Basic Scientific Research to Support Forensic Science for Criminal Justice Purposes

Characterization of Designer Drugs: Chemical Stability, Exposure, and Metabolite Identification

Final Summary Overview

Submitted via Grants.gov to:
U.S. Department of Justice
Office of Justice Programs
National Institute of Justice
810 Seventh St., NW
Washington, DC 20531

Prepared by:
Megan Grabenauer, Katherine N. Moore, Brian F. Thomas
RTI International
3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

April 30, 2015

Administrative Point of Contact:
Alicia D. Brown
aliciab@rti.org
Phone: 919-541-8826
Fax: 919-541-6624

NIJ Award No. 2012-R2-CX-K001
RTI Proposal No. 020281200.475
RTI Project No. 0213484.000

RTI INTERNATIONAL

Purpose

Designer drugs such as synthetic cannabinoids and cathinones have become increasingly prevalent, as have their health and societal consequences. Most forensic laboratories are not equipped with the analytical research capabilities required to keep up with the rapid turnover of designer drugs being marketed for recreational use. Currently, little is known about the pharmacological and toxicological profiles of these products; the consequences of long-term usage have yet to be studied, and behavioral and metabolic studies have only been performed on a relatively limited number of compounds. Detection of these designer drugs remains a challenge because as bans on specific compounds go into effect, manufacturers rapidly substitute closely related analogs for the newly banned substances, creating a constantly moving analytical target.

The objective of this research is to gain a more thorough understanding of designer drugs with respect to their chemical exposure profiles and biological elimination pathways. The goals of this project were to 1) determine the stability of currently popular designer drugs and identify major degradation products, including pyrolysis products, and 2) identify their major metabolites. In January 2014 the original scope was expanded to include CB1 and CB2 receptor binding studies to determine activity and efficacy of select synthetic cannabinoids containing a tetramethyl cyclopropyl group and their common ring open degradant forms.

Project Design

The project initially focused on compounds from the JWH and AM series of synthetic cannabinoids and expanded to include emerging designer drugs as they became prevalent. Compounds for analysis were chosen by monitoring multiple sources including online user forums, the Forendex Forums, Designer Drugs Online News, which provides notifications on new compounds discovered by European customs agencies, and through discussions with local law

enforcement and forensic laboratory practitioners. For a complete list of compounds analyzed and their structures see Table A-1 in the appendix. Additional identifying information about each compound is listed in Table A-2.

Designer drug compounds were procured from multiple sources (see methods section) and GC-MS spectra were acquired and uploaded to Forendex (http://forendex.southernforensic.org). Compounds underwent one or more of the following analyses: herbal formulations prepared and smoke condensate analyzed, automated pyrolysis at 800˚C, automated pyrolysis at variable temperatures (200-800 ˚C), stability assessment/forced degradation, in vitro metabolism, in vivo metabolism and characterization of urinary metabolites, and cannabinoid (CB1 and CB2) receptor binding. See Table A-3 for a list of which analyses were completed for each compound.

Methods

Additional methodological details are in the appendix.

Stability/Forced Degradation

Stock solutions were prepared in acetonitrile or methanol at concentrations of 0.5-1 mg/mL. Stability samples were prepared by mixing stock solution with water, acidic, basic, and oxidizing solutions (1:1), which were then placed in an environmentally-controlled stability chamber at 25 °C/60 % relative humidity for 24, 48, 72, and 96 hours. Additional samples of the same composition in water were created and stored under elevated temperatures (50 °C ±5).

Metabolism

In vitro

Compounds (10 µM) were incubated at 37 °C in cryopreserved human hepatocytes (pool of 10 donors), with the exception of PB-22 and 5F-PB-22, which were incubated at 100 µM, and JWH-018 and AM2201 at 50 µM . An aliquot of 100 µL was removed and quenched with acetonitrile

containing 0.2% acetic acid at 0, 15, 120, and 180 min (JWH-018, AM2201, PB-22 and 5F-PB-22 stopped at 120 min). A portion of each aliquot was hydrolyzed with Abalone β-D-glucuronidase (≥ 5,000 units).

In vivo

Mice dosed as part of an independent behavioral study were placed in metabolism cages and urine was collected over 24 hrs. Urine from animals dosed with the same compounds was pooled together and extracted using a salting out liquid-liquid extraction (SALLE) method prior to analysis. A portion of each urine pool was hydrolyzed using Abalone β-D-glucuronidase (≥ 5,000 units).

