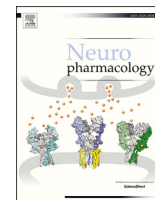




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Invited review

Intoxication from the novel synthetic cannabinoids AB-PINACA and ADB-PINACA: A case series and review of the literature

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ABSTRACT

Synthetic cannabinoids (SC), are a novel class of designer drugs which emerged as a drug of abuse in the late 2000's. We report a case series of 6 patients who may have smoked a synthetic cannabinoid product in a remote wilderness setting. They presented with varying degrees of altered mental status, agitation, and seizures. Two were confirmed to have AB-PINACA, ADB-PINACA and their respective pentanoic acid metabolites in biological specimens via liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS). Both compounds had DEA Schedule I classification at the time of case presentation, and 22 SCs are currently temporary or permanent DEA Schedule I. More than 150 SCs are known to date, and new compounds are appearing at a rapid rate on darknet and surface web e-commerce websites, marketed as "research chemicals" or "legal highs." The scale and rapidity of SC evolution make legal control and analytical detection difficult. Nontargeted testing with liquid chromatography high resolution mass spectrometry (LC-HRMS), examining both parent compounds and metabolites, is the ideal method for novel SC identification and confirmation. Due to full agonism at the cannabinoid receptors CB1 and CB2, clinical effects are more severe than marijuana, which is a partial cannabinoid receptor agonist. They include agitated delirium, lethargy and coma, seizures, tachycardia, hypertension, and hallucinations, among other findings. Treatment is primarily symptomatic and aimed at airway protection and control of agitation and seizures. SCs do not appear to be abating anytime soon and require the cooperation of law enforcement, analytical scientists, and clinicians to adequately control.

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Abbreviations

AAPCC	American Association of Poison Control Centers
AKI	Acute kidney injury
BP	Blood pressure
cAMP	cyclic adenosine monophosphate
CBC	complete blood count
CBP	US Customs and Border Protection
CDCR	California Department of Corrections and Rehabilitation
CK	creatinine kinase
CNS	Central nervous system
DAWN	Drug abuse warning system
GC-MS	Gas chromatography mass spectrometry
ECG	electrocardiogram
ED	emergency department
EMS	emergency medical services
GCS	Glasgow coma scale

HEIA	Homogenous enzyme immunoassay
HEMS	Helicopter emergency medical services
HR	Heart rate
ICU	Intensive care unit
LC	liquid chromatography
LC-HRMS	Liquid chromatography high resolution mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LC-TOF/MS	liquid chromatography time-of-flight mass spectrometry
MCI	Mass casualty incident
PKA	protein kinase A
RR	respiratory rate
SC	Synthetic cannabinoid
Temp:	temperature
THC	tetrahydrocannabinol
US:	United States

1. Introduction

Since their detection in the late 2000's, synthetic cannabinoids (SC) have been proliferating at a rapid rate, with more than 150 compounds now identified (UNODC, 2015). Initially developed as full cannabinoid receptor agonists for biomedical research, synthetic cannabinoids are now widely used as drugs of abuse. We report a case series presenting to our local emergency medical services (EMS) system, with confirmation of a novel combination of SCs in biological specimens. We also review the history, legal status, epidemiology, pharmacology, analytical techniques, and care of the patient intoxicated by SCs.

2. Case series

2.1. Clinical details

A mass casualty incident (MCI) was called for a reported overdose of heroin in a remote location of the Sequoia foothills of California, involving a California Department of Corrections and Rehabilitation (CDCR) wildland firefighting crew. Six patients were evaluated and transported by helicopter emergency medical services (HEMS) to three local hospitals for further evaluation and treatment. Blood products were available on five of six patients for testing by LC-TOF/MS (liquid chromatography time-of-flight mass

spectrometry). We report the clinical presentation of three of the six who presented at our hospital. Upon questioning, all three refused to report what substance they had used.

2.1.1. Case one

A 27 yo male CDCR inmate was observed to be acting strangely and confused after picking up a cigarette butt off the ground and smoking it while in a remote wilderness location as firefighter. The patient was alert and oriented but slow to answer questions and occasionally answered incorrectly by HEMS report. He had no complaints on arrival to the emergency department (ED). Review of Systems and physical examination was unremarkable; the patient was alert, oriented, and acting appropriately. Vital signs on arrival were: Blood Pressure (BP) 104/62 mm Hg; Heart Rate (HR) 88 beats per minute; Temperature (Temp) 36.5 °C; Respiratory Rate (RR) 23 breaths per minute; and oxygen saturation of 98% on room air. Routine ED laboratory studies including complete blood count (CBC), and chemistry panel were within normal limits with exception of blood glucose of 184 mg/dL. AST and ALT were elevated [50 U/L (normal: 10–41 U/L) and 109 U/L (normal: 12–60 U/L), respectively]; Ammonia was 52 µmol/L (normal <35 µmol/L). Alcohol and a urine drug screen for amphetamines, barbiturates, benzodiazepines, cannabis, cocaine, opiates and phencyclidine were negative. An electrocardiogram (ECG) had a normal sinus rhythm at 81 beats per minute with normal axis, intervals, ST

segments, and T waves. Troponin was negative. A chest x-ray was unremarkable. The patient was observed in the ED for 6 h with no bizarre behavior or alteration in level of consciousness or orientation noted. Vital signs remained unchanged.

