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Designer Benzodiazepines

New Drugs Challenge Laboratories

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Benzodiazepines are a class of drugs used as minor tranquilizers. The core chemical structure contains the fusion of a benzene and diazepine ring. Most also contain a phenyl ring attached to the diazepine ring (Figure 1).

Benzodiazepines produce central nervous system depression by enhancing the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA). The $GABA_A$ receptor is a ligand-gated, chloride-selective ion channel, and benzodiazepines potentiate the receptor by binding to it and acting as a positive modulator. The binding of a benzodiazepine to the $GABA_A$ receptor increases the frequency of the chloride ion channel opening, which ultimately decreases neuronal activity by hyperpolarizing the cell membrane potential.

Benzodiazepines are indicated for medical use in the treatment of anxiety, insomnia, alcohol withdrawal symptoms, and muscle spasms. They have a dose-dependent effect on central nervous system depression that ranges from sedation to anesthesia to respiratory depression and death (1,2).

Benzodiazepine Use Increases

Benzodiazepines are among the most prescribed drugs in the United States (1). Per 100 people, 37.6 benzodiazepine prescriptions were written in the U.S. in 2012 (3). This large number of prescriptions has raised public health concerns over possible increased dependency and overdose mortality. From 1996 to 2013, the increase in benzodiazepine prescriptions in the U.S. paralleled the rise in the number of deaths, with most deaths involving the use of other substances in addition to the benzodiazepines (4). Because of this, in 2016, the Food and Drug Administration (FDA) announced that healthcare professionals should exercise caution when prescribing opioid pain medicine along with benzodiazepines (5).

Medicaid expenditures on benzodiazepines rose by 30%, or nearly $40 million, between 1991 and 2009, even as the average price per prescription fell (3). This growth highlights the possible hidden role of substance dependence, a trend also seen in other countries, such as Australia (6).

Many clinicians may not be fully aware of the addictive potential of benzodiazepines. Landmark neurological studies have made it clear that benzodiazepines operate on neural networks similar to those affected by other drugs of abuse (7). Some of the common consequences of misuse, overuse, and extended use of benzodiazepines include memory impairment, accidental injuries, increased motor vehicle accidents, increased hospital admissions, more emergency room visits, and worsening of the symptoms for which the benzodiazepine was initially prescribed (3,7,8).

Even more alarming, the failure to address the overprescribing of benzodiazepines could have a high potential impact on society and the healthcare system. This concern is highlighted by the current U.S. opioid epidemic. This crisis may have been fueled by overprescribing of FDA-approved opioids, which led to an increase in heroin and illicit fentanyl use (3). Further, the epidemic of opioid addiction and overdose has reduced the resources available to address inappropriate prescribing of benzodiazepines and treatment of dependency (7). The focus on the opioid epidemic could open the door to a new epidemic, in this case of benzodiazepines.

Benzodiazepines on the Illicit Market

Alprazolam (Xanax), clonazepam (Klonopin), lorazepam (Ativan), and diazepam (Valium) are

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Benzodiazepines

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among the most commonly used benzodiazepines in the U.S. and are also the most frequently found on the illicit market (3,9,10).

Benzodiazepines that are legal in some countries but not approved by the U.S. FDA can also be found on the illicit market (9). For example, etizolam belongs to a class of compounds known as thienodiazepines and is prescribed in Japan, Korea, and Italy (2,11). Etizolam is often sold in the illicit market as "high-quality" product under one of its brand names, Etizest (9). Most of the etizolam in the online illicit market is shipped from the U.S. As another example, phenazepam is commonly used medically in Russia (12). There is a high risk of overdose with illicit use of phenazepam (12–14).

There are still other benzodiazepines available that have not been approved for medicinal use in any country. Many of these drugs can be purchased online and sent directly to the buyer's address in total anonymity (9,12).

Designer Benzodiazepines

There is an insidious trend that illicit—and potentially deadly—new forms of benzodiazepines, called "designer drugs," are available on the dark net, a computer network with restricted access used primarily for trafficking illicit products (2,9,15). Designer benzodiazepines are synthetic functional or structural analogs of benzodiazepines. Sold as "research chemicals" and labeled "not for human consumption," they are often described as "legal highs."

