

Flualprazolam: Report of an Outbreak of a New Psychoactive Substance in Adolescents

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Flualprazolam is a nonregistered drug in the benzodiazepine family and constitutes a new psychoactive substance (NPS). Since 2014, a growing number of designer benzodiazepines have become available over the Internet and on the counterfeit drug market. In June 2019, a cluster of patients intoxicated with flualprazolam was identified by the Oregon Poison Center. As an emerging drug of abuse, the clinical characteristics of flualprazolam have been poorly characterized thus far. Over a one-week period, 6 teenagers presented to local emergency departments after ingesting illegally obtained counterfeit alprazolam, which led to sedation. Other symptoms included slurred speech, confusion, and mild respiratory depression. All 6 patients had resolution of their symptoms within 6 hours of ingestion. Blood and urine samples, as well as a tablet fragment, were obtained from 3 patients. The tablet and biological samples were analyzed by using liquid chromatography–quadrupole time-of-flight mass spectrometry and were found to contain the NPS flualprazolam without other drugs or intoxicants. With this case series, we add to the medical literature a clinical description of an emerging drug of abuse. Flualprazolam appears to share the clinical properties of other benzodiazepines. As flualprazolam and other NPSs become more common, physicians must be aware of their availability and characteristics. Sedation lasting <6 hours was observed in 6 of 6 patients exposed to flualprazolam. No effects that would be unexpected from benzodiazepine intoxication were seen among the patients. Specifically, none developed prolonged symptoms or required intubation and mechanical ventilation, ICU admission, or antidotal therapy.

In June 2019, the Oregon Poison Center identified an outbreak of central nervous system (CNS) depression among adolescents. The patients developed symptoms at school and required evaluation and treatment at local emergency departments. Further assessment revealed intoxication with a new psychoactive substance (NPS) colloquially referred to as “Hulk,” which on analysis, was revealed to be the benzodiazepine flualprazolam.

The benzodiazepine class of drugs bind to γ -aminobutyric acid A (GABA-A) receptors, leading to CNS depression. Unlike barbiturates, which stimulate the GABA-A receptor directly, benzodiazepines enhance the effect of GABA on its receptor through allosteric modulation. This leads to enhanced chloride permeability in the postsynaptic neuron, subsequent hyperpolarization, and diminished probability of generating an action potential.¹ Whereas overdose of opioids or barbiturates often leads to

abstract

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Dr Blumenberg conceptualized, researched, wrote, and edited the manuscript; Dr Hughes helped conceptualize and wrote sections of the manuscript; Mr Ellison and Mr Reckers analyzed and interpreted analytes, wrote sections of the manuscript, and helped edit the manuscript; Dr Gerona analyzed and interpreted analytes, wrote sections of the manuscript, researched, and helped edit the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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respiratory depression or apnea, the classic benzodiazepine toxidrome is a coma with normal vital signs. Chlordiazepoxide, the first benzodiazepine, was synthesized because of demand for a sedative with less risk of respiratory depression. Subsequent benzodiazepines, such as diazepam, were created via modification of chlordiazepoxide's molecular structure.² Death from isolated benzodiazepine toxicity is rare; however, in combination with alcohol, opioids, or other sedatives, death from respiratory depression or aspiration is more common.³

Flualprazolam is a nonregistered drug in the benzodiazepine family and constitutes an NPS.⁴ Since the early 2000s, there has been a rise in the rate of NPS exposures worldwide because of Internet purchasing. There have been >800 unique NPSs reported to the United Nations Office on Drugs and Crime between 2009 and 2017 and 22 tons of NPS seized by law enforcement in 2016 alone.⁵ Typically, NPSs are synthesized by simple structural modification or substitution of existing recreational drugs, pharmaceuticals, or their active metabolites.⁶ In the United States, the incidence of exposures to designer benzodiazepines in particular has been rising since 2014.⁷

