Up in smoke: Uncovering a lack of evidence for proton pump inhibitors as a source of tetrahydrocannabinol immunoassay false positives

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Abstract:

Objective: It is recommended that positives in immunoassay drug screens be followed up with more specific confirmatory testing. The drug package insert for pantoprazole mentions reports of false-positive urine screening tests for tetrahydrocannabinol in patients receiving proton pump inhibitors, but no method details or data are given, referenced, or found in literature searches. Thus, we investigated this using our laboratory's assay. **Methods:** A spiked sample and samples from 32 patients taking a proton pump inhibitor were analyzed using the EMIT II Plus Cannabinoid assay with a 20 ng/mL cutoff. Additionally, we examined urine samples from 50 patients with false-positive or low-positive screens for evidence of a proton pump inhibitor. To determine whether O-desmethyl pantoprazole sulfate, the major metabolite, shares any structural or electrostatic similarity to suggest a basis for cross-reactivity in the immunoassay, we used computational techniques for analyses. Molecular electrostatic potential energy (MEP) maps were calculated for the global minimum conformers, and the maximum common substructure Tanimoto similarity was calculated for the modeled compounds. **Results:** Neither the spiked sample nor the patient samples were found to screen positive. None of the false-positive or low-positive screens were found to contain a proton pump inhibitor. Computational studies showed very little similarity in shape or electrostatics between the two molecules. **Conclusions:** We find no supporting evidence of pantoprazole as the cause of false positives in the EMIT II Plus Cannabinoid assay and caution the use of proton pump inhibitors as an explanation for tetrahydrocannabinol immunoassay false positives.

Keywords: Drug Screen | Tetrahydrocannabinol (THC) | Proton Pump Inhibitor (PPI) | Metabolite | False Positive | Pantoprazole

Article:

Introduction

Since the early 2000s, Wyeth Pharmaceuticals has added a statement of precaution to its pantoprazole drug package insert in a subsection titled *Laboratory Tests* [1]. The statement reads as follows:

> There have been reports of false-positive urine screening tests for tetrahydrocannabinol (THC) in patients receiving most proton pump inhibitors, including pantoprazole. An alternative confirmatory method should be considered to verify positive results.

As a result, many laboratories continue to answer questions from physicians about this potential interference as a cause for unexpected positive results; however, there are little to no data in the literature to authenticate these claims and no mention of specific assays identified to have this interference. Most papers continue to cite references that only refer to the pantoprazole drug package insert, which gives no data or references to studies [2]. Recently, a case report was published speculating that a false-positive THC urine drug screen was due to pantoprazole in a 13-year-old with cyclic vomiting syndrome; however, no mention of assay details such as vendor or cutoff used or confirmatory test method was given [3]. Further, no THC assay was done before or after pantoprazole was out of the system, and the patient was also administered two other drugs. Thus, it is still debatable whether pantoprazole was truly the cause of this supposed THC false positive. To our knowledge, no THC immunoassay product insert includes data to test pantoprazole interference in their assay. The CEDIA DAU THC assay (ThermoFisher) package insert states that the proton pump inhibitor (PPI) omeprazole tests negative at concentrations of up to 1,000,000 ng/mL. However, there are many other PPIs on the market today. Additionally, drug metabolites may also be responsible for cross-reactivity in urine drug screens.

The highest percentage of false-positive THC screens reported in the literature is found in infants and is believed to be due to certain infant body washes [4] or unique metabolites that may be cross-reacting but not measured in typical confirmatory testing [5]. Some THC metabolites are known to cross-react in immunoassays such as the EMIT II Plus cannabinoid assay, which primarily detects the 11-nor-Δ9-carboxy-THC metabolite. However, the degree of cross-reactivity varies from assay to assay.