Many of the designer benzodiazepines were synthesized as drug candidates by pharmaceutical companies and are now being rediscovered and produced in clandestine laboratories.

The first designer benzodiazepines—diclazepam, flubromazepam, and pyrazolam—entered the illicit market around 2012 (2). They were joined later by clonazolam, deschloroetizolam, flubrazolam, nifoxipam, meclonazepam, desalkylflurazepam, and 4-chlorodiazepam. None of these compounds are approved for medicinal use in any country.

The designer benzodiazepines often have structural similarities to regulated benzodiazepines (Figure 1). For example, flubromazepam is structurally similar to phenazepam, but with the chlorine atom on phenazepam replaced with a fluorine atom. Other designer benzodiazepines are hybrid compounds of two regulated benzodiazepines. For example, the designer molecule clonazolam is a hybrid molecule of clonazepam and alprazolam. Still others are metabolites of regulated benzodiazepines. For example, nifoxipam is a metabolite of flunitrazepam (16) and 3-hydroxyphenazepam is a metabolite of phenazepam (2). (Note that flunitrazepam and phenazepam are not approved in the U.S.)

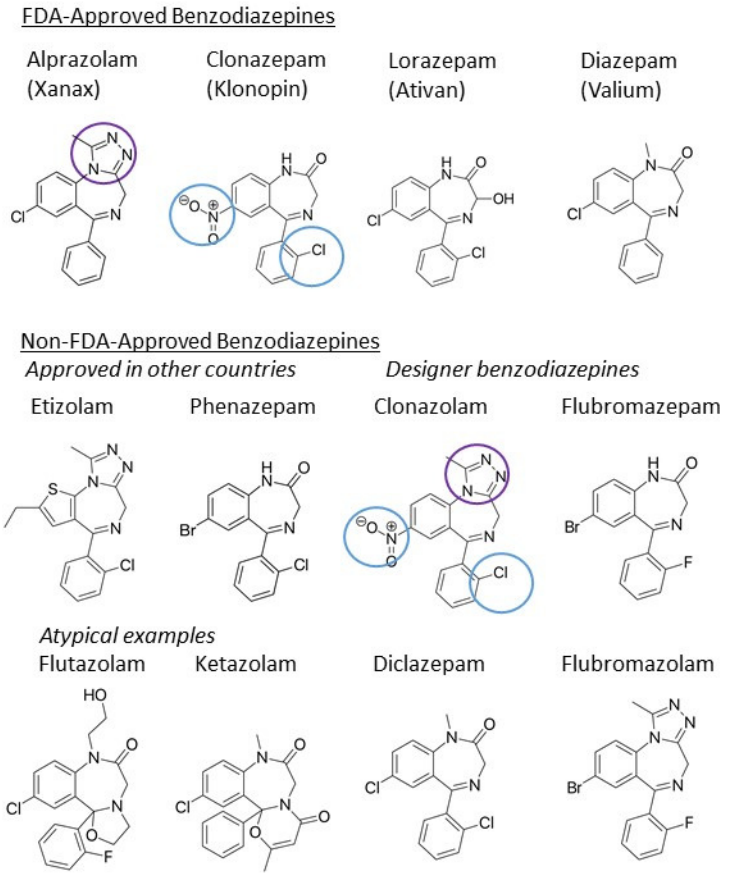

Figure 1. Structures of the most common prescription (FDA-approved) benzodiazepines and non-FDA–approved benzodiazepines. Non-FDA–approved benzodiazepines can be further divided into benzodiazepines approved in other countries and designer benzodiazepines not approved in any country. The circles illustrate that clonazolam is a hybrid structure of alprazolam and clonazepam. Flutazolam and ketazolam are non-FDA–approved benzodiazepines with an atypical structure and are not well-recognized by immunoassays.

Finally, designer benzodiazepines can be prodrugs of regulated benzodiazepines. For example, cloniprazepam has a cyclopropylmethyl side chain located on the nitrogen of the diazepine ring; when the side chain is removed via metabolism, the product is clonazepam (17).

The pharmacokinetics and side effects of designer benzodiazepines are difficult to predict because there are no clinical and pharmacological studies available.

