Failure of Amoxicillin to Produce False-Positive Urine Screens for Cocaine Metabolite

Gary M. Reisfield1, Judella Haddad1, George R. Wilson1, Laura M. Johannsen3, Kathryn L. Voorhees3, Chris W. Chronister4, Bruce A. Goldberger4, James D. Peele5, and Roger L. Bertholf2,*

Departments of 1Community Health and Family Medicine and 2Pathology, University of Florida Health Science Center, Jacksonville, Florida; 3Shands Jacksonville Medical Center Clinical Laboratories, Jacksonville, Florida; 4Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, Florida; and 5Baptist Medical Center Clinical Laboratories, Jacksonville, Florida

Abstract

Amoxicillin has been causally linked in the lay and medical literature to false-positive urine drug screens for cocaine metabolites. An exhaustive search of the peer-reviewed medical literature revealed no data to support this link. We hypothesized that amoxicillin does not cause false-positive urine drug screens for cocaine metabolites. To test this hypothesis, we examined the urine of 33 subjects administered a course of amoxicillin, subjecting the specimens to four common urine screening immunoassays. Thirty-one specimens were negative for the cocaine metabolite, benzoylecgonine (BE), by all four screening methods; two were positive for BE by all four screening methods. Both positive specimens were confirmed by gas chromatography-mass spectrometry (GC-MS) for the presence of BE at > 150 ng/mL. Three specimens that screened negative, but produced absorbance values that were intermediate between negative and positive controls, were submitted for GC-MS analysis; BE was detected in all three specimens at concentrations of 54, 94, and 119 ng/mL. Twenty-eight specimens produced screening results indistinguishable from negative controls. Within the limitations of the study design, we conclude that amoxicillin is unlikely to produce false-positive urine screens for cocaine metabolites.

Introduction

Urine drug testing (UDT) is increasingly being used in clinical medicine as a monitoring tool for patients prescribed controlled substances. Interpretation of test results, however, is technical and complex. We and others have shown that physicians are not adept at UDT interpretation (1-3). False-positive test results may result in patients being incorrectly labeled as drug abusers or addicts, with potential negative consequences such as the withholding of important prescription medications, dismissal from physicians' practices, referral for drug counseling, and identification to law enforcement (1).

Amoxicillin has received significant attention as a putative cause of false-positive urine drug screens for cocaine metabolites. This assertion is ubiquitous on the worldwide web from such diverse sources as university-based health information websites (4-6); diagnostic laboratory services (7,8); law firms (9); think tanks (10); manufacturers of consumer drug testing kits (11); manufacturers of urine adulterants, synthetic urines, and body cleansers (12-21); dentist and physician websites (22,23); discussion groups (24); and newspaper columns (25). This claim has recently appeared in the medical literature as well (26).

Through comprehensive review of the medical literature—including, but not limited to, the peer-reviewed literature—we were unable to retrieve any experimental data to support the veracity of this claim. We tested the hypothesis that amoxicillin administration does not produce (false) positive urine screening assays for cocaine metabolites. The importance of this issue lies in the prevalence of both amoxicillin prescriptions and cocaine abuse in our society and the potentially serious consequences of false-positive screens.

Materials and Methods

Research protocol

The study was conducted at a primary care clinic at the University of Florida Health Science Center–Jacksonville. The protocol was approved by the University of Florida–Jacksonville Institutional Review Board. Waiver of informed consent was granted on the basis of minimal subject risk and non-collection of individual subject identifying information. Subjects were recruited into the study by their primary care physicians. Subjects were paid for their participation.
Study participation was offered to all patients 18 years of age or older who received a prescription for amoxicillin from a provider in the Community Health Clinic between September 15, 2007, and October 10, 2007. There were no exclusion criteria. Participation was not contingent upon specific amoxicillin dosages or frequencies of administration.

Thirty-three subjects were recruited, and all completed the study. Subjects were told only that their urine would be used to investigate a possible interference of their antibiotic with a laboratory test and that no personal identifying information would be collected. Primary care providers answered all questions and provided prospective subjects with informational fliers. Interested patients were instructed to return to the clinic, with their labeled amoxicillin prescription container, after taking at least five doses of the antibiotic. On arrival, patients identified themselves as amoxicillin study subjects and were brought to the laboratory. A study investigator checked the prescription bottle and verified medication adherence by subject report. All prescriptions were noted to fall in the range of 750–1000 mg/d in divided doses. Participation consisted of provision of 1. a urine specimen of at least 30 mL and 2. a list of all current medications, including prescription, over-the-counter, and herbal. The specimen and medication list were labeled with a unique subject identification number. Urine specimens were immediately transported to the laboratory for analysis and frozen storage.