Reports of cross-reactivity with other compounds in THC urine screens have been published. A 1986 report on urine testing for drugs of abuse mentions that Syva notified laboratories that ibuprofen and other nonsteroidal anti-inflammatory agents (NSAIDs) could interfere with their EMIT assay at the 20 ng/mL cutoff [6]. However, the company eliminated the problem by altering a reagent [7]. A study by Brunk showed that a supposed false-positive EMIT result (d.a.u. cannabinoid 100 ng assay) in a patient taking ibuprofen was a true positive. Their study utilized an alternate confirmation method and showed that ibuprofen concentrations in excess of 250 mg/mL caused disappearance of the THC-COOH peak by Gas chromatography-mass spectrometry (GC-MS) [8]. Today, manufacturers of urine drug screens routinely include NSAIDs in their interference testing, and package inserts state that there is no interference by these compounds.

In order to determine if PPI use was a potential explanation for unexpected THC immunoassay results, we designed studies to investigate if pantoprazole or other PPIs would contribute to a positive result using our laboratory's EMIT II Plus Cannabinoid assay. Since cross-reactivity

could result from either the parent drug or a metabolite that forms in vivo, a THC-negative urine sample was spiked with pantoprazole, and urine samples collected from recruited patients taking pantoprazole or another proton pump inhibitor (PPI) were also examined. As an additional predictive tool, we used computational techniques to examine possible similarities between the major urine metabolite of PPI, O-desmethyl pantoprazole sulfate, and the specific cannabinoid recognized in most THC immunoassays.

Methods

Urine Screen

The THC urine screen in our laboratory was performed using the EMIT II Plus Cannabinoid Assay (Beckman Coulter) on a Beckman AU5810. This assay detects the 11-nor-Δ9-carboxy-THC metabolite (THC-COOH) with a 20 ng/mL cutoff. Quality control samples of 50% cutoff (10 ng/mL) and 150% cutoff (25 ng/mL) were prepared from Siemens Calibrator Levels 0 and 3. Samples below the cutoff are reported as negative. For this study, samples close to or above the cutoff were submitted for confirmation using a clinically validated liquid chromatography–tandem mass spectrometry (LC-MS/MS assay).

Spiked Sample

A THC-negative urine sample was spiked with pantoprazole (10,000 ng/mL) and analyzed using the screen method described above.

Samples from Patients Taking a PPI

Volunteers were recruited from Associated Regional and University Pathologists, Inc. (ARUP) Laboratories and the University of Utah under Institutional Review Board No. 00007275. Samples were de-identified prior to the analyses described above. Volunteers completed a questionnaire indicating what PPI they were taking, dose taken, time of last dose, and list of additional drugs being taken. Additional samples were obtained from patient specimens submitted to ARUP Laboratories for a drug screen in which a list of drugs expected was also provided.

LC-MS/MS Confirmation

This was performed using an in-house developed and validated method currently in use at ARUP Laboratories. Samples were prepared by addition of a deuterated THC-COOH internal standard followed by base-catalyzed hydrolysis (55 °C for 20 minutes). The samples were purified using Strata X-C solid phase extraction cartridges (Phenomenex) and a series of washes, followed by elution into a 96-well plate. Samples were dried under nitrogen. They were then derivatized to the butyl ester, dried again, and resuspended in acetonitrile prior to injection on the instrument. Sample analysis was completed on a Waters Acquity UPLC-MS/MS system using an electrospray ionization source in positive mode with multiple-reaction monitoring for identification and quantitation. The UPLC column was an HSS C18 (1.8 µm particle size [2.1 ×

50 mm] kept at 25 °C). A gradient of 5 mM ammonium formate (pH 3.0) and 0.05% formic acid (v/v) in methanol was used for elution. Data analysis was performed using QuanLynx software.