2.1.2. Case two

A 29 yo male CDCR inmate was transported from the MCI site for agitation and depressed level of consciousness (Glasgow Coma Scale (GCS): 7). He was paralyzed and intubated with ketamine and vecuronium prior to HEMS transport from the remote firefighting site. On arrival to the ED, the patient was hypothermic (Temp 32.9 °C). Other vital signs were: BP 152/0 palpated; HR 75 beats per minute; RR 14 breaths per minute; Oxygen Saturation 100% on 100% inhaled oxygen via endotracheal intubation and conventional ventilation. History and review of systems were unobtainable. Physical examination was unremarkable with the exception of a depressed level of consciousness (GCS 3T, eye subscore 1, verbal subscore 1T, motor subscore 1). Laboratory evaluation including CBC and chemistry panel were within normal limits. Urine drug screen was positive for benzodiazepines and opiates (obtained after initiation of midazolam and morphine for sedation by prehospital providers). Creatine kinase (CK) was elevated at 229 U/L (normal: 38–174 U/L). Chest x-ray was unremarkable. ECG showed Osborne waves and a prolonged QRS at 124 msec. Troponin was negative. The patient was admitted to a monitored critical care setting with supportive care and rewarming initiated. Approximately 12 h following arrival, his mental status improved to normal and was successfully extubated and discharged 3 h later.

2.1.3. Case three

A 33 yo male CDCR inmate was found acting bizarrely and then became unresponsive after a witnessed generalized tonic-clonic seizure while at a remote firefighting site. HEMS crew intubated him for airway protection and to facilitate safe transport prior to departure. On arrival to the ED, the patient was hypertensive (174/127 mm Hg). HR was 85 beats per minute; Temp 33.7 °C; RR 14 breaths per minute; and oxygen saturation of 100% on 100% inhaled oxygen via endotracheal intubation and conventional ventilation. Further history and review of systems were unobtainable. Physical examination was unremarkable with the exception of bilateral lower extremity clonus and depressed level of consciousness (GCS 3T, eye 1, verbal 1T, motor 1). Laboratory studies were remarkable for an elevated white blood cell count of 20.3×10^3 (normal: $3.9\text{--}11.7 \times 10^3$ /mL). Chemistry panel was notable for an elevated K of 5.7 mmol/L (normal: 3.5–5.1 mmol/L) and glucose of 145 mg/dL (normal: 70–99 mg/dL). CK was mildly elevated at 104 U/L and troponin was 0.05 ng/mL (upper limit of normal 0.04 ng/mL). The troponin peaked at 1.228 ng/mL. A urine drug screen was positive for benzodiazepines (obtained after initiation of midazolam for sedation by prehospital providers). An ECG, Computed Tomography of the head, and chest x-ray were unremarkable. An echocardiogram revealed no wall motion abnormalities or significant valvular lesions. Following admission to a monitored critical care setting, the patient had posturing/jerking of the right upper extremity. An electroencephalogram (EEG) was performed revealing a pattern consistent with “mild bilateral cerebral dysfunction.” Nine hours following initial presentation, the patient's mental status improved and was successfully extubated to room air. He was transferred to a non-critical care bed and observed for an additional 24 h prior to discharge without any complications.

2.2. Analytical methods and results

2.2.1. Sample preparation

Each 1 mL aliquot of serum sample was combined with 2 mL of

saline and 0.8 mL of 10% acetic acid, followed by gentle vortexing. Samples were then combined with 5 mL of 9:1 hexane:ethyl acetate solution, capped and rocked for 30 min. The slurry was then centrifuged in a Beckman GH 3.8 swing bucket rotor for 15 min at 2800 rpm. The solvent layer was carefully decanted into clean tubes and evaporated to dryness with nitrogen at 45 °C. Samples were then reconstituted with 125 µL of 1:1 acetonitrile:water solution and transferred to injection vials.

2.2.2. Instrumentation

The LC-TOF/MS system consisted of an Agilent 1260 Infinity Quaternary Liquid Chromatography system containing a quaternary pump, autosampler, and temperature controlled column compartment connected to an Agilent 6230 LC-TOF/MS equipped with an Agilent Jet Stream Electrospray Ionization (AJS ESI) source. The time-of-flight was set to operate in extended dynamic (2 GHz) 3200 m/z mass range in the positive ion mode. The ion source was set to 3500 V. Nitrogen was used as a nebulizer, sheath and drying gas at 35 psig and 250 °C.

Liquid chromatography (LC) was performed using an Agilent Poroshell 120, EC-C-18 column (3.0 × 50 mm, 2.7 µm) and operated at 0.8 mL/min for 9 min. Mobile phase A consisted of 0.1% formic acid in water and phase B consisted of 0.1% formic acid in methanol. The LC step was run isocratically at 5% phase B for 1 min, followed by a gradient from 5% to 95% phase B in 4 min, and held at 95% phase B for 4 min. The injection volume was 5 µL and column Temp was held constant at 35 °C. Instrument control, data acquisition, and data analysis were performed using Agilent Masshunter software for 6200 series TOF. Compound search and identification was based on mass, isotope abundance, isotope spacing, and retention time scoring. Standards for AB-PINACA (N- (1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide), AB-PINACA pentanoic acid metabolite (3-[[[1-(aminocarbonyl)-2-methylpropyl]amino]carbonyl]-1H-indazole-1-pentanoic acid), ADB-PINACA (N- [1-(aminocarbonyl)-2,2-dimethylpropyl]-1-pentyl-1H-indazole-3-carboxamide), and ADB-PINACA pentanoic acid metabolite (5-(3-((1-amino-3,3-dimethyl-1-oxobutan-2-yl) carbamoyl)-1J-indazol-1-yl) pentanoic acid) were purchased from Cayman Chemical Company.

2.2.3. Analytical results

Using LC-TOF/MS, AB-PINACA, ADB-PINACA, and their respective pentanoic acid metabolites were detected and confirmed in cases two and three from the patients presenting to our hospital (Fig. 1).