Flualprazolam is structurally related to the US Food and Drug Administration–approved pharmaceuticals alprazolam and triazolam, differing in chemical composition by the presence of a fluorine atom on the ortho position of the phenyl moiety, as seen in Fig 1. All 3 compounds are triazolobenzodiazepines.⁸ As a benzodiazepine, expected clinical effects of flualprazolam would include sedation, anxiolysis, amnesia, and potentially respiratory depression. In overdose, alprazolam appears to have greater toxicity than other pharmaceutical

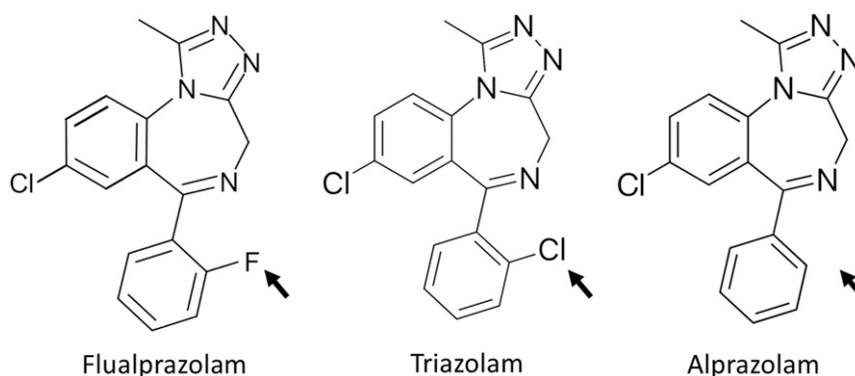


FIGURE 1
Comparative structures of flualprazolam, triazolam, and alprazolam.

benzodiazepines.⁹ Although flualprazolam has not been studied directly, rat studies of fluorinated diazepam analogues vary in potency from one-half to 10-fold¹⁰; therefore, the pharmacodynamic effects of fluorinated alprazolam cannot be surmised on the basis of chemical structure alone.

Mei et al¹¹ identified flualprazolam in postmortem blood; however, at present, there are no data on the clinical effects or pharmacology of flualprazolam. Correlations between dose and response, duration of action, metabolism, and onset of action are unknown. In this case series, we add to the medical literature the first description of confirmed clinical flualprazolam intoxication.

CASES

Over a 7-day period, 6 teenagers were transported to local emergency departments from a single high school after ingesting an illegally obtained substance colloquially named Hulk. All 6 received the drug as a free sample from a single student, believing that the substance was commercial Xanax. The clinical characteristics and laboratory results for the patients who were exposed are summarized in Table 1. Five of the patients were boys; their ages ranged from 14 to 16 years. Lethargy and slurred speech were the most

common reported clinical findings. One individual (patient 3) developed mild respiratory depression (respiratory rate of 10 breaths per minute) that was unresponsive to 0.4-mg naloxone, which was given empirically because of the unknown identity of the drug. Two of the 6 patients (patients 2 and 6) were initially drowsy but asymptomatic at the time of evaluation. All patients who were symptomatic recovered within 6 hours, and all were discharged from the hospital's emergency department.

ANALYSIS

A urine immunoassay (MEDTOX PROFILE-V; MEDTOX Diagnostics, Inc., Burlington, NC) for common drugs of abuse was performed in 5 patients, all of whom tested positive for benzodiazepines (detection threshold 150 ng/mL nordiazepam). The results of a urine test for patient 1 were also positive for cannabinoids (detection threshold 50 ng/mL; 11-nor-9-carboxy- δ -9-tetrahydrocannabinol). A pale green tablet in the possession of patient 3 had the markings "S 90 3," which is intended to identify the tablet as 2 mg of alprazolam. An analysis of a tablet fragment (Fig 2) in the possession of patient 6 revealed that the tablet contained 2.77 mg/g of flualprazolam, or ~2.75 to 3 mg of flualprazolam per intact tablet. No