LC-TOF-MS Screen

Fifty de-identified patient samples submitted to ARUP Laboratories for urine drug screens that screened positive for THC by immunoassay but failed to confirm by LC-MS/MS, or confirmed with very low concentrations of THC, were selected for further examination by liquid chromatography–time of flight mass spectrometry (LC-TOF-MS). This test was an in-house research assay to detect eight common PPIs or histamine H2 antagonists. Specimens were diluted 1:50 in initial mobile phase conditions prior to analysis on an Agilent 1,290 liquid chromatograph coupled to an Agilent 6550 QTOF run in TOF mode. Chromatographic separation was achieved on a Phenomenex F5 column (2.1 × 50 mm) and used a gradient of 5 mM ammonium formate buffer, pH 3.5, and methanol ramped from 5% B to 95% B over three minutes. A standard containing cimetidine, omeprazole/esomeprazole, famotidine, lansoprazole, nizatindine, pantoprazole, rabeprazole, and ranitidine was run to establish retention times. Patient specimens were analyzed and compared with a compound library using mass match, isotope ratios, and chromatographic retention time as criteria for a positive result.

Molecular Modeling

O-desmethyl pantoprazole sulfate (M2) and 11-nor-Δ9-carboxy-THC were built in Maestro v10.4 (Schrödinger, LLC, New York, NY, USA). Tautomeric states were determined using Epik v3.4 (Schrödinger, LLC, New York, NY, USA) in water at a pH of 7 +/-2. Of the four tautomeric states found for M2, three were within 2.6 kcal/mol of each other, while the fourth was 14.5 kcal/mol higher. A conformational search was performed on the three lower energy tautomers. A 25-K step mixed torsional/low mode conformational search was performed in the OPLS3 forcefield with an 8.0 Å extended nonbonded cutoff, 20.0 Å electrostatic cutoff, and 4.0 Å hydrogen bond cutoff, and the generalized Born/surface area (GB/SA) continuum solvation model for water as available in Macromodel (Schrödinger, LLC, New York, NY, USA). In each conformer search, local energy minima were minimized to a gradient of 0.1 kcal/(mol·Å) and checked for uniqueness with a maximum atom deviation of 0.5 Å. The electrostatic potential energy was calculated for the global minimum conformer using the OPLS3 forcefield in Macromodel and mapped onto the Connolly surface of each ligand with a probe radius of 1.4 Å and a 0.3-Å resolution grid. A rainbow spectrum from red through yellow, green, blue, to purple defines the electron-rich regions (red) and the electron-poor regions (blue/purple), with the range set from –0.25 to 0.25 kcal/mol. Both compounds were submitted to ChemMine SDWorkBench (http://chemmine.ucr.edu; accessed January 28, 2017) in sdf format, and the maximum common substructure (MCS) Tanimoto similarity score was calculated [9].

Results and Discussion

The urine sample spiked with 10,000 ng/mL pantoprazole gave a negative result in our THC urine screen, implying that the parent drug does not cross-react in this immunoassay. To examine possible cross-reactivity from pantoprazole metabolites and other PPI or PPI metabolites, urine samples were obtained from 32 volunteers or patients known to be taking a PPI (pantoprazole,

N = 6; omeprazole, N = 17; esomeprazole, N = 3; lansoprazole, N = 2; dexlansoprazole, N = 4). None exceeded the required optical density (OD) cutoff (100) to be classified as positive for THC. Figure 1 shows the results for these samples as well as that of a negative control (synthetic urine) and a 50% cutoff control (synthetic urine spiked with 10 ng/mL THC-COOH). One patient sample, from someone taking omeprazole, generated a result close to the positive cutoff. This was sent for confirmation testing in our laboratory and found to contain THC-COOH at a concentration of 7.3 ng/mL.