3. Discussion

The mass casualty nature of this case series posed distinct challenges from a clinical standpoint due to the number of patients simultaneously presenting in a remote wilderness environment. High-resolution mass spectrometry (HRMS) enabled the detection of AB-PINACA and ADB-PINACA in these patients, the combination of which has not yet been reported in human exposures.

3.1. History and legal status of synthetic cannabinoids

Roger Adams synthesized the first synthetic cannabinoids in the 1940's, through side chain modification of tetrahydrocannabinol (THC) (Adams et al., 1948). The SCs HU-210 and CP-47,497 were then synthesized in the 1980's, followed by WIN55, 212-1 in the early 1990's. This progress in the field led to the work of John W Huffman, who undertook extensive research to delineate structure-function relationships of hundreds of synthetic cannabinoids synthesized in his lab to the cannabinoid receptors CB1 and CB2 (Wiley

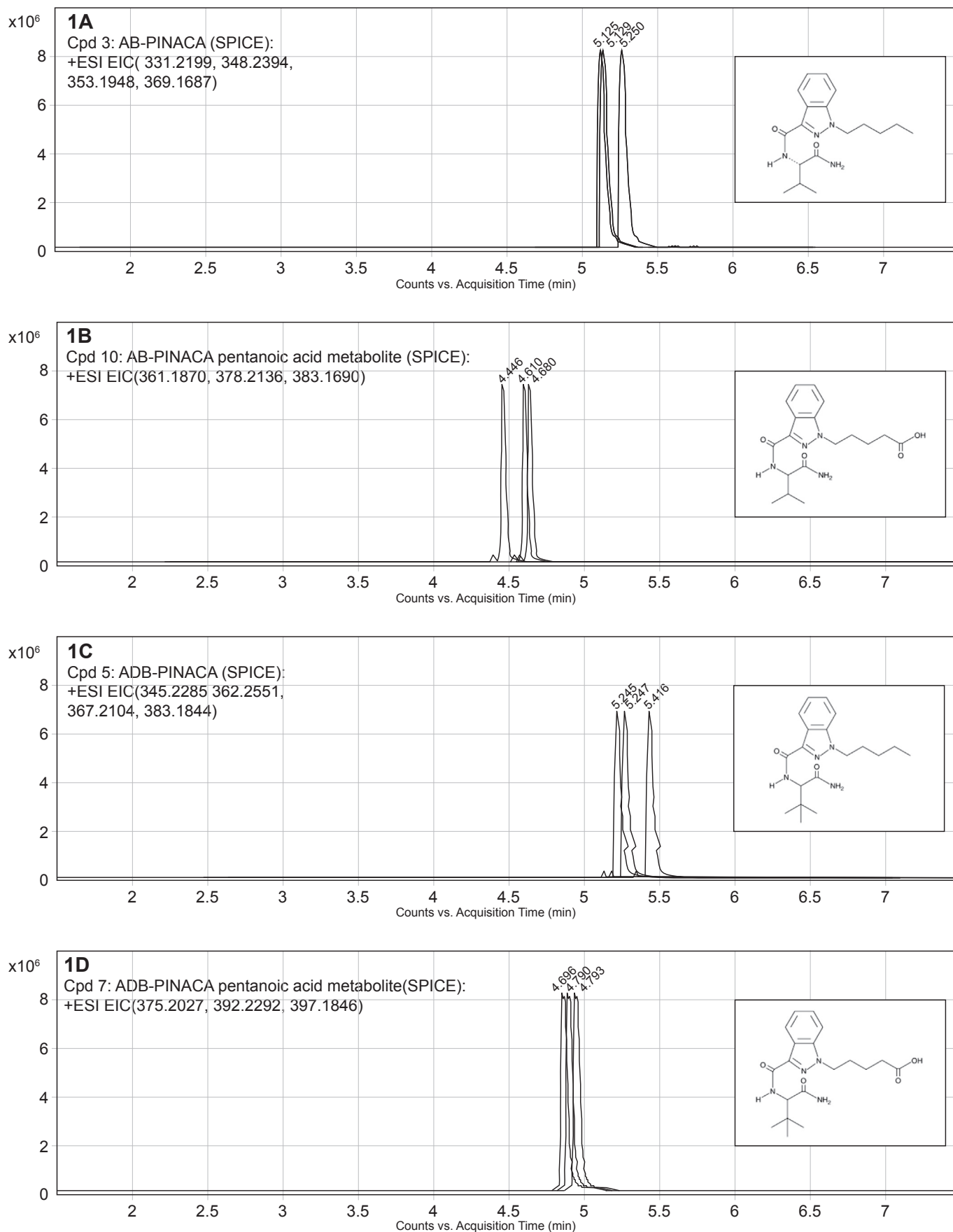


Fig. 1. The extracted ion chromatograms and chemical structures for synthetic cannabinoids and metabolites detected in patient serum with LC-TOF/MS. A) AB-PINACA, left to right: Case 2, Case 3, Standard. B) AB-PINACA pentanoic acid metabolite, left to right: Standard, Case 2, Case 3. C) ADB-PINACA, left to right: Case 3, Case 2, Standard. D) ADB-PINACA pentanoic acid metabolite, left to right: Case 3, Case 2, Standard.

et al., 2011). From the late 1980's to 2010, Huffman synthesized multiple synthetic cannabinoids, with names starting in JWH, for the purposes of scientific research.