Detection of Nonapproved Benzodiazepines

As non-FDA–approved benzodiazepines become more popular, testing becomes more important for detection and identification. Consumers often cannot know what is in a product they buy. For example, etizolam has been found in illicitly manufac-

tured alprazolam (Xanax) pills in the U.S. (18) and phenazepam has been identified as a constituent of an herb (kronic) in New Zealand (19). Buyers on the dark net are often misled. For example, sellers often refer to pyrazolam as the active ingredient of Xanax, when it is actually alprazolam (9).

Immunochemical Tests

Immunochemical tests in clinical and forensic laboratories can detect most of the non-FDA–approved benzodiazepines with sufficient sensitivity. Bergstrand et al. tested the reactivity of 13 of these drugs in several benzodiazepine immunoassays: CEDIA, EMIT II Plus, HEIA, and KIMS II (20). By spiking parent drug standards in blank urine and testing authentic urine samples from intoxication cases, they found that these assays in general have a high reactivity for non-FDA–approved benzodiazepines. KIMS II and CEDIA immunoassays showed the highest, and EMIT II Plus the lowest overall reactivity. The lowest reactivity (4–41%, depending on the assay) was found for flutazolam, the most chemically distinct benzodiazepine tested in the study.

A study using spiked urine to test a somewhat different set of 15 non-FDA–approved benzodiazepines confirmed high reactivity with the CEDIA assay (21). With the exception of ketazolam (reactivity of 8%), the tested benzodiazepines showed a reactivity of 99% or greater. Similar to flutazolam, ketazolam has a rather atypical benzodiazepine structure.

Non-FDA–approved benzodiazepines can also be detected in blood using an ELISA assay (Immunalysis Benzodiazepine Kit). Reactivity ranged from 69% to 126% for six tested compounds (22).

Although the parent compound may show good reactivity with an immunoassay, the metabolite(s) may not. For example, in a single-subject, self-administration study of flubromazepam, urine immunoassay results were predominantly negative. As there was good reactivity for parent flubromazepam, this was likely due to low reactivity for the monohydroxylated metabolite (23).

Confirmation Techniques

The mass spectrometric methods needed for confirmation do not generally cover the latest designer benzodiazepines, resulting in apparent false-positive results (a positive benzodiazepine immunoassay and negative confirmation result). A study in Sweden showed that out of 390 "false-positive" urine samples, 40% contained benzodiazepines not approved for medical use (24). Ideally, toxicology laboratories should include these drugs in their confirmatory methods. However, the lack of reference materials, the breadth and structural variability of designer benzodiazepines, and the constantly changing market of designer drugs make this an extremely difficult task.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) as well as high-resolution mass spectrometry (LC-HRMS) methods have been developed (2,21,24). LC-HRMS using quadrupole time-of-flight technology has the advantage that method development is simpler and faster, because there is no need to develop compound-dependent parameters when collecting data in an untargeted manner. This is a clear advantage when toxicology laboratories are trying to keep up with new designer drugs. In addition, untargeted data collection allows for identification of unexpected compounds or compounds outside of the library. In addition, compounds can be preliminarily identified using accurate mass and isotope pattern, and later be retrospectively confirmed after analysis of a reference standard (25).

Designer Benzodiazepine Metabolism

Benzodiazepines are mainly metabolized by cytochrome P450 enzymes, which perform oxidation, hydroxylation, and *N*-dealkylation reactions, and by glucuronosyl transferases, which conjugate a glucuronide group. Most benzodiazepines are primarily excreted as metabolites. Detection of benzodiazepine use therefore depends on analysis of the correct metabolites. Researchers have begun identifying the metabolites of designer benzodiazepines (Table 1).