TABLE 1 Clinical and Laboratory Characteristics of Patients Intoxicated With Flualprazolam

Patient No.	Age	Sex	Clinical Presentation at Emergency Department	Urine Immunoassay for Drugs of Abuse	Flualprazolam Concentration (LC-QTOF/MS)
1	16	Male	Lethargy and slurred speech	Positive for benzodiazepines and cannabinoids	Urine: 72.1 ng/mL
2	15	Male	Asymptomatic	Not obtained	Not obtained
3	16	Male	CNS depression, slurred speech, and mild respiratory depression unresponsive to 0.4-mg intravenous naloxone	Positive for benzodiazepines	Urine: 19.4 ng/mL Blood: 14.6 ng/mL
4	16	Female	Lethargy and slurred speech	Positive for benzodiazepines	Urine: 3.0 ng/mL
5	14	Male	Lethargy and confusion	Positive for benzodiazepines	Not obtained
6	14	Male	Asymptomatic	Positive for benzodiazepines	Not obtained

alprazolam or other drugs were detected in the tablet. Blood from patient 3 and urine from patients 1, 3, and 4 were analyzed by using liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QTOF/MS). The concentrations of flualprazolam in the urine were 72.1 ng/mL in patient 1, 19.4 ng/mL in patient 3, and 3.0 ng/mL in patient 4. The concentration of flualprazolam in the serum of patient 3 was 14.6 ng/mL, within the therapeutic range of alprazolam (10–100 ng/mL) and above the peak range of triazolam (1.7–9.4 ng/mL). The urine and blood tested with LC-QTOF/MS contained no antipsychotics, antiemetics, antimicrobial agents, antidepressants, opioids, or unaccounted benzodiazepines.

Alprazolam is metabolized by cytochrome P450 CYP3A to the active metabolites 4-hydroxyalprazolam and



FIGURE 2 Recovered pill fragment of flualprazolam with a penny for scale.

α -hydroxyalprazolam and the inactive metabolite 2-(3-(hydroxymethyl)-5-methyl-4-triazolyl)-5-chlorobenzophenone.^{12,13} Triazolam is metabolized by P450 CYP3A to the chlorinated analogues of expected alprazolam metabolites.^{14,15} Urine of patients 1, 3, and 4 had formula matches to the predicted fluorinated analogues of expected alprazolam metabolites.

Analyses of the drug product and patient biological samples were performed by using LC-QTOF-MS (Agilent LC 1260-QTOF/MS 6550; Agilent, Santa Cruz, CA). The drug product was pulverized and extracted in methanol. The serum sample was prepared for analysis via protein precipitation (95:5 vol/vol acetonitrile/methanol). Urine samples were deconjugated by using *Helix pomatia* β -glucuronidase type H3 (500 U/0.5 mL reaction vol) before running at 1:5 and 1:25 dilutions. The drug product and serum samples were evaporated and reconstituted at 9:1 (acetonitrile/water). Chromatographic separation of analytes before mass spectrometry was achieved by using an Agilent Poroshell 120 EC-C18 column (2.7 μ M, 2.1 \times 10 mm) with gradient elution between mobile phase A (water with 0.05% formic acid and 5 mM ammonium formate) and mobile phase B (acetonitrile with 0.05% formic acid). The elution gradient was as follows: 0 to 0.5 minute = 5%B, 1.5 minutes = 30%B, 4.5 minutes = 70%B, 7.5 minutes =

100%B, 7.5 to 10 minutes = 100%B, and 10.01 to 12 minutes = 5%B. The LC-QTOF/MS ionized the analytes in positive polarity with an electrospray ionization source at 2 GHz in extended dynamic range and auto MS/MS modes. The concentration was quantified by using the area ratio of flualprazolam to the internal standard (paroxetine-d6). The lower limit of quantitation of flualprazolam in all 3 matrix types (drug product, serum, and urine) was 1 ng/mL. The representative intra- and interday precision of the method ranged between 1.76% coefficient of variation (CV) and 7.36%CV and 0.88%CV to 6.58%CV for low (5 ng/mL), medium (20 ng/mL), and high (200 ng/mL) quality control levels, respectively.