These compounds were not known outside of the research setting until 2004, when they appeared in Europe as a legal substitute for cannabis (Deluca et al., 2012; EMCDDA, 2009). Initially known as “spice” and marketed as herbal incense, mixtures of dried herbal material were initially thought to cause the physical and psychological effects experienced by users. In 2008, SCs were first identified in the “spice” mixtures in Europe, with a CP-47,497 analog (Cannabicyclohexanol, C8 homologue of CP-47,497) identified by German researchers (Auwärter et al., 2009). Analogs of older SCs have thus been a part of the synthetic cannabinoid story from the beginnings of their recreational use.

Evolution of these compounds has largely been shaped by legislation. In the end of 2010, the first human cases were primarily due to the JWH compounds JWH-018, JWH-250, JWH-073, as well as CP-47,497 and its C8 homologue. At that same time, the US DEA (Drug Enforcement Agency) announced a notice of intent to place those compounds into Schedule I classification, which went into effect March 2011 (Table 1) (DEA, 2011). By then, a second-generation SC, AM-2201, and JWH-210 and JWH-122 were being detected with the initial 5 scheduled compounds no longer appearing in forensic specimens. In 2012, third-generation SCs XLR-11 and UR-144 were causing outbreaks. Sixteen cases of acute kidney injury were reported in 6 states in March 2012, due to XLR-11 (CDC, 2013a). In May 2013 these compounds, as well as AKB-48, entered temporary Schedule I classification (DEA, 2013). Another novel indole SC, PB-22, and the indazoles ADB-PINACA and AB-FUBINACA were detected in the second half of 2013 to the beginning of 2014. Temporary scheduling of these 4 compounds in February 2014 led to a dropoff in cases (DEA, 2014). Until this point, a majority of the SCs had been developed by research or pharmaceutical laboratories for legitimate scientific purposes. Illicit laboratories are now responsible for the new, emerging SCs with synthetic chemists altering existing compounds at an alarming rate to produce products that have not been tested on animals or humans. The indazole SCs AB-CHMINACA and ADB-CHMINACA,

first detected in early 2014, are part of this group of compounds that seems to have been synthesized solely for recreational use. AB-CHMINACA, AB-PINACA, and THJ-2201 became Schedule I in January 2015 (DEA, 2015). This pattern is repeating, with newer SC compounds continuously appearing in forensic specimens as soon older SCs are scheduled and an increasing total number of cases (Table 1) (Riederer et al., 2016).

Street slang and packaging have also evolved, likely in order to evade authorities. Initially marketed as herbal incense “spice” or “K2” in colorful packaging, SCs were sold at head shops and other brick and mortar retail establishments. Herbal blends are not the only way to purchase these substances anymore, as they are available in powder format, labeled with specific compound names rather than brand names. E-commerce is now a major proportion of sales of novel SCs, driven primarily by “research chemical” and “legal high” websites. Any number of illicit drugs can be bought and sold on the dark web (darknet), using cryptocurrencies such as Bitcoin (Armenian et al., 2017 This issue). As concerning is the prevalence of these compounds on the surface web, sold using currencies such as credit cards, Western Union, PayPal, Moneygram, and bank transfers. Most of these e-commerce “research chemical” websites are based in China or the United Kingdom, with Chinese pharmaceutical factories driving the synthesis of new compounds. In a recent study, multiple “research chemicals” including the indazole SCs AB-CHMINACA and PX-2, were purchased in late February–March 2015 from Internet vendors and shipped to the US. 82% were found to be the advertised compound. Of note, AB-CHMINACA was Schedule I as of 1/30/15 in the US (Armenian et al., 2015).

Common among all forms of SC sales is the disclaimer “not intended for human consumption.” This is wording intended to circumvent the Federal Analog Act, a section of the US Controlled Substances Act, passed in 1986. It established that any analog of a controlled substance could also be considered scheduled in that manner. Its chemically vague wording describes that a compound with a “chemical structure substantially similar to the chemical structure of a controlled substance in Schedule I or II” and an “effect on the central nervous system substantially similar to or greater than” the controlled substance or a “person intends to have a central nervous system effect similar to or greater than” the controlled substance is also now considered Schedule I or II. The law clarifies that it excludes “any substance to the extent not intended for human consumption before such an exemption takes effect with respect to that substance.” (Federal Analogue Act, 1986) No further mention is made of what chemical structures or pharmacodynamic interactions would be considered “substantially similar.” Due to the vagueness of this law, only 1 case using it has been successfully argued in court (United States v. Washam, 2002). In it, 1,4-butanediol was deemed to be a controlled analog of GHB (gamma-hydroxybutyrate) due to structural similarities and since 1,4-butanediol is metabolized to GHB in the human body. To date, no synthetic cannabinoid analog cases have been brought to court. The disclaimer “not for human consumption” is currently used to not only circumvent the Federal Analog Act, but also as a covert slang term to indicate to potential recreational drug users, that this compound is, in fact, intended for human use.

The Synthetic Drug Abuse Prevention Act of 2012 added multiple synthetic drugs to Schedule I of the Controlled Substances Act, including 15 SCs (Synthetic Drug Abuse Prevention Act of 2012). Interestingly, this law attempts to become more explicit regarding what is scheduled, stating that cannabimimetic agents, defined as CB1 receptor agonists, and their salts, isomers, and salts of isomers are also considered scheduled. It lists 5 specific SC structural classes which are also scheduled: 2-(3-hydroxycyclohexyl)phenols, 3-(1-naphthoyl)indoles or 3-(1-naphthylmethane)indoles, 3-(1-

Table 1
Scheduled synthetic cannabinoids.