Liquid Chromatography-Mass Spectrometry (LC-MS)

Samples were analyzed using a Waters Synapt G2 HDMS quadrupole time of flight (Q-TOF) mass spectrometer interfaced to a Waters Acquity ultra performance liquid chromatography (UPLC) system (Waters, Milford, MA). Leucine enkephalin was used as a lockmass to correct for mass shifts during acquisition. Liquid chromatography was performed on an Acquity BEH C18 column (1.7 µm 2.1 x 50 mm) connected to a Vanguard BEH C18 pre-column (1.7 µm x 2.1 X 5 mm) and held at 30 °C. Acquired data was analyzed using Waters MassLynx 4.1 with the aid of the Metabolynx application manager. Parent and metabolite reference standards were used for verification when available.

Pyrolysis

An Agilent 7890 gas chromatograph coupled to a 7001B MSD mass selective detector (Agilent Technologies; Santa Clara, CA) controlled by Agilent Masshunter (version B.05.02.1032) software was used for all analyses. A CDS Analytical 5250T pyrolysis autosampler (Oxford, PA) plumbed with independent supplies of helium and another of compressed air (zero grade) was employed to pyrolyze each sample and transfer the products to the gas chromatograph.

An Agilent DB-5MS capillary column (30 m x 0.25 mm x 0.25 μm, Agilent Technologies) was used to separate volatile analytes with a helium carrier gas flow of 1.0 mL/min. Pyroylsis at 800 ˚C was carried out by setting a starting temperature of 50 ˚C. After one second the pyrolysis probe was ramped at 20˚C/sec to 800˚C. An alternate pyrolyzer method was developed to perform pyrolysis at variable temperatures ranging from 200 to 800 ˚C.

Receptor Binding

Affinity at CB_1 and CB_2 receptors was measured using competitive displacement of [^{3}H]-CP55,940. Efficacy was determined using G-protein coupled signal transduction (GTP-γ-[^{35}S]) binding assays.

Results

Detailed results will be disseminated through peer-reviewed publications. For completeness, overall summary tables are provided in the appendix to this Final Summary Overview.

Stability/Forced Degradation

The Stability/Forced degradation results are summarized in Table A-5. The data suggest that for compounds with a tetramethyl cyclopropyl group (e.g. UR-144) exposure to elevated temperatures is a pivotal factor in the ring-opening conversion of the cyclopropyl ring. Synthetic cannabinoid compounds with ester linkages (e.g., PB-22) are susceptible to rapid hydrolysis and transesterification proceeds rapidly in the presence of methanol.

Pyrolysis

Full pyrolysis results at 800˚C are summarized in Table A-6. Of the synthetic cannabinoids studied, only four retained more than 80% of the original dose after pyrolysis: JWH-018 (91%), JWH-018 adamantyl analog (85%), JWH-019 (84%), and THJ-018 (98%). All ester containing synthetic cannabinoids underwent significant thermally-induced structural changes resulting in less

than 5% retention of the original dose: PB-22 (0%), 5F-PB-22 (0%), BB-22 (0%), A-834735 (2%), FDU-PB-22 (3%), and FUB-PB-22 (0%). For all synthetic cannabinoids containing a teramethylcyclopropyl ring substituent, greater than 50% of the original dose was converted to a ring opened form of the parent compound. Dehalogenation was commonly observed for halogen containing compounds. It is likely that users are unknowingly being exposed to novel synthetic cannabinoid structures from thermal degradation and pyrolysis, which may have unique pharmacological properties from the original chemical entities.

Metabolism

ADBICA, ADB-FUBINACA, AB-FUBINACA, ADB-PINACA, AB-PINACA, 5F-AB-PINACA, and 5Cl-AB-PINACA in vitro metabolites