DISCUSSION

In this case series, we add to the medical literature the first description of clinical toxicity from flualprazolam with identification of flualprazolam in tablet form and in the urine and sera of 5 patients without other medications or drugs. Flualprazolam appears to have led to CNS depression, which is an expected effect of benzodiazepines. All patients had sufficient clinical improvement within 6 hours such that they could be discharged from the hospital.

Urine samples from all patients who underwent LC-QTOF/MS analysis had detectable concentrations of flualprazolam as well as positive

urine immunoassay results. Several articles have been published on the high cross-reactivity of benzodiazepine analogues to benzodiazepine assays in urine drug screens contrary to other NPSs, such as synthetic cannabinoids and cathinones, that evade detection from urine drug screens.^{16,17} This makes it challenging to distinguish cases of benzodiazepine analogue intoxication from those of prescription benzodiazepines, especially if no unusual signs and symptoms are observed. Hence, intoxications from benzodiazepine analogues may be underreported.¹⁸

Formula matches to predicted fluorinated analogues of alprazolam and triazolam metabolites were detected in the urine of all patients who underwent advanced testing. Given that both alprazolam and triazolam have the same metabolic pathway, it is likely that flualprazolam is metabolized by P450 CYP3A; however, this requires experimental confirmation. Meanwhile, patients intoxicated with flualprazolam should be monitored for prolonged symptoms in the setting of exposure to a P450 CYP3A inhibitor, such as clarithromycin, ritonavir, or ketoconazole.

The flualprazolam tablets were identical in appearance and labeling to 2-mg tablets of alprazolam. This indicates an intentionally counterfeit product entering the drug supply chain. There are multiple previous examples of counterfeit alprazolam tablets containing potentially deadly adulterants, such as fentanyl or the opioid U-47700.^{19,20} It is possible that the patients believed the tablets to be a legitimate pharmaceutical product. Given the dynamic nature of any given NPS's entrance and exit from the global drug market, it is likely that physicians will again encounter patients with flualprazolam intoxication.⁵

Clinical findings of other designer benzodiazepines (such as etizolam and clonazolam) were similar to those of registered benzodiazepines, namely drowsiness, lethargy, slurred speech, and respiratory depression.⁷ As of July 2019, there have been no published reports on clinical intoxication with flualprazolam.

In these 6 patients, flualprazolam displayed the expected clinical effects of a benzodiazepine. The molecular structure is similar to that of alprazolam and triazolam, and it likely has an identical mechanism of action. As an emerging NPS, flualprazolam may be encountered more frequently in the future.⁶ In March 2018, the Center for Forensic Science Research and Education identified flualprazolam in biological samples.²¹ Although flualprazolam intoxication cannot be clinically differentiated from that of other benzodiazepines without advanced testing, patient management should be the same. For mild to moderate intoxication, patients should be treated with close monitoring and supportive care until symptom resolution. The benzodiazepine antidote flumazenil may be considered a safe and effective antidote in pediatric patients with significant CNS or respiratory depression.²² In patients for whom there is a concern of benzodiazepine dependence and flumazenil-induced seizures, airway protection and mechanical ventilation may be considered.³

CONCLUSIONS

Sedation lasting <6 hours was observed in 6 of 6 patients exposed to flualprazolam. No effects that would be unexpected from benzodiazepine intoxication were seen among the patients. Specifically, none developed prolonged symptoms or required intubation and mechanical ventilation, ICU admission, or antidotal therapy.

ABBREVIATIONS

CV: coefficient of variation
CNS: central nervous system
GABA: γ -aminobutyric acid
LC-QTOF/MS: liquid chromatography–quadrupole time-of-flight mass spectrometry
NPS: new psychoactive substance

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