**Specimen analysis**

_Screening assays._ Specimens were screened for the presence of benzoylecgonine (BE) by four immunoassays: OnLine DAT Cocaine II on a Modular Analytics P (Roche Diagnostics GmbH, Mannheim, Germany); Cocaine Metabolite homogeneous immunoassay on a SYNCHRON LX-20 Clinical System (Beckman-Coulter, Fullerton, CA); CEDIA Cocaine Assay (Microgenics, Fremont, CA) on a Hitachi 717 (Boehringer Mannheim, Indianapolis, IN) automated chemistry analyzer; and Triage TOX Drug Screen (Biosite, San Diego, CA). All of the screening immunoassays employed a 300 ng/mL benzoylecgonine cutoff concentration. All of the OnLine DAT Cocaine II assays were performed on the fresh specimen within a few hours of collection. Specimens were frozen at −20°C for up to 35 days prior to screening by the Syva EMIT II Plus Cocaine Metabolite method and the CEDIA Cocaine Assay. Triage TOX Drug Screen assays were performed on some of the specimens immediately after collection, and others were assayed after freezing at −20°C for 2–3 weeks. Frozen specimens were thawed at room temperature for 2–3 h prior to analysis.

_Confirmatory assays._ Specimens that screened positive for BE were submitted for confirmation by gas chromatography–mass spectrometry (GC–MS). Methanol, acetonitrile, methylene chloride, isopropanol, potassium phosphate monobasic, hydrochloric acid, potassium hydroxide, sodium hydroxide, and ammonium hydroxide were ACS grade and purchased from Fisher (Fairlawn, NJ). N-Methyl-N-(tert-butylidimethylsilyl) trifluoroaceticamide with 1% t-butylidimethylchlorosilane (MTBSTFA) was purchased from United Chemical Technologies (Bristol, PA). Phosphate buffer (0.1 M, pH 6) was prepared from a mixture of potassium phosphate monobasic and 5 M potassium hydroxide. The cocaine elution solvent, methylene chloride/isopropanol/ammonium hydroxide solution (78:20:2, v/v/v), was prepared daily. Cocaine (1.0 mg/mL), cocaethylene (1.0 mg/mL), benzoylecgonine (1.0 mg/mL), cocaine-d3 (100 μg/mL), cocaethylene-d3 (100 μg/mL), and benzoylecgonine-d3 (100 μg/mL) were purchased from Cerilliant (Round Rock, TX).

CLean Screen® (ZSDAU020, United Chemical Technologies, Bristol, PA) solid-phase extraction columns were used. Two milliliters of phosphate buffer and 0.2 mL of 0.1 M sodium hydroxide were added to 1 mL of urine. Columns were conditioned with 3 mL of methanol, 3 mL of DI water, and 2 mL of phosphate buffer. Specimens were then applied to the column, followed by 2 mL of water, 2 mL of 0.1 M hydrochloric acid, 3 mL methanol, and 1 mL acetonitrile. Columns were dried under full vacuum, and analytes were then eluted with 4 mL of elution solvent. The eluant was evaporated to dryness at 50°C under a stream of nitrogen followed by derivatization with 75 μL of MTBSTFA at 70°C for 45 min.

GC–MS analysis was performed with a Hewlett-Packard 5890A series II GC and 7673B automatic liquid sampler interfaced with a Hewlett-Packard 5972A mass selective detector (MSD, Hewlett-Packard, Little Falls, DE). The gas chromatograph was equipped with an Rxi|5ms, Crossbond® 5% diphenyl/95% dimethyl polysiloxane fused-silica capillary column (30 m x 0.25-mm i.d., 0.25-µm film thickness, Restek, Bellefonte, PA). The analysis was performed in the selected ion monitoring (SIM) mode. The following ions were monitored at a dwell time of 20 ms (ions in italics were used for quantitation): cocaine: m/z 182, 272, and 303; cocaine-d3: m/z 185 and 306; cocaethylene: m/z 196, 272, and 317; cocaethylene-d3: m/z 199 and 320; benzoylecgonine: m/z 282, 346, and 403; and benzoylecgonine-d3: m/z 349 and 406. Analytes were identified based upon comparison of retention times and ion ratios with the corresponding values of calibrators assayed in the same run. Ion ratios were calculated by dividing the ion peak-area of the confirming ion by the ion peak-area of the quantitative ion. Quantification of analytes was based upon the ratios of the integrated ion peak-areas to the corresponding trideuterated standard analogues.