The metabolism of flubromazolam has been rather well-established. The major metabolites in human urine are α-hydroxy-flubromazolam-*O*-glucuronide and *N*-glucuronide-flubromazolam (26). Parent flubromazolam is generally found in a relative abundance of up to 20%, but can be as high as 50–60% (26). As preparation for mass spectrometry analysis of urine specimens, hydrolysis to cleave the glucuronide is recommended because of the abundance of glucuronidated forms. After hydrolysis, good targets for urine drug testing are parent flubromazolam (~40% abundance) and α-hydroxy-flubromazolam (~60% abundance) (26). The metabolic pathway of pyrazolam is similar to that of flubromazolam in that α-hydroxy and 4-hydroxy metabolites (with or without glucuronide) are formed, as well as the *N*-glucuronide form of the parent drug (26). Pyrazolam is mainly excreted as the unchanged parent drug, with a relative abundance generally greater than 80% (26). Therefore, the best urine drug screening target is parent pyrazolam and hydrolysis is likely not required. A designer drug similar to flubromazolam, flubromazepam may be mainly excreted as 3-hydroxy-flubromazepam-glucuronide, as observed in a single-subject, self-administration study (23). Only small amounts of parent flubromazepam were detected in urine (23).

The major metabolites of the nitro-containing designer benzodiazepines clonazolam, meclonazepam, and nifoxipam are 7-amino and 7-acetamino forms (27). For nifoxipam, the amount of parent-glucuronide form may be substantial (up to a relative abundance of 50–60%) (27). Unmetabolized parent forms of these nitro-containing designer benzodiaze-

Table 1. Metabolites and Possible Urine Drug-Screening Targets for Designer Benzodiazepines, Etizolam, and Phenazepam

Parent drug	Metabolites[#]	Comments	Possible target(s)	References
3-Hydroxy-phenazepam	no known metabolites	only in vitro HLM^ data available	from in vitro data: likely 3-hydroxy-phenazepam (hydrolysis may or may not be required)	(17)
Clonazolam	7-amino- (gluc.) 7-acetamino- (gluc.) desmethyl-* hydroxy- (gluc.)		7-amino-clonazolam and 7-acetamino-clonazolam (hydrolysis likely not required)	(27, 28, 32)
Cloniprazepam	hydroxy- (3 forms) (gluc.)* dihydroxy- * 3-keto- * 7-amino- * clonazepam* hydroxy-clonazepam* 7-amino-clonazepam* 3-hydroxy-7-amino-clonazepam*	only in vitro HLM / cytosolic fraction study data available	only in vitro data available	(17, 30)
Deschloroetizolam	hydroxy-* (three forms) dihydroxy-* (one form)	only in vitro HLM study data available	from in vitro data: likely hydroxy-deschloroetizolam	(28, 32)
Diclazepam	desmethyl- (gluc.) = delorazepam hydroxy- (gluc.) = lormetazepam desmethyl-hydroxy- (gluc.) = lorazepam	single-subject, self-administration study	after hydrolysis: delorazepam, lormetazepam, and lorazepam may allow for a longer window of detection	(28, 29)
Etizolam	α-hydroxy- (on 9-methyl group) 8-hydroxy- (on 2-ethyl group)		α-hydroxy-etizolam (hydrolysis may or may not be required)	(11, 28, 31)
Flubromazepam	3-hydroxy- (gluc.) desbromo-* 3-hydroxy-desbromo- (gluc.)	HLM study and single-subject, self-administration study	after hydrolysis: 3-hydroxy-flubromazepam	(23, 28)
Flubromazolam	α-hydroxy- (gluc.) 4-hydroxy- (gluc.) (α, 4)-dihydroxy- *N*-glucuronide-	(α, 4)-dihydroxy-found in human urine only after hydrolysis (35)	after hydrolysis: flubromazolam and α-hydroxy-flubromazolam	(26, 28, 32, 35-37)
Meclonazepam	7-amino- 7-acetamino-		7-amino-meclonazepam and 7-acetamino-meclonazepam (hydrolysis likely not required)	(27, 28, 32, 38)
Metizolam (desmethyletizolam)	hydroxy- (on 2-ethyl group) (gluc.) *N*-hydroxy- hydroxy-* dihydroxy-*	HLM and single-subject, self-administration study	hydroxy-metizolam (on 2-ethyl), hydrolysis may or may not be required	(17, 33)
Nifoxipam	7-amino- 7-acetamino- glucuronide- denitro-*		7-acetamino-nifoxipam (hydrolysis likely not required), in addition after hydrolysis: nifoxipam	(27, 28)
Phenazepam	3-hydroxy- ABPH^ QNZ^		limited human data available, likely 3-hydroxy-phenazepam (hydrolysis may or may not be required)	(34)
Pyrazolam	α-hydroxy- (gluc.) 4-hydroxy- (gluc.) *N*-glucuronide-	mainly excreted as unchanged parent drug	pyrazolam (hydrolysis likely not required)	(26)