Synthetic Cannabinoid	DEA (US)	Date ^a
JWH-018	Schedule I	3/1/2011
JWH-073	Schedule I	3/1/2011
JWH-200	Schedule I	3/1/2011
CP-47,497	Schedule I	3/1/2011
Cannabicyclohexanol (CP-47,497 C8 Homologue)	Schedule I	3/1/2011
UR-144	Schedule I	5/16/2013
AKB48 (APINACA)	Schedule I	5/16/2013
XLR-11	Schedule I	5/16/2013
PB-22 (QUPIC)	Schedule I	2/10/2014
AB-FUBINACA	Schedule I	2/10/2014
ADB-PINACA	Schedule I	2/10/2014
5F-PB-22	Schedule I	2/10/2014
AB-CHMINACA	Schedule I	1/30/2015
AB-PINACA	Schedule I	1/30/2015
THJ-2201	Schedule I	1/30/2015
MAB-CHMINACA	Schedule I	2/5/2016
5F-ADB (5F-MDMB-PINACA)	Schedule I	4/10/2017
5F-AMB	Schedule I	4/10/2017
5F-APINACA (5F-AKB48)	Schedule I	4/10/2017
ADB-FUBINACA	Schedule I	4/10/2017
MDMB-CHMICA (MMB-CHMINACA)	Schedule I	4/10/2017
MDMB-FUBINACA	Schedule I	4/10/2017

^a Date of temporary scheduling order. Substances that receive temporary scheduling have all had final scheduling within 2 years.

naphthoyl)pyrroles, 1-(1-naphthylmethylene)indenes, and 3-phenylacetylindoles or 3-benzoylindoles. The United Kingdom was also banning specific SCs as they entered the market, but passed the Psychoactive Substances Act 2016 to control the production and sale of “legal highs” which would include a variety of compounds. In this law, a psychoactive substance is considered anything that could alter the central nervous system, with the exceptions of alcohol, tobacco, nicotine, and caffeine. SCs are not yet scheduled by the United Nations Convention on Psychotropic Substances or the Single Convention on Narcotic Drugs.

3.2. Epidemiology of synthetic cannabinoids

The first cases of SC toxicity reported to the United Kingdom National Poisons Information Service were in the first quarter of 2009, mostly in young males, with a rise in cases since then (Waugh et al., 2016). In November 2008, United States (US) Customs and Border Protection (CBP) seized the first “spice” shipment containing JWH-018 (DEA, 2011). The first SC cases reported to the American Association of Poison Control Centers (AAPCC) were 14 incidents in the last quarter of 2009. By 2010, that number had risen to 1206 cases due to SCs alone, with an initial peak of 6968 cases in 2011 (Bronstein et al., 2011, 2012). In 2010, cases were primarily in the Midwest and Southern regions of the US, with expansion to the entire country by 2011. A significant number of SC-related US ED visits occurred in 2010, as reported by the Drug Abuse Warning Network (DAWN) (Center for Behavioral Health Statistics and Quality, 2012). The 11,406 ED visits in 2010 rose to 28,531 in 2011, the most recent year for which data are available. Exposures reported to the AAPCC dropped off by 2013. However, another peak with 7792 cases was noted in 2015, the most recent year for which data are available (Mowry et al., 2016). The resurgence of SC cases in 2015 was primarily in the month of April and due to MAB-CHMINACA. The epicenter of that outbreak was in Mississippi, but many incidents were also noted in New York, the District of Columbia, Maryland, Alabama, and Arizona. 721 suspected cases and 9 deaths were reported from this outbreak and poison control centers fielded 1501 cases in April 2015 (Kasper et al., 2015; Law et al., 2015; Mowry et al., 2016).

Other occasional outbreaks in the US due to specific SCs have sporadically occurred since 2012. In Wyoming and other states in March 2012, 16 cases of acute kidney injury were attributed to XLR-11 (CDC, 2013a). From August to September 2013, 221 probable cases of SC toxicity presented to hospitals in Denver and Colorado Springs, CO (CDC, 2013b). In August 2013, a separate cluster of 22 cases in Georgia was attributed to ADB-PINACA (Drenzek et al., 2013). Anchorage, Alaska had an outbreak of 535 affected persons in July 2015. 40% of those patients were homeless, and toxicity was attributed to AB-CHMINACA, MAB-CHMINACA, and 5F-AMB (Springer et al., 2016). Most recently, in July 2016, 33 people in New York City had “zombielike” behavior, thought to be due to AMB-FUBINACA (Adams et al., 2017).

Synthetic cannabinoid users tend to be young and male, as evidenced by the outbreaks mentioned above, other case reports, and epidemiological survey data gathered in Europe and the US (Loeffler et al., 2016). The most common age group is teens to early 20's, with 2–3 times more males than females. In general population surveys conducted in Europe, lifetime prevalence of SC use was as high as 4% in Hungary in 2013. In high school age adolescents, lifetime prevalence was as high as 18.6% in Poland in 2010. Among 8th–12th grade students in the US, past 12-month SC use prevalence was the highest in 12th graders, with 11.4% in 2011 and 11.3% in 2012. Lifetime marijuana use was the sociodemographic variable most associated with SC use in US high school students (Palamar and Acosta, 2015). Last 12 month use prevalence somewhat

declines in college students (8.5%), but is higher in non-students of the same age group (15.5%) (Loeffler et al., 2016). A US Department of Defense survey revealed lifetime SC use at 4.7% and 1.1% in the past 12 months for military personnel (Jeffrey et al., 2013). In active duty military personnel, 1.4% of random urine specimens were positive for SCs from 2011 to 12 (Castaneto et al., 2014; Wohlfarth et al., 2015b). SC use is especially of interest in the military because it is not routinely detected in workplace urine drug screening tests and so users are able to evade detection from the US military's zero tolerance policy for illicit drugs. In our case series, the 6 subjects who received medical care were inmates with the CDCR, and it is unclear how they had access to the substances while incarcerated.