**Results**

Thirty-three urine specimens were collected; 31 were negative for BE by all four screening methods; two were positive by all four methods. The positive specimens were confirmed for the presence of BE (> 150 ng/mL) by GC–MS. Cocaine was detected by GC–MS in both positive specimens, and cocaethylene was detected in one of the positive specimens. Three specimens that screened negative, but produced absorbance values (by the OnLine DAT Cocaine II and CEDIA methods) that were intermediate between negative and positive controls, were submitted for GC–MS analysis. BE was detected in all three specimens at concentrations of 54, 94, and 119 ng/mL. Twenty-eight of the specimens produced screening results that were indistinguishable from negative controls.
The significance of these results was assessed statistically by means of a binomial distribution model with an unknown \( p \), the probability of a false-positive cocaine screen in a patient taking amoxicillin. Binomial distributions are applicable to trials that have only two possible outcomes. In this study, the two outcomes were false positive versus not false positive, and the probability of a false positive was unknown. The two other variables that determine the binomial distribution are \( n \), the number of trials, and \( r \), the number of times one or the other of the two possible outcomes is observed. Therefore, in this study, the \( n \) was 33, and the \( r \) (number of false positives) was zero. Statistical modeling based on the binomial distribution therefore predicts the limits within which the probability of a false positive, \( p \), must fall. The results of this study produced a one-tailed 95% confidence interval for \( p \) between 0.0 and 0.11, corresponding to a predicted probability of a false positive as small as zero, but not exceeding 11%.

**Discussion**

The Internet is replete with claims of false-positive urine drug screens caused by a variety of prescription and over-the-counter medications. In the present case, entering the terms “amoxicillin,” “cocaine,” “false positive,” and “urine drug test” into the Google™ search engine yielded thousands of hits. We were interested in investigating the amoxicillin-cocaine claim because 1. amoxicillin is frequently prescribed and cocaine is frequently abused and 2. the claim has reached the medical literature, albeit without attribution.

Amoxicillin is a \( \beta \)-lactam antibiotic that bears little structural resemblance to cocaine or its metabolites, so immunological cross-reactivity between amoxicillin and cocaine (and/or metabolites) would seem unlikely. Occasionally, however, antibody cross-reactivities are unpredictable, and it is conceivable that an amoxicillin metabolite with epitopic similarities to cocaine could appear in the urine. About 50% of an amoxicillin dose is excreted unchanged in the urine. Penicilloic acid has been identified as a urinary metabolite of amoxicillin, but it likewise has few structural similarities to cocaine (27) (see Figure 1).

This study generated no false-positive cocaine metabolite (BE) screening results among 33 patients who were administered a course of amoxicillin. We did find, however, true-positive cocaine screens in two subjects (6%) and intermediate absorbance values in three other subjects, all five of which were confirmed as positive for cocaine metabolite by GC–MS. Therefore, even in specimens that produced screening data indicating a signal above background, but below the threshold for a positive screen, BE was detected, albeit below the SAMHSA-specified positive threshold concentration of 150 ng/mL. Importantly, our results provided no evidence that BE may be falsely detected in the urine of patients on amoxicillin therapy, even at concentrations below the customary screening thresholds.

Statistical analysis of our data indicates there is a 5% probability that the interference occurs with a frequency as high as 11%. The number of trials necessary to effectively exclude the possibility of an interference (probability < 1%) is approximately 1000, which was impractical with our study design and subject pool. Therefore, we interpret the results of this study as having failed to demonstrate the existence of interference from amoxicillin, but acknowledge that our study does not completely eliminate the possibility that such interference exists. Our results indicate that such an interference is very unlikely, however. Interestingly, among our 33 subjects administered amoxicillin, there was a relatively high prevalence of recent cocaine use, with definitive evidence of cocaine metabolites in the urine specimens of 5 (15%) subjects.

**Conclusions**

Among 33 subjects administered a course of amoxicillin, there were no instances of false-positive urine cocaine metabo-
lite screens using four common immunoassay methods. We did observe true-positive cocaine screens—confirmed by GC–MS—in two subjects (6%) and confirmed the presence of BE in an additional three specimens that had elevated but sub-threshold screening results. Within the limitations inherent in a study of this size, we conclude that it is unlikely that amoxicillin is a cause of false-positive urine cocaine screens. In the absence of published, verified cases of false-positive cocaine screening results in patients taking amoxicillin, we question the validity of the widely made claim that the interference exists.

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References