[#] Locations of hydroxyl-group are predictions for pyrazolam, flubromazepam, and cloniprazepam. (gluc.) indicates the metabolite may be glucuronidated.
* Found only in human liver microsomes (HLM)
^ ABPH = 5-bromo-(2-chlorophenyl)-2-aminobenzophenone; HLM = human liver microsomes, QNZ = 6-bromo-(2-chlorophenyl) quinazoline-2-one

pines are found in low concentrations or are undetectable (27).

Diclazepam's metabolism is interesting because its three metabolites—delorazepam, lormetazepam, and lorazepam—are pharmaceutical drugs in themselves, although only lorazepam has been approved by the FDA (28,29). In a single-subject, self-administration study, parent diclazepam was found in low concentrations. As previously mentioned, cloniprazepam is also a prodrug for an FDA-approved benzodiazepine, clonazepam (17,30).

Less than 0.3% of administered etizolam is excreted as the unchanged parent compound and its major metabolite is α-hydroxy-etizolam (31). Two analogs of this thienodiazepine—descholoretizolam and metizolam—follow similar metabolic routes. Its major metabolites are the mono-hydroxylated forms (17,28,32,33). Analogous to etizolam, only about 0.3% of metizolam is excreted as the unchanged parent compound in the first 24 hours (33).

Finally, 3-hydroxy-phenazepam, the major metabolite of phenazepam (34) and a designer benzodiazepine in itself, appears not to be further metabolized (17).

Conclusion

The current opioid crisis may be masking what could ultimately become a benzodiazepine epidemic. As the number of prescriptions for benzodiazepines has steadily risen over the years, recreational use has become more and more popular. Recreational users have found sources in illicit markets, such as the dark net and street dealers. This use is risky, however, because fentanyl in counterfeit Xanax tablets has caused multiple fatalities in unsuspecting users (18). Designer benzodiazepines have also gained popularity, possibly because they are marketed as "research chemicals" and widely discussed on internet forums such as Reddit and Bluelight.

With a wide variety of non-FDA–approved benzodiazepines on the illicit market, analytical testing for these drugs is challenging. Because immunochemical screens can generally recognize these benzodiazepines, clinical and forensic toxicology laboratories should consider including these novel drugs, and most importantly also their metabolites, in their mass spectrometry confirmatory methods. Although LC-HRMS instruments are expensive, this technique has many advantages in the evolving world of designer drugs.

Learning Objectives

After reading this article, the reader will be able to describe the evolving complexity of the non-FDA –approved benzodiazepines and the role of the illicit market. The reader will also be able to list methods and drug-screening targets, such as metabolites, for detection of these designer benzodiazepines.

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Imatinib Mesylate

Should Cancer Drug Be Added to the List of Therapeutic Drugs to Monitor?

By Laura Smy, PhD, MLT

Commercial therapeutic drug monitoring (TDM) began in the 1970s when concepts of pharmacokinetics were employed to reduce dose-related adverse drug events and optimize dosing by establishing therapeutic ranges and toxic thresholds (1). Some additional reasons to provide TDM include monitoring adherence, evaluating drug–drug interactions, evaluating changes in drug formulations, evaluating a dosing regimen, and guiding withdrawal of therapy. Using TDM to tailor a patient's drug regimen promotes personalized patient care and can improve outcomes.

Over the past decade, a body of literature has grown in support of adding imatinib mesylate (marketed under trade names such as Gleevec) to the list of drugs that can benefit from TDM.

Imatinib belongs to a class of drugs called tyrosine kinase inhibitors. The most notable disease for which imatinib is a first-line therapy is chronic myelogenous leukemia (CML).

CML was first described clinically in the 1840s, but it took until the 1960s for scientists to discover the cause is a chromosome abnormality (2). This abnormality, called the Philadelphia chromosome, is a translocation between chromosomes 9 and 22 that produces a fusion gene transcript named *BCR-ABL* for the genes that are fused together, the breakpoint cluster region (*BCR*) gene and the Abelson (*ABL)* gene. The *BCR-ABL* fusion gene produces an oncoprotein known as BCR-ABL1, a tyrosine kinase with abnormally increased activity that results in the development of cancer.