Aside from the major motivators curiosity and the desire to get high, another reason for sustained SC use is to evade detection. In a sample of 396 patients entering a residential substance use treatment facility, 80% reported SC use in the past 12 months, with 71% citing “get high without having a positive drug test” as a motive for use (Bonar, et al., 2014). In the general population, avoiding detection was a motivator for use in 8–57% of SC users (Loeffler et al., 2016). Overall, synthetic cannabinoids remain a significant public health problem and are likely to remain present in drug culture for the foreseeable future.

3.3. Clinical pharmacology of synthetic cannabinoids

To date, more than 150 SCs have been synthesized (UNODC, 2015), some of which have never undergone any in vitro or in vivo studies in animals or humans. For that reason, receptor affinities, metabolic mechanisms and pharmacodynamic patterns are unknown for many SCs. We present a broad summary of published literature, with the idea that similarities exist within the drug class.

3.3.1. Cannabinoid receptor interactions

Although frequently referred to as synthetic marijuana or marijuana alternative, synthetic cannabinoids have different clinical presentations and more adverse outcomes than natural marijuana. This is primarily due to the fact that Δ^9 -THC, the active compound in marijuana, is a partial agonist at the cannabinoid receptors, CB1 and CB2, whereas SCs are full agonists at those same receptors. Δ^9 -THC also has lower CB1 and CB2 receptor affinities than many SCs. CB1 receptors are primarily located in the central nervous system (CNS), especially in the amygdala, cingulate cortex, prefrontal cortex, hypothalamus, hippocampus, nucleus accumbens, ventral tegmental area, and cerebellum (Le Boisselier et al., 2017; Miliano et al., 2016). Via G_i proteins, they inhibit cAMP (cyclic adenosine monophosphate) formation, thus decreasing protein kinase A (PKA) dependent phosphorylation. Due to their inhibitory action, end effects are primarily retrograde, regulating presynaptic function and suppressing neurotransmitter release. CB1 receptors also inhibit some Ca^{2+} channels and activate A-type and inward rectifying K^+ channels. Although primarily located in the CNS, CB1 receptors are also found in peripheral tissue to a far less degree. CB2 receptors, on the other hand, are mostly located in peripheral tissue, particularly in immune cells and the hematopoietic system such as the spleen and lymph nodes. There is some evidence that CB2 receptor activity controls basic immune system functions such as cytokine release and immune cell migration, perhaps modulating inflammatory pain (Le Boisselier et al., 2017; Harris and Brown, 2013). In some animal studies, dopamine release was elicited in the nucleus accumbens through CB1 and CB2 agonism (Chen et al., 1993; Cheer et al., 2004). This system is involved in the rewarding and reinforcing properties of addictive drugs. Multiple animal studies with SCs demonstrate their influence on dopaminergic transmission in the mesolimbic pathway and on conditioned

behaviors (Miliano et al., 2016).

Most SCs are nonspecific for CB1 and CB2 receptors, and bind to them with varying affinities. Δ^9 -THC binding affinities (K_i) range from 5 to 80 nM for CB1 and 3–75 nM for CB2 (Pertwee, 2008). Although some SCs of the JWH and AM classes have similar affinities, there are some with higher binding affinities. For example, JWH-210 has a K_i of 0.46 nM for CB1 and 0.69 nM for CB2. AM-694, JWH-122, JWH-182, and AM-2232 also have $K_i < 1$ nM for CB1 receptors (Debruyne and Le Boisselier, 2015). In a Chinese hamster ovary cell study examining binding affinities and functional activity of SCs at human CB1 receptors, JWH-210 had a K_i of 1.01 nM, ADBICA 1.3 nM, CP-55,940 0.67 nM, and ADB-PINACA 0.59 nM. Functional activity (EC_{50}) was 111, 0.38, 0.30, and 0.024, respectively and each had near 100% maximum effect, indicating full agonism at CB1 (Gatch and Forster, 2016). Compared to the full agonist CP-55,940, AB-PINACA and AB-CHMINACA had enhanced receptor efficacy and CB1/CB2 K_i of 2.87/0.88 nM and 0.78/0.45 nM, respectively (Wiley et al., 2015). AB-FUBINACA, ADB-FUBINACA, AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, ADBICA, and 5F-ADBICA all had greater potency than Δ^9 -THC at CB1 and CB2 receptors (Banister et al., 2015).

3.3.2. Metabolism

SC metabolism pathways are known for some, but not all SCs due to the number and rate of synthesis of new compounds. Two of the oldest SCs, JWH-018 and AM-2201 are known to undergo first pass metabolism in the liver, primarily by oxidation via the CYP2C9 and CYP1A2 isoforms of the P450 system (Chimalakonda et al., 2012). CYP2C9 is present in the liver and intestine, thus may also be involved in intestinal metabolism of JWH-018 from oral routes of exposure. CYP2C19 and CYP2D6 are also involved in JWH-018 and AM-2201 metabolism, to a far lesser degree. All of these CYP isoforms exhibit genetic polymorphisms, which would result in differential enzymatic activity among individuals. The major hydroxylated metabolites of JWH-018 and AM-2201 are active, binding to CB1 receptors with great affinity (Chimalakonda et al., 2012). The most common initial metabolites for JWH-018, JWH-015, JWH-073, and JWH-250 are all monohydroxylated and often glucuronidated. Over time, the dihydroxylated or ω -carboxylated metabolites predominate (Moran et al., 2011; Chimalakonda et al., 2012). In order to detect and confirm the presence of these compounds, it is important to know which metabolite may be present at different times post-exposure.