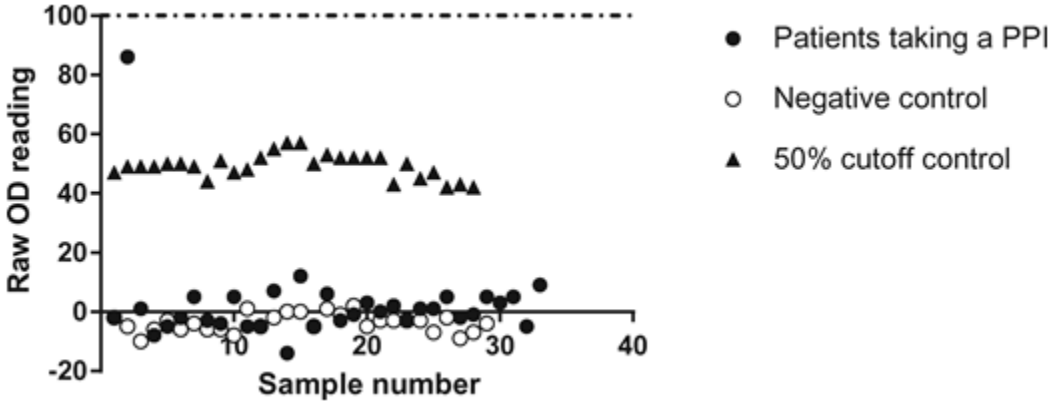

Figure 1. Comparison of raw data for negative control, patients taking a proton pump inhibitor, and a positive (50% cutoff, 10 ng/mL) control. Urine screen results for tetrahydrocannabinol (THC) are shown for several patients taking a proton pump inhibitor, blank urine samples, and positive THC quality control samples. The value for a positive cutoff is shown using a dotted and dashed line.

Table 1. Liquid chromatography–time of flight mass spectrometry screen results from tetrahydrocannabinol immunoassay–positive urine samples (N = 50)

THC-COOH by LC-MS/MS, ng/mL	Number of Samples	LC-TOF Screen Results for Proton Pump Inhibitors
<5	7	None found
6–10	14	None found
11–15	4	None found
16–20	20	None found
21–25	4	None found
Unable to quantify	1	None found

LC-MS/MS = liquid chromatography–tandem mass spectrometry; LC-TOF = liquid chromatography–time of flight mass spectrometry; THC-COOH = 11-nor-Δ9-carboxy-THC metabolite.

Since recruitment was limited and only 32 urine samples were available from patients self-reporting to be currently taking a PPI, we obtained additional urine samples that screened positive for THC by the immunoassay and confirmed negative by LC-MS/MS (<5 ng/mL). During the time frame of the analysis, only seven of these occurred. Since various labs have different cutoff values for their confirmation testing and certain federal guidelines use a higher cutoff (15 ng/mL) for confirmation, we also included samples that screened positive for THC and confirmed at low concentrations (6–25 ng/mL). Using a LC-TOF-MS screen developed in our laboratory for detection of PPIs or H2 antagonists, we searched for evidence of PPIs in these urine samples. None of the 50 samples tested was found to contain a PPI (Table 1). One sample

contained an H2 antagonist, ranitidine. Of note, the package insert for our screening assay indicates that 1,000 µg/mL (1,000,000 ng/mL) of ranitidine showed a negative response when using a 100-ng/mL cutoff, but ours used a 50-ng/mL cutoff.

Molecular modeling studies were designed to examine whether or not there were similarities in the three-dimensional structure or electrostatics of THC, and pantoprazole that might suggest possible cross-reactivity toward the antibody used in the immunoassay screen. As pantoprazole is known to be unstable in acid environments and the main metabolite (M2) is primarily eliminated in urine, this metabolite was compared with THC-COOH, which is detected by most THC immunoassays. The M2 metabolite has four tautomers, with three that are all predicted to be less than 2.6 kcal/mol apart involving aromatic nitrogen protonation-deprotonation, and the fourth predicted 14.5 kcal/mol higher and involving sulfate protonation. The most populated and lowest energy tautomer was used for further comparisons with THC-COOH. This tautomer of M2 has the pyridine N unprotonated and a negative charge on the sulfate group. As such, it would have the same overall negative charge expected for THC-COOH.

