Detection and Interpretation of Lysergic Acid Diethylamide Results by Immunoassay Screening of Urine in Various Testing Groups

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Abstract

A total of 2259 urine samples were assayed for lysergic acid diethylamide (LSD) using radioimmunoassay (RIA, Coat-a-Count, Diagnostics Products) and a premarket cloned enzyme donor immunoassay (CEDIA, Boehringer Mannheim). Urine samples were obtained from patients admitted to the emergency room, patients in drug rehabilitation programs, and adults and juveniles in criminal probation programs. An overall incidence of positive results was 0.80% for CEDIA (500-pg/mL cutoff) and 0.89% and 0.18% for RIA at cutoffs of 250 and 500 pg/mL, respectively. Of the CEDIA-positive samples, only 17 and 11% were positive by RIA at 250 and 500 pg/mL, respectively, whereas among RIA-positive samples, only 10% of those > 250 pg/mL and only 25% of those > 500 pg/mL were positive by CEDIA. Moreover, only 2 of 25 of samples positive by one of these screening assays were confirmed by gas chromatography-mass spectrometry (GC-MS). It is likely that discrepancies in results between immunoassays are due to differences in antibody specificities used to detect LSD metabolites. In addition, immunoassays may be more sensitive than GC-MS for detecting LSD use as current confirmation assays are targeted towards detection of the parent drug only. The interpretation of results for LSD analysis must be made with knowledge of the limitations for each assay.

Introduction

Lysergic acid diethylamide (LSD) is a powerful hallucinogenic drug in the serotonin class of psychedelics (1). Surveys have shown that high school and college students report the highest usage of LSD. Of subjects 18–25 years old, 11.1% reported having used LSD at least once, and 3.3% have used it within the past year (2). The corresponding figures for subjects 26–34 years of age are 12.4% and 0.5%. One group of investigators reported an LSD prevalence among high school students of 5.6% in 1992 (3), whereas among college students, a prevalence as high as 17% in a southern university has been reported (4). In drug-related emergency department (ED) visits, a low but steady frequency of LSD usage has been documented by The Drug Abuse Warning Network (DAWN) (5). From 1988 to 1993, the prevalence of LSD-related ED visits ranged from 0.0015 to 0.0018%.

The active form of LSD is the d-isomer that has a molecular weight of 323 Daltons. LSD has a half-life of about 3–4 h and a volume of distribution of 0.28 L/kg (1). Approximately 90% of LSD is bound to plasma proteins. Blood and urine concentrations of LSD following confirmed use have been studied by a number of investigators. Aghajanian et al. (6) found that after a 2-μg/kg dose, blood concentrations of LSD peaked at 5 ng/mL after 1 h and declined to 1 ng/mL after 8 h. Vu-Duc et al. (7) used RIA and found positive urine results for LSD at a cutoff of 0.1 ng/mL for 3 days after a 50-μg dose. Using HPLC with fluorescence detection, McCarron et al. (8) found positive urine results exceeding 0.5 ng/mL for at least 11 h after use. The LSD values in urine ranged from 0.2 to 7.7 ng/mL. These latter two studies suggest that urine is a reasonable specimen for detecting recent LSD use by the donor.

Sensitive assays for LSD and its metabolites have been developed using liquid chromatography–mass spectrometry and gas chromatography–tandem mass spectrometry (GC–MS–MS) (9,10). However, routine GC–MS assays are targeted for detection of the parent compound alone. Commercial isotopic and nonisotopic immunoassays have been available for several years. Antibodies used in these assays are raised against the parent drug, but, like other immunoassays for drugs of abuse, the LSD assay is likely to be sensitive to a variety of different unidentified metabolites. The purposes of this study were to examine the prevalence of LSD usage in various subject populations and to compare results of immunoassays with each other and with GC–MS.

Experimental

Assays

The Coat-A-Count radioimmunoassay (RIA) for LSD was obtained from Diagnostic Products Corporation (DPC, Los Angeles, CA). This assay was performed in the quantitative...
mode using calibrators ranging from 0 to 3000 pg/mL. LSD
cutoff concentrations of 250 and 500 pg/mL were evaluated in
this study. This solid-phase RIA makes use of 125I-labeled LSD,
which competes with LSD from the urine sample for binding
onto an immobilized LSD-specific antibody. After a 2-h incu-
bation, the radioactivity of bound LSD tracer was measured
using a γ-counter (model 1277, Wallace, Gaithersburg, MD). A
premarket cloned enzyme donor immunoassay (CEDIA) was
obtained from Boehringer Mannheim (BMC, Concord, CA).
Subsequent to this study, this assay received approval by the
Food and Drug Administration and is now commercially avail-
able. The general principles of CEDIA were presented previ-
ously (11). The CEDIA LSD assay was performed in the quali-
tative mode with a cutoff concentration of 500 pg/mL. The
threshold calibrator was set at a zero rate. This assay requires
the use of three reagents and was adapted for automated anal-
ysis onto the BMC/Hitachi 911 analyzer (BMC, Indianapolis,
IN).

