td>Not tested</td>
<td>Positive (154)</td>
<td>Negative (&lt;5)</td>
<td>N N N</td>
</tr>
<tr>
<td>7</td>
<td>Chlorpromazine</td>
<td>Not tested</td>
<td>Positive (110)</td>
<td>Negative (&lt;5)</td>
<td>N N N</td>
</tr>
<tr>
<td>8</td>
<td>Chlorpromazine</td>
<td>Not tested</td>
<td>Positive (161)</td>
<td>Negative (&lt;5)</td>
<td>N N N</td>
</tr>
<tr>
<td>9</td>
<td>Chlorpromazine</td>
<td>Not tested</td>
<td>Positive (137)</td>
<td>Negative (&lt;5)</td>
<td>N N N</td>
</tr>
</tbody>
</table>

* LC-PDA indicates liquid chromatography with photodiode array detection; EMIT-A, EMIT II Plus amphetamines assay; C, calibrator compound; M, d-methamphetamine calibrator; A, d-amphetamine calibrator; N, “negative” result using the point-of-care assays; P, “positive” result using the point-of-care assays. See Table 1 for calibrator compounds and concentrations.

† The numbers in parentheses are the percent enzyme activity relative to the cutoff calibrator.
Three inmates (17%) had amphetamine detected in their urine. No false-positive results were seen when less than 50 mg/day promethazine was administered. In this study, we demonstrated that false-positive results by EMIT-MAM are caused by promethazine use. The higher false-positive frequency observed in this study may be explained by the differences in the subject population. All subjects in this study were patients admitted to the emergency department in whom serum and urine toxicology testing was ordered, and no limit was placed on the amount of promethazine the patients had received.

A previous study by Smith-Kielland et al. indicated that preparations of promethazine up to 3000 mg/L tested negative by the EMIT-MAM assay for amphetamine. In addition, the Syva package insert states that promethazine shows a negative response. This suggests that a promethazine metabolite(s) is responsible for the false-positive results described in Table 2. Chlorpromazine metabolites cross-react in the EMIT-MAM assay.3,4 Promethazine and chlorpromazine possess similar phenothiazine chemical structures, supporting the hypothesis that a promethazine metabolite(s) interferes with the EMIT-MAM assay. The metabolism of promethazine has not been thoroughly investigated in humans, but it is extensively metabolized. The major metabolites are promethazine sulfoxide, norpromethazine, and monodesmethy promethazine sulfoxide.10 Primary standards of promethazine metabolites are not currently available so we were unable to definitively determine which of the promethazine metabolites are responsible for the cross-reactivity in the EMIT-MAM assay. Manufacturers cannot test for all potential cross-reactants in drugs of abuse assays, and laboratories and clinicians should be encouraged to report false-positive results in these assays. We also suggest that manufacturers expand the information included in package inserts to include whether a compound exhibits significant cross-reactivity compared with a ‘blank’ solution. We believe this extra information will aid investigators studying suspected false-positive results.

Nine samples with false-positive amphetamine results by EMIT-MAM assay were examined by 5 alternate immunoassays (Table 1) to compare cross-reactivity profiles. All 5 methods had reduced interference to both promethazine and chlorpromazine use. Eight of the 9 urine samples tested by EMIT-A had enzyme rates indistinguishable from the blank calibrator (ie, <5%). However, the ninth sample had a rate 22% above the blank. The ninth sample also had the highest degree of interference in the EMIT-MAM assay, and generated a positive result by TesCard 9 methamphetamine assay. The EMIT-A assay can be made more sensitive by using either of the available 300 and 500 μg/L cutoff calibrators rather than the 1000 μg/L cutoff used in this study. Using a lower cutoff of 300 μg/L, the ninth sample had a rate 89% of the cutoff calibrator, extremely close to the rate that would be judged positive, raising the possibility that some cross-reactivity caused by phenothiazine metabolites may still remain in the EMIT-A assay. No false-positive results were seen with the Triage amphetamine or methamphetamine assay or the TesCard 9 amphetamine assay, indicating improved specificity compared with EMIT-MAM. The 2 positive results obtained by TesCard 9 methamphetamine assay may represent false-positive results. As an alternative explanation, the manufacturer of TesCard 9 claims a lower detection limit for methamphetamine than the other assays, so it may be detecting amphetamine concentrations that the other immunoassays cannot. However, the negative LC-PDA finding argues against this alternate explanation.

Although the sample size is limited, evidence strongly indicates that the EMIT-A, Triage, and TesCard 9 assays provide superior specificity compared with EMIT-MAM in samples from patients receiving promethazine or chlorpromazine. A recent comparison of 6 urine amphetamine immunoassay systems found the EMIT-A to be superior in specificity to both EMIT-MAM and the other 4 systems studied.11 Our data also suggest that EMIT-A has superior specificity to EMIT-MAM. However, the sensitivity of EMIT-A should be considered. The manufacturer (Syva Corp) states the concentrations of MDMA (“Ecstasy”) necessary to yield a positive result for the EMIT-MAM and EMIT-A assays (using a 1000 mg/L cutoff) as 9140 and 34 274 mg/L, respectively. The concentrations illustrate that the EMIT-A assay is less sensitive for detection of MDMA use than the EMIT-MAM assay. We and others11 have observed discordant EMIT amphetamine-type results, in which the urine EMIT-MAM is positive and the EMIT-A is negative. The patients at our hospital with discrepant results had only MDMA in their serum, consistent with the manufacturer’s data that EMIT-A is less sensitive to MDMA detection. In our emergency department population, the frequency of phenothiazine use exceeds that of MDMA. In addition, our serum toxicology assay has a limit of detection of 70 μg/L for MDMA. Changing from the EMIT-MAM to the EMIT-A assay for urine testing at our institution would improve specificity with little cost to sensitivity. In different patient populations, a switch from the EMIT-MAM to the EMIT-A assay may have different consequences. The National Academy of Clinical Biochemistry recently recommended that, for patients in emergency departments, highly specific amphetamine immunoassays directed at the illicit amphetamines (such as MDMA and methamphetamine) are less useful compared with assays sensitive to a broad spectrum of sympathomimetic, stimulant, and illicit amines.12

We conclude that promethazine use can cause false-positive results in EMIT-MAM urine amphetamine assays. Clinicians should understand this limitation when interpreting urine drug screens from laboratories employing this method. We urge that promethazine be added to the EMIT-MAM package insert listing of compounds that may cause false-positive results. Reduced interference by phenothiazines was observed using these alternative drugs of abuse amphetamine immunoassay platforms; EMIT-A, Triage, and TesCard 9.

References

6. Puopolo PR, Volpicelli SA, Johnson DM, Flood JG. Emergency toxicology testing (detection, confirmation, and quantification) of basic drugs in serum by