In the next generation of SCs, analogs with fluorine substitution at the 5-pentyl position of the pentylindole/indazole compounds were created. For example, the non-fluorinated UR-144, PB-22, and AKB-48, are usually monohydroxylated and glucuronidated on the terminal carbon of the alkyl group, similar to JWH-018 (Gandhi et al., 2015). Their fluorinated analogs XLR-11, 5F-PB-22, and 5F-AKB-48 preferably metabolize to 5-hydroxypentyl and pentanoic acid metabolites. A few studies have described the metabolism of the newer generation of pentylindazole SCs, which include the PINACA series. AB-PINACA primarily undergoes hydrolysis to AB-PINACA carboxylic acid, carbonyl-AB-PINACA, hydroxypentyl AB-PINACA, and AB-PINACA pentanoic acid. Its fluorinated analog 5F-AB-PINACA is primarily metabolized to AB-PINACA pentanoic acid and 5-hydroxypentyl-AB-PINACA, similar to the aforementioned SCs (Wohlfarth et al., 2015a). Hydrolysis, mono- and dihydroxylation metabolites were also demonstrated in a series of human liver microsome experiments (Thomsen et al., 2015).

3.4. Analytical detection

SCs are not routinely screened for in workplace or standard hospital drug tests, and so their true prevalence in the general

population is unknown. The chemical structures of SCs are distinct from Δ^9 -THC, therefore do not cross-react with routine cannabis urine immunoassay tests, which detect Δ^9 -THC and other THC metabolites. Some commercially available SC urine immunoassays are available, but have only been externally validated for the older compounds JWH-018, JWH-250, JWH-073, and AM-2201 (Barnes et al., 2014, 2015; Spinelli et al., 2015; Wohlfarth et al., 2015b; Kronstrand et al., 2014). Immunalysis has developed a urine homogenous enzyme immunoassay (HEIA) kit for detecting newer SCs in the PINACA, FUBINACA, ADBICA groups with their metabolites at a 10 ng/mL cutoff. Randox Toxicology also includes these newer indazole SCs for a total of 106 SCs and their metabolites in their Drugs of Abuse V panel utilizing a semiquantitative biochip immunoassay technique. Neither of these immunoassays is externally validated.

The commercially available immunoassays use 10 ng/mL as their usual detection cutoff. Validation studies note that a lower cutoff of 5 ng/mL improves test sensitivity (Barnes et al., 2015). The immunoassays with the highest sensitivity and specificity, as compared to the gold standard, mass spectrometry confirmation, were able to detect parent drug as well as at least 2 metabolites (Wohlfarth et al., 2015b). Metabolite detection is key, since SC parent compounds are unstable and degrade, especially if stored at room temperature or subjected to multiple freeze-thaw cycles (Wohlfarth et al., 2015b). It may also be that parent drug is not excreted into urine for all SCs, making urine detection difficult. Because SCs are a rapidly moving target, with multiple new drugs appearing on the market every year, it is difficult to develop immunoassay tests fast enough to keep up with the changing compounds. In addition, they do not undergo uniform metabolism, making it difficult to predict all the metabolites that are needed for analytical confirmation.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) and liquid chromatography high resolution mass spectrometry (LC-HRMS) are necessary for confirmation of SCs. Gas chromatography mass spectrometry (GC-MS), although sometimes utilized, may lead to occasional misidentification because some analogs are conformation isomers or regioisomers, with similar mass spectra. Interestingly, cyclopropyl SCs are unstable in heat, degrading when injected into the GC port (Namera et al., 2015). Most importantly, in order to identify a compound by GC-MS or LC-MS/MS, spectra needs to be compared to already existing databases and characterized compounds. These modalities aren't useful when trying to detect a novel compound. Nontargeted testing can be done with the various LC-HRMS modalities, including LC-TOF/MS and LC-QTOF/MS (liquid chromatography-quadrupole time-of-flight mass spectrometry).

In this case report, our lab's SC panel did not include any newer generation SCs, and the possible compounds AB-PINACA and ADB-PINACA were identified in serum through nontargeted testing. Standards of these 2 compounds as well as their pentanoic acid metabolites were obtained and used to confirm their presence using LC-TOF/MS. Even with LC-HRMS, metabolites are ideally needed for confirmation of the SC. In this case, metabolites were already available from a reference compound company. However, that is not always the case, especially with the newest SCs. Another concern is the convergence of metabolic pathways, which would be a problem if a test only looked for one metabolite. For example, AB-PINACA N-Pentanoic Acid is a metabolite of both AB-PINACA and 5F-AB-PINACA. LC-HRMS is ideal to look for multiple parent drugs and their metabolites simultaneously. Other advantages of LC-HRMS include less labor to include new analytes, smaller number of validation experiments and flexibility in methodology (Kronstrand et al., 2014). Unfortunately, this testing modality is not available in real-time in hospitals and is only available at a few specialized commercial and research laboratories.