GC–MS was performed on a subset of urine samples that were
positive by immunoassay. In some cases, an insufficient
volume of urine prohibited confirmation analysis. All quanti-
tative GC–MS analyses were performed at MedTox Laboratories
(St. Paul, MN). The assay was based on a modification of the
LSD assay described by Francom et al. (12). Standards, controls,
and unknowns were alkaliniwed and extracted into butyl chlo-
ride. A trimethylsilyl derivative was prepared and analyzed by
GC–MS (M+ = 395). Quantitation for controls and unknowns is
obtained from the standard–internal standard linear least-
squares regression line. Unknown specimens greater than or
equal to the limit of quantitation and meeting retention time
and qualifying ion ratio criteria were reported as positive. The
limits of detection and quantitation are 0.10 and 0.25 ng/mL,
respectively. LSD metabolites were not identified or quanti-
tated in this confirmation assay.

Urine samples

The protocol for the use of urine samples was reviewed and ap-
proved by the respective Institutional Review Boards at Hartford
Hospital and the University of Texas Health Science Center. All
subjects were identified by number, and informed consent was
deemed unnecessary. A total of 2259 urine samples were tested in
this study. Of these, 640 samples were obtained from consecutive
patients admitted to the Emergency Department of Hartford
Hospital (Hartford, CT) for emergency drug screen analysis that
included cocaine, barbiturates, benzodiazepines, opiates, canna-
bainoids, and amphetamines. Both negative and positive samples for these drugs were used in the analysis for LSD. For ED
patients, the only criterion for entry into this study was an order
for a urine drug screen that was due to a suspicion of drug use
and enough leftover urine present for the LSD analysis. A total of
570 samples were also obtained from the drug rehabilitation
program administered by Hartford Hospital’s psychiatric unit
(The Institute of Living, Hartford, CT). Urine specimens (350)
were obtained from adults and juveniles who were in criminal
probation programs in the state of Connecticut. These latter
samples were originally tested for drugs of abuse at the Graham
Massey Laboratory (Bridgeport, CT). The remaining 699 samples
were submitted to the Hermann Hospital (Houston, TX) for drug
analysis and include patients from the emergency department,
obstetrical patients, and those enrolled in drug rehabilitation
programs. The assay for LSD was not part of the normal testing
protocol for any of these subject groups.

After routine testing for the ordered drug tests, urine speci-
mens were stored for up to one week at refrigerated tempera-
tures and then kept frozen at -20°C for up to six months before
testing. All samples were thawed and tested by both RIA and
CEDIA on the same day. Positive samples were then refrozen
and sent in a batch to MedTox Laboratories for GC–MS confir-
mation analysis.

### Results

The within-run and day-to-day precision of the CEDIA assay are shown in Table I. The table shows the standard deviations and coefficients of varia-
tion (CV) were well within manufacturer’s specifications. Table II lists the prevalence of LSD in various subject populations. The

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<tr>
<th>Table I. Assay Precision for the CEDIA LSD Assay*</th>
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<td>Material</td>
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<tr>
<td>Control 1 (300 pg/mL)</td>
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<td>Control 2 (700 pg/mL)</td>
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* Studies conducted at Hartford Hospital (n = 20 for within run and n = 89 for between run).

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<tr>
<th>Table II. Prevalence of LSD-Positive Urine Samples by CEDIA and RIA*</th>
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<td>Assay</td>
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<td></td>
</tr>
<tr>
<td>CEDIA</td>
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<tr>
<td>RIA (≥250 pg/mL)</td>
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<td>RIA (≥500 pg/mL)</td>
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* Samples obtained and tested at Hartford Hospital, Hartford, CT, except where noted.
† Samples obtained and tested at Hermann Hospital, Houston, TX.
highest incidence of LSD positive results was observed in the
two hospitalized patient categories (1.1%). Lower incidences were observed in subjects enrolled in drug rehabilitation and 
probation programs. For CEDIA, the absorbance rate values for positive results ranged from 2 to 69 ΔmAU/min. Positive RIA values ranged from 254 to 14,900 pg/mL. For RIA, lowering the cutoff concentration increased the overall positive detection rate from 0.18% to 0.89%, as would be expected.

The percentage concordance among positive samples between CEDIA and RIA at either cutoff concentration was poor. For the 18 urine specimens positive for CEDIA, only 2 (11%) and 3 (17%) were positive by RIA using the 500- and 250-pg/mL cutoff concentrations, respectively. Likewise among the 4 and 20 urine samples positive by RIA (500 and 250 pg/mL cutoffs, respectively), only 1 (25%) and 2 (10%) were positive by CEDIA.