Clinical Response to Imatinib

CML treatment response is monitored by quantitative real-time polymerase chain reaction measure-

ment of the *BCR-ABL1* oncoprotein transcript in relation to the *BCR* transcript. A major molecular response (MMR) is defined as a decrease of 3 logs in the *BCR-ABL1*-to-*BCR* ratio, which is a result of <0.1% on the currently used International Scale (3,4). A decrease of 4.5 logs equates to an undetectable *BCR-ABL1*, which is known as a complete molecular response (3,5).

Imatinib's efficacy for treating CML was shown in the International Randomized Study of Interferon and ST1571 (IRIS) phase 3 clinical study. The final paper on the IRIS study, published in March 2017 and reporting on the 10-year outcomes, found that patients who achieved an MMR after 12 months of treatment had an estimated 91.1% overall survival rate at 10 years, compared with only 85.3% among patients who did not.

CML can also be monitored by cytogenic response, in which the patient's cells are assessed for the presence of the Philadelphia chromosome. When no metaphase cells contain the Philadelphia chromosome, it is considered a complete cytogenic response. When up to 35% of metaphase cells contain a Philadelphia chromosome, it is referred to as a major cytogenic response (4).

Reasons for Imatinib TDM

Three major findings support the use of TDM for imatinib. First, imatinib pre-dose (trough) concentrations above 1000 ng/mL are statistically associated with better outcomes. Second, trough concentrations can vary widely in patients given the same dose. Third, lack of adherence to therapy is associated with a suboptimal response or loss of response (6,7).

The first study in 2007 found that a trough imatinib concentration of 1002 ng/mL was 77% sensitive and 71% specific for an MMR with a strong odds ratio of 7.8 (8). A 2008 study showed that patients who achieved a complete cytogenic response, which was 84.6% of patients, had higher trough concentrations of imatinib (4). Several other studies have found similar results and are summarized by Verheijen et al. in an article providing practical recommendations for TDM of kinase inhibitors (9).

Studies Support TDM

In 2014, Gotta et al. published two studies in support of imatinib TDM. The first was a randomized controlled study of 56 patients allocated to either routine imatinib TDM or rescue TDM (in which TDM was used when the patient was experiencing clinical concerns, such as potential adherence problems, suboptimal efficacy or tolerance, or possible drug–drug interactions) (10). If necessary, recommendations were made for dose adjustment according to a predetermined protocol to achieve a trough concentration between 750–1500 ng/mL in patients in either arm of the study. Although only 50% of the patients in the routine TDM group were given the recommended dose, these patients had fewer adverse events.

The authors recognized several other study limitations, yet they recommended TDM for imatinib because those who followed the dosage recommendations achieved the target trough concentration with a combined outcome of efficacy, tolerance, and persistence. TDM may have also led to discontinuation of imatinib by identifying patients who were intolerant at the recommended trough concentrations, allowing for transition to another medication.

The group's second study was an observational study of 2478 patients with CML who had received imatinib TDM for at least two years (7). The authors used data from these patients to develop a population pharmacokinetic model. After stratification by sex, the results did not confirm that a trough concentration of >1000 ng/mL was associated with a better rate of MMR. However, they did report that certain patient populations were at risk of altered pharmacokinetics that would affect imatinib concentrations and, thus, possibly their disease response, making them good candidates for TDM. For example, their model estimated that, over two years, the clearance of imatinib decreased by 15.2% in females and by 23% in patients aged 40 to 80 years. Table 1 shows the potential effects of drug–drug interactions.

Adherence Issues

Adherence issues with imatinib are associated with patient age (lower among younger patients), duration of therapy, and adverse events that are more than mild, for example, nausea, anemia, rash, fatigue, or cytotoxicity (5,10). The root issues of adherence appear to be intolerance and poor response at the expected milestones, which can result in a decline in adherence within the first two years.