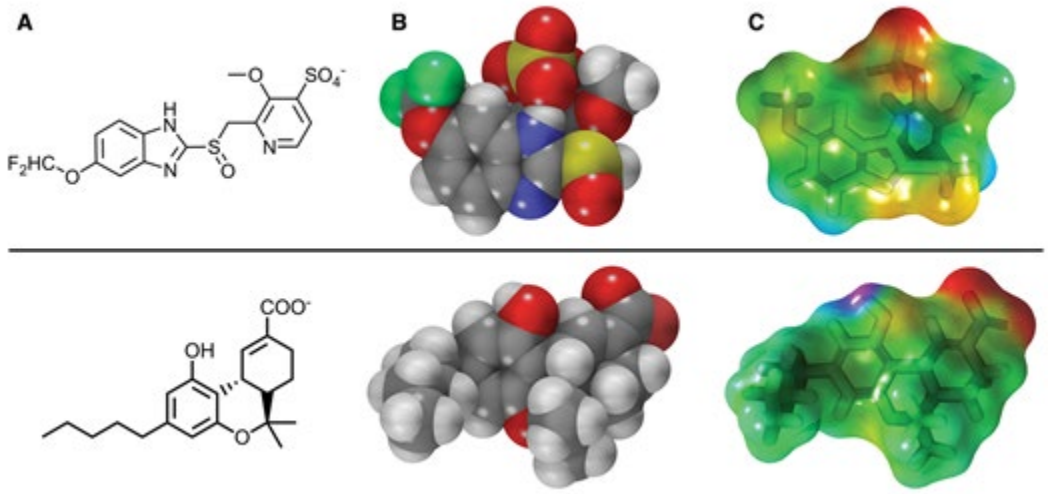

Figure 2. Molecular modeling comparison of metabolites of tetrahydrocannabinol (THC) and pantoprazole (M2). Panel (**A**) shows simple line drawings of M2 and the THC metabolite recognized in the urine screen immunoassay. Panel (**B**) shows space-filling models of the two metabolites using the typical CPK coloring scheme, with each in its lowest energy conformation. Panel (**C**) shows molecular electrostatic potentials mapped to the surface area of each metabolite, respectively. Red areas indicate strong negative electrostatic potential, while green areas represent neutral electrostatic potential.

Figure 2 shows results from the molecular modeling studies. Panel A shows line-drawing representations with expected charges of each metabolite. Panel B uses space-filling models to represent the lowest energy conformations of M2 (top) and THC-COOH (bottom). Typical CPK colors are used for the atoms. Panel C shows molecular electrostatic potentials mapped to the surface area of each molecule respectively. The red areas are the strongly negative electrostatic potential, green represents neutral electrostatic potential, and purple/blue represents positive electrostatic potential. The global electrostatic surface map of each is quite different. Thus, the MEP maps suggest that cross-reactivity of these compounds by the same antibody would not be

expected. As many other minima exist for the M2 metabolite, the MEP map comparison is far from definitive; therefore, we also calculated MCS Tanimoto similarity score [10]. This score, 0.16, also suggests very low likelihood of cross-immunoreactivity for these two structurally dissimilar compounds.

Conclusion

Evidence for cross-reactivity of pantoprazole and other proton pump inhibitors in causing false positives for THC in drug urine screens continues to be lacking. In our studies, we examined a spiked sample and urine from 32 patients taking a PPI and found no false positives. In addition, we used a mass spectrometry–based method to identify PPIs in urine samples from patients who screened positive for THC and found that none of these contained PPIs. Additionally, computational modeling studies do not suggest any structural or electrostatic similarities that would be suggestive of possible cross-reactivity. Our study is limited by the use of only one screening assay, a fairly small number of samples, and a fairly short half-life for pantoprazole. However, multiple approaches were used for the investigation. In conclusion, we find no convincing evidence to support that pantoprazole, its main metabolite, or other PPIs tested cause false positives in the EMIT II Plus Cannabinoid assay, and we suggest caution regarding PPI ingestion as an explanation for an unexpected THC-positive screen. As not all drug screens have the same limitations, communication with the lab director or toxicologist when encountering unexpected results is highly encouraged.

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Conflicts of interest: None.

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