Of the 35 samples positive by one or more of the immunoassays, 25 were tested again by GC–MS. Of this group, only two were positive for LSD at a cutoff of 200 pg/mL. Table III lists results of urine samples for which data using all three methodologies were available.

Discussion

In this study, an overall LSD prevalence of 0.8–0.9% was reported in different testing populations using an immunoassay for screening of urine, which is roughly consistent with national surveys of recent LSD use among subjects aged 18 to 34. When considering the association of LSD use and ED visits, these results are considerably higher than values reported by the DAWN (5). The DAWN data are falsely low because hospital emergency departments do not routinely screen urine for the presence of LSD; thus, the true incidence is largely unknown.

The hitherto absence of an automated nonisotopic immunoassay has limited the ability of EDs and laboratories to perform such testing in real time. This study showed that LSD had a higher prevalence among hospitalized patients than what was previously reported for PCP (0.15%) and had a prevalence equal to that of amphetamines (0.86%) (13).

There have been several other studies comparing the performance of nonisotopic immunoassays for LSD with RIA, producing varying degrees of agreement among positive results. In one study of 35 urine samples, 88% concordance was observed between CEDIA and GC–MS–MS, and 100% concordance was observed between RIA and CEDIA (14). In that study, only samples with positive GC–MS results were compared; thus, they all had high concentrations of the parent drug. In the study by McNally et al. (15), there was an 82% concordance rate among positive samples between a commercial RIA assay and a nonisotopic immunoassay developed by the same manufacturer (Roche Diagnostics, Somerville, NJ). Although the antibodies used in these assays were not identical, they were raised against structurally similar immunogens, and a high concordance rate was not unexpected. In the study of Cassells et al. (16), a concordance rate of 85% was observed between RIA (DPC) and a microplate enzyme immunoassay. In contrast with these studies, Cody et al. (17) found significant differences between two RIA assays (DPC and Roche) when nonhuman primates were given a 2 μg/kg dose of LSD.

A major concern with interpreting results of different LSD immunoassays is the absence of available GC–MS confirmation procedures that are targeted toward LSD metabolites. LSD has a biological half-life of about 3 h and is metabolized by N-demethylation (N-demethyl–LSD), N-deethylation (N-desethyl–LSD), and hydroxylation (13-hydroxy-LSD and 14-hydroxy-LSD) to inactive metabolites. Recently, two new metabolites of LSD, lysergic acid ethylvinylamide and 2-oxo-LSD, were identified by capillary electrophoresis–tandem mass spectrometry (18). Only a small amount of LSD is excreted into the urine unchanged (19). Thus, even with very sensitive assays, the window for detecting LSD use is very short. LSD metabolites have a longer biological half-life; therefore, it is not surprising that GC–MS confirmation results were negative on samples that were initially positive by immunoassay. The low rate of GC–MS confirmation observed in this study was not due to degradation of storage because LSD has shown to be very stable when frozen (20)

It may be possible that the mode of immunoassay testing (i.e., qualitative CEDIA and quantitative RIA) had a role in producing these discordant results. Data in Table III showed, however, that in the majority of cases, specimens with positive results by CEDIA did not have quantitative results that were at

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<th>Table III. Results of LSD Analysis for Samples with GC–MS Results*</th>
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<tr>
<td>CEDIA rate (ΔmAU/min)</td>
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* For CEDIA, the rate for the cutoff calibrator (500 ng/mL) was set at zero. For RIA, positive quantitative results are set to a cutoff of 250 pg/mL.


or near the RIA cutoff concentration. Likewise, specimens with positive RIA results did not have ΔmAU/min rates that were near the cutoff values for CEDIA.

The lack of concordance between immunoassays most likely occurred because the immunoassays cross-react to different LSD metabolites. In crossreactivity studies conducted by the manufacturer, common therapeutic drugs and drugs of abuse and LSD-like substances such as ergotamine, α-ergocryptine, lysergic acid, and serotonin did not produce any significant crossreactivities (13). However, because the metabolism of LSD is not well-defined, crossreactivity studies to LSD metabolites have not been fully documented.

Conclusion

With the development of more automated immunoassay methods for screening, consideration should be given for routine LSD testing in urine of select populations. The LSD assay could be added to a “stimulants and hallucinogens” panel which might include amphetamines, phencyclidines, and marijuana and may be helpful in explaining unusual or hallucinogenic behavior. For drug rehabilitation, this assay would give a more complete evaluation of the effectiveness of the program. In forensic toxicology, testing for LSD may be helpful in identifying drug use as a contributor to death. For workplace testing, the combination of the screening assay with GC–MS would allow for detection of positive samples only if urine is sampled reasonably soon after drug use. When the metabolism of LSD in humans is further defined, more useful confirmation assays could be developed that are targeted to specific metabolites, thereby enabling more effective confirmation of screening results.

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References


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