Major metabolic transformations of all of the 1-amino-3-methyl-1-oxobutan-2-yl (AB) and 1-amino-3,3-dimethyl-1-oxobutan-2-yl (ADB) indazole carboxamide compounds studied are hydrolysis of the distal amide and hydrolysis of the distal amide followed by glucuronide conjugation. (See Table A-7). ADBICA was the only 1-amino-3,3-dimethyl-1-oxobutan-2-yl (ADB) carboxamide compound for which the amide hydrolysis and its glucuronide conjugate were not observed. Unlike the other compounds in Table A-7, ADBICA contains an indole moiety rather than an indazole moiety. It is unknown if this may contribute to the lack of amide hydrolysis as we do not have data on any other indole carboxamide compounds for comparison. Unlike with other metabolites identified in this study, the metabolite resulting from amide hydrolysis elutes after the parent compound while its glucuronide conjugate elutes earlier than the parent compound. Also, n-dealkylation was observed with ADBICA but not the other carboxamide compounds. Again, it is unclear if the indole versus indazole moiety plays a role in this. N-dealkylation was not observed with the compounds in Table A-10 (THJ type compounds) and these also contain an indazole moiety. Compounds with a halogen group (Cl or F) on the terminal alky chain underwent

dehalogenation forming non-unique metabolites. AB-FUBINACA and ADB-FUBINACA were similar to other 4-fluorobenzyl compounds FDU-PB-22 and FUB-PB-22 in that no defluorination was observed.

In vitro metabolites of PB-22, 5-F-PB-22, BB-22, FDU-PB-22, and FUB-PB-22 and in vivo metabolites of PB-22 3-carboxyindole

Major metabolites for all 3-indole ester-linked compounds studied were the 3-carboxyindole and its glucuronide conjugate (see Table A-8). For PB-22, 5-F-PB-22 and BB-22 the presence of 3-carboxyindole may provide evidence that a compound from this class was ingested, but care should be taken to look for other distinctive metabolites because of other compounds that could lead to the 3-carboxyindole metabolite. An example of this is shown with FDU-PB-22 and FUB-PB-22 where the same 3-carboxyindole is identified as a metabolite. Interestingly, FDU-PB-22 and FUB-PB-22 do not undergo defluorination as seen with compounds with terminal fluoroalkyl side chains. They also do not hydroxylate on the 4-fluorobenzyl indole moiety as seen in similar compounds. 5-F-PB-22 undergoes defluorination subsequently forming several metabolites in common with PB-22 metabolites. PB-22 4-hydroxypentyl and PB-22 5-hydroxypentyl co-eluted with the LC method utilized, but at least one of these metabolites was observed for both PB-22 and 5F-PB-22 (defluorinated).

SDB-006, JWH-018 adamantyl analog and JWH-018 adamantyl carboxamide in vitro metabolites

In vitro metabolites for the compounds listed above are summarized in Table A-9. For all compounds, hydroxylation and hydroxylation followed by glucuronide conjugation were the primary metabolic transformations. Both JWH-018 adamantyl analog and JWH-018 adamantyl carboxamide are hydroxylated and glucuronide conjugated on both the adamantly moiety and the 1-pentyl-1*H*-indole moiety, with hydroxylation on the adamantly moiety being the dominant metabolite. Note that beyond hydroxylation, the metabolites for JWH-018 adamantyl analog were low intensity and

characteristic fragment ions were not observed. SDB-006 was the only compound in this table to form a dihydrodiol. This was a minor metabolite and fragment intensity in the high energy mass spectrum was not sufficient to determine the exact location of the modification. SDB-006 was also the only compound to form 3-carboxamide indole.

THJ, 5F-THJ, THJ-018, THJ2201, and AM2201 benzimidazole analog in vitro metabolites and in vivo metabolites for AM2201 benzimidazole analog excreted in urine

All five of these compounds underwent hydroxylation and glucuronide conjugation (Table A-10) which is common among this class of new designer cannabinoids as well as in previous reports for the original JWH type compounds. These THJ type compounds all also form dihydrodiols, regardless of whether they are naphthalene or quinonline type compounds. This is similar to reports in the literature for the original JWH type compounds that typically have a naphthalene moiety. However, in the THJ type compounds, these are only minor metabolites. Like all of the compounds in this study, the parent compound was detected in the in vitro samples even at 3h. The fluorinated compounds underwent defluorination forming THJ and THJ-018 metabolites. Carboxylation of THJ was not observed, even when searching for common fragment ions, but it was observed in 5F-THJ after defluorination. Interestingly, for THJ2201 and AM2201 benzimidazole analog we observe what could possibly be saturation at the carbonyl, which was not detected for any of the other compounds with similar structures. More investigation into this is needed. Also, N-dealkylation of the pentyl chain was not observed for these compounds. It may be due to the presence of an indazole moiety versus an indole moiety, or since it is typically a minor uncharacteristic metabolite, the intensity may be too low to observe in these cases.

Cyclopropyl ketone indole in vitro metabolites for degradant and non-degradant compounds and in vivo metabolites for the degradant compounds.