However, a longitudinal study of patients with a complete cytogenic response demonstrated its importance: Adherence was an independent predictor of MMR and complete molecular response, and adherence of ≥90% was associated with higher probabilities of MMR and complete molecular response after six years (5). The authors acknowledge that they were unable to determine whether poor initial response was due to lack of adherence or vice versa.

Table 1. Effects of Concomitant Drugs on Imatinib Metabolism

Concomitant Drug	Change in Trough Concentration
Cytochrome P450 3A4 inducer	-24.8%
Cytochrome P450 3A4 inhibitor	+21.7%
P-glycoprotein inducer	-17.6%
Human organic cation transporter-1 inducer	+8.2%

Applying imatinib TDM in early treatment may help to answer that question.

Imatinib TDM, which is performed by measuring imatinib by competitive immunoassay in a plasma sample, is not widely available today. The lack of availability is consistent with the overall limited use of TDM in oncology, but an increased availability of testing is likely to improve understanding of appropriate use. CML patients may not be the only ones who could benefit from imatinib TDM. Researchers have also raised the possibility that imatinib TDM could be useful for patients treated for gastrointestinal stromal tumors (11). In the future, TDM for the other tyrosine kinase inhibitors, such as nilotinib, dasatinib, or bosutinib, may also be warranted.

In summary, evidence is growing to support imatinib TDM. TDM can aid clinicians in providing the best care for their patients by personalizing therapy, particularly for patients at risk of altered pharmacokinetics due to gender, age, or coadministered medications. Additionally, TDM provides a direct method for monitoring adherence to therapy.

Learning Objectives

After reading this article, the reader will be able to describe the reasons for and the evidence supporting therapeutic drug monitoring of imatinib mesylate. The reader will also be able to list the factors that affect adherence to imatinib therapy and explain how adherence affects therapy response.

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Fentanyl

Common Drugs Used in Pregnancy Can Cause Positive Immunoassays

By Beerinder S. Karir, MD, and Nicholas Heger, PhD

Fentanyl is a synthetic opioid analgesic used for management of chronic pain or in combination with sedative hypnotic agents for the induction of anesthesia. Because fentanyl is much more potent than heroin, the adulteration of street preparations of heroin with fentanyl has resulted in many overdoses and deaths.

At our institution, initial toxicology testing with immunoassay is followed by more definitive testing, as necessary, in patients who screen positive for nonprescribed or illicit substances. Here, we describe a case of a false-positive fentanyl immunoassay result due to a commonly prescribed medication.

A 36-year-old pregnant female at 18 weeks gestational age presented to the OBGYN clinic with features of preeclampsia, including high blood pressure and proteinuria. Her medical history was significant for chronic hypertension that was managed by oral labetalol in 400-mg tablets taken three times per day. During her OBGYN visit, she had persistent high blood pressure that required additional intravenous labetalol infusions.

Positive Immunoassay

A urine specimen was sent to the laboratory for a routine urine toxicology screen (amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, ethanol, fentanyl, marijuana, methadone, opiates, and oxycodone). It tested positive for fentanyl only. The patient's OBGYN physician consulted laboratory professionals and requested a toxicology consultation in light of the unexpected positive fentanyl result.

The urine specimen was sent to a reference laboratory for definitive testing by liquid chromatog-

raphy-tandem mass spectrometry (LC-MS/MS). The results did not identify fentanyl nor the metabolite norfentanyl (limit of detection, 0.5 ng/mL). As such, the positive in-house fentanyl immunoassay was deemed to be a false positive.

A review of the package insert for the FDA-cleared Immunalysis SEFRIA fentanyl urine enzyme immunoassay revealed that the assay is designed to produce a positive test if it reacts with fentanyl at concentrations of 1.0 ng/mL and above. The assay also cross-reacts with other fentanyl analogs such as butyryl fentanyl and acetyl fentanyl, but is insensitive to the fentanyl metabolite norfentanyl. The package insert identified several common drugs that can also cross-react with the assay, including haloperidol (1250 ng/mL), risperidone (2500 ng/mL), trazodone (10,000 ng/mL), and labetalol (15,000 ng/mL).