3.5. Care of the poisoned patient

3.5.1. Clinical presentation

SCs cause more significant clinical adverse events than marijuana. Similar to both is the anxiety and paranoia that can occasionally occur. Clinically though, it is best to characterize SCs as stimulants, since they lead to tachycardia, hypertension, hyperthermia, diaphoresis, generalized tonic clonic seizures, agitation, and delirium, similar to other CNS stimulant drugs. If agitation and seizures are uncontrolled they can lead to hyperthermia and rhabdomyolysis, which can cause end-organ damage to the brain, kidneys, liver and coagulation system (Tait et al., 2016; Castaneto et al., 2014). Even without prolonged agitation, acute kidney injury (AKI) has been noted with some specific compounds, such as the 16 cases of AKI from XLR-11 in 2012 (CDC, 2013a). In that case cluster, 15/16 patients were male and 12/16 had abdomen/back/flank pain. Peak serum creatinine ranged from 3.3 to 21 mg/dL (median: 6.7 mg/dL; normal: 0.6–1.3) and 5/16 required hemodialysis. In other reported outbreak clusters, the most common symptoms were lethargy, agitation, intermittent lethargy and agitation, aggressive behavior, confusion, tachycardia, hypokalemia, hyperglycemia, vomiting, and tonic-clonic seizures (CDC, 2013b; Drenzek et al., 2013; Kasper et al., 2015; Law et al., 2015; Springer et al., 2016). During the 2015 case increase, US poison control centers fielded 1501 cases regarding SCs, of which 81% were male and 11% were noted to have a major adverse event. Males or those more than 30 years old were more likely to have a severe outcome (Law et al., 2015). In these case clusters, intensive care unit (ICU) admission rates were from 8 to 27%.

Physical symptoms and exam findings also may include mydriasis, injected conjunctivae, nausea, vomiting, slurred speech, shortness of breath, and chest pain. Rarely, myocardial infarction and ischemic strokes have been reported. 8 cases of myocardial infarction with troponin elevation and abnormal ECGs have been reported to date, all in adolescent boys ranging from 14 to 17 years old (Clark et al., 2015; McKeever et al., 2015; Mir et al., 2011; Zaleta et al., 2016). These cases were attributed to the synthetic cannabinoid K2, and none of them included confirmatory laboratory testing for SCs. In our case, the patient had a normal ECG and echocardiogram despite a high troponin level. Laboratory findings are generally nonspecific and may include mild leukocytosis, hypokalemia, and hyperglycemia, which can all be seen with stimulant drugs (Castaneto et al., 2014). Deaths from SC are rare, but have been reported in the literature, due to dysrhythmias, status epilepticus, liver failure, renal failure, and from traumatic injury (i.e. jumping from a building, self-injury) (Tait et al., 2016). A withdrawal syndrome has been described among daily users, characterized by increased craving of the drug, tachycardia, hypertension, nausea, diaphoresis, nightmares and muscle twitches. No severe morbidity or mortality has been reported from withdrawal (Castaneto et al., 2014; Zimmermann et al., 2009).

There are only a few clinical reports specific to the drugs found in our patients. In 2013, 263 possible cases with 76 patients presenting to local teaching hospital EDs in Colorado were attributed to ADB-PINACA (Monte et al., 2014). Available product was analyzed and no biological specimens were tested in this case series. Altered mental status, tachycardia followed by bradycardia, and seizures were the primary clinical manifestations with 7 patients admitted to ICUs. In a case series of 9 patients, 7 were confirmed to be exposed to ADB-PINACA through biological specimen testing. These patients primarily presented with agitated delirium, psychosis, aggression and seizures. Four required endotracheal intubation and ICU admission, of which one had an ST-elevation myocardial infarction requiring balloon angioplasty of the left anterior descending coronary artery (Schwartz et al., 2015).

In a 10 month old girl with respiratory depression requiring intubation, AB-PINACA and its pentanoic acid metabolite were identified (Thornton et al., 2015).

3.5.2. Treatment

There is no specific antidote for SC intoxication. Rather, treatment is primarily symptomatic, with airway protection and control of agitated delirium the key initial steps in caring for the intoxicated patient. Two of the three patients treated at our hospital were intubated, and both extubated within 12 h. Similar to intoxications from stimulant drugs, SC intoxication is primarily treated with benzodiazepines as the first-line agent (Cooper, 2016). Midazolam, lorazepam, or diazepam may be used, preferably IV, with larger doses given IM if an IV is unavailable. Clinically, we have noticed that occasional use of an antipsychotic agent such as haloperidol offers control of agitation and psychosis when large doses of benzodiazepines are given without effect. IV fluids may be used to correct acidosis and hyperglycemia. Supplemental potassium may be needed to correct hypokalemia. For the patient with tachycardia or chest pain, an electrocardiogram (ECG) is recommended. Antiemetics may be needed to control nausea and vomiting. In the case of seizures, we recommend nonspecific sedative-hypnotic agents such as benzodiazepines, barbiturates and propofol. Specific anti-epileptic medications such as phenytoin or levetiracetam, may not affect drug-induced seizures (Chen et al., 2016). After acute intoxication, mental status typically improves within a few hours, sometimes as long as 24 h. There is no set observation period for these patients, but once they are back to their baseline mental status and have normal vital signs, it is acceptable to discharge them. Treatment of withdrawal is also supportive, with benzodiazepines, antiemetics, and other symptomatic care (Castaneto et al., 2014; Zimmermann et al., 2009).

4. Conclusion

The novel combination of AB-PINACA and ADP-PINACA were identified in this case series of patients who manifested typical signs of SC toxicity, including agitation, confusion, lethargy, seizure, and hypertension. Tachycardia, hyperthermia, and psychosis are also common symptoms of SC toxicity from full CB1 and CB2 receptor agonism. LC-TOF/MS and knowledge of SC metabolites was necessary for analysis, since these compounds are not included on any standard toxicology testing and at the time, were not included in our laboratory SC panel due to their novel nature. New compounds are proliferating at a rapid rate, with the legal, law enforcement, analytical, and medical communities struggling to keep up. All parties involved must maintain a high level of vigilance in order for proper diagnosis, analysis, and law enforcement to take place.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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