As summarized in Table A-11, all of these compounds undergo hydroxylation followed by glucuronidation. Other oxidative metabolites such as dihydroxlation, and carboxylation were also observed. Hydoxylation occurs on the 1-(tetrahydro-2*H*-pyran-4-ylmethyl)-1*H*-indole unlike the 1-(4-fluorobenzyl)-1-*H*-indole compounds (e.g. ADB-FUBINACA, AB-FUBINACA, FDU-PB-22 and FUB-PB-22) that do not form hydroxylations on that moiety. UR-144, XLR-11, and their degradants underwent N-dealkylation of the alky pentyl and 5-fluoro alky pentyl side chain. Care must be taken when choosing distinctive biotransformations to monitor for the presence of these compounds since XLR-11 and its degradant undergo defluorination forming UR-144 and UR-144 degradant specific metabolites.

Receptor Binding

CB_1 and CB_2 receptor affinities of the ring-open degradants of XLR-11 and UR-144 were determined. These ring-opened analogs retained nM affinity and act as full agonists at both the CB_1 and CB_2 receptors, as do their non-degraded, parent compounds. However, the thermal degradation product and biological metabolite observed with PB-22 (1-pentyl-1H-indole-3-carboxylic acid) was unable to alter GTP-γ-S binding at doses up 1000-fold higher than active concentrations of PB-22. The XLR-11 degradant displaced [^{3}H]CP55,940 from the CB_1 receptor, with an apparent Ki of 5.0 ± 0.57 nM and from the CB_2 receptor with an apparent Ki of 0.9 ± 0.13 nM. The UR-144 degradant displaced [^{3}H]CP55,940 from the CB_1 receptor with an apparent K_i of 11.23 ± 2.54 nM and from the CB_2 receptor with an apparent K_i of 1.54 ± 0.21 nM. Notably, the degradants of each compound showed at least 2-fold lower affinity for both cannabinoid receptors than shown previously for the parent compounds, which in turn, had at least 2-fold lower affinity than Δ^9-THC. Because smoking or vaporizing results in increased exposure to the ring opened degradant compounds compared to the

parent compounds, cannabimimetic potency is predicted to be greater in human users than the CB_1 affinities of XLR-11 and UR-144 would suggest.

Scholarly Products

Detailed results have been presented within 8 conference presentations. See table A-12 in the Appendix for full list of these presentations.

Publications

Wiley, JL, Marusich, JA, Lefever, TW, Grabenauer, M, Moore, KN, Thomas, BF. 2013. Cannabinoids in Disguise: Δ^9-Tetrahydrocannabinol-Like Effects of Tetramethylcyclopropyl Ketone Indoles. *Neuropharmacology*. 75, 145-154

Planned Publications

- Wiley, JL, Marusich, JA, Lefever, TW, Antonazzo, KR, Wallgren, MT, Cortes, RA, Patel, PR, Grabenauer, M, Moore, KN, Thomas, BF. 2015 All That Glitters Is Not (Spice) Gold: Dissociation Between Affinity and Potency of Novel Synthetic Cannabinoids in Producing Δ^9-Tetrahydrocannabinol-Like Effects in Mice. Submitted 4/2015 to Journal of Pharmacology and Experimental Therapeutics.

- Manuscript joint with Jenny Wiley's group with mouse in vivo metabolites of JWH-073, JWH-018, JWH-081, JWH-391, JWH-210, AM-2201, JWH-167, combined with behavioral data from the dosed animals.

- Manuscript on 5F-AKB48 found in Spice samples from Richland County. Homogeneity of packages of samples. This manuscript is in the final stages of editing prior to submission to the Journal of Forensic Sciences (JFS).

- Manuscript on pyrolysis survival and binding affinity for UR-144, XLR-11, PB-22 (content of poster presented at SOFT). This manuscript will be targeted for a high profile journal with a more general audience such as the Journal of the American Chemical Society (JACS).

- Manuscript on metabolites of structurally related synthetic cannabinoids (content of poster presented at SOFT) This manuscript will be targeted for the forensic toxicology community specifically, with a planned submission to the Journal of Analytical Toxicology (JAT).

- Manuscript comparing mouse in vivo metabolites and human hepatocyte in vitro metabolites. This manuscript will be targeted for a more general analytical chemistry audience such as the readership of Analytical Chemistry (Anal Chem).

Implications for