Labetalol Use

Labetalol is one of the safest and most commonly prescribed drugs for hypertension-related disorders of pregnancy, including chronic hypertension with superimposed preeclamsia. Our hospital sees many patients with pregnancy complications, and labetalol is given routinely for preeclampsia. The commonwealth of Massachusetts recommends screening pregnant women with a history of alcohol and substance use through a questionnaire, along with urine toxicology testing if indicated. It is further recommended that test results be disclosed to the mother and her response documented.

Mandated reporters are required to file a report with the state for all positive test results, which may require investigation and intervention, if necessary, including removing the child from the mother's custody after delivery. Given the grave consequences of these actions, screening results should always be confirmed with a definitive method, such as LC-MS/MS.

Review of Screening Results

A retrospective review of unexpected positive urine fentanyl screening results in the past 12 months at our institution identified 32 cases in which definitive tests by LC-MS/MS were negative for fentanyl and norfentanyl. Of these cases, 53% came from patients who were prescribed labetalol, 9% who were prescribed trazodone, and 6% who were prescribed risperidone. The remaining 32% were not prescribed any drugs listed as possible cross-reactants on the assay package insert. These remaining unexplained false-positive results may be attributed to nonspecific cross-reactivity by prescribed or unprescribed drugs that were not explored during specificity testing by Immunalysis.

Laboratorians should be familiar with the sensitivity and specificity information in the package inserts of urine drug-screening immunoassays. They should pay particular attention to cross-reactivity with commonly prescribed drugs that can cause false positives. A false-positive drug screening result that is not confirmed with a more sensitive and specific method (such as mass spectrometry) can lead to serious consequences for the patients. Laboratorians should also be a resource to clinicians and other providers for consultation, interpretation of toxicology results, and assistance in test selection.

Learning Objectives

After reading this article, the reader will be able to recognize the limitations of urine immunoassay drug screening with respect to cross-reactivity and false-positive results. The reader will also be able to describe the potential implications of false-positive results, especially for pregnant patients.

Suggested Reading

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The authors have nothing to disclose.

Contaminated Synthetic Cannabinoids Lead to Deaths and Bleeding in Illinois

The Illinois Department of Public Health has issued warnings about an outbreak of health problems and deaths linked to synthetic cannabinoids, some of which appear to contain the rat poison brodifacoum.

According to its website, the department has received reports of 164 cases of people suffering severe bleeding after using synthetic cannabinoids. There have been four deaths.

These products are also known as herbal or liquid incense and are sold under many brand names, including K2, Spice, Black Mamba, Bombay Blue, Genie, and Zohai.

For more information: http://www.dph.illinois.gov/topics-services/prevention-wellness/medical-cannabis/synthetic-cannabinoids and https://www.washingtonpost.com/news/to-your-health/wp/2018/04/03/synthetic-marijuana-leaves-two-dead-and-dozens-with-severe-bleeding/?noredirect=on&utm_term=.8a1b3d32f130.

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Learning Objectives

Learning objectives vary by article, but in general, after reading *CFTN*, the reader will be able to:

- Describe emerging and changing drug-abuse trends.
- Identify potential analytes of clinical significance.
- Evaluate methodologies' utility and limitations.
- Discuss relevant regulations.
- Explain analytical and regulatory issues.
- Describe the medical implications of drug abuse.

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States Consider Laws to Ban the Sale Of Fake Urine Used to Beat Drug Tests

In response to the sale of "clean urine samples" in places like truck stops and over the internet, many states are considering legislation to ban the sale of fake urine, according to a recent report in *The Washington Post*.

"Laws making it illegal to sell or use synthetic urine or cheat on a drug test are on the books in at least 18 states, according to the National Conference of State Legislatures. Indiana and New Hampshire banned synthetic urine last year. Bills to do so were introduced this year in Missouri and Mississippi," writes Katie Zezima.

Commercially sold synthetic urine is a relatively new tactic employed by illicit drug users who are trying to beat their drug tests. The concoctions are sold under names such as "Monkey Whizz" and "UPass."

For more information: https://www.washingtonpost.com/national/states-move-to-ban-fake-urine-a-new-challenge-for-drug-testing-amid-an-abuse-epidemic/2018/04/07/05cad026-1cd8-11e8-ae5a-16e60e4605f3_story.html?utm_term=.040d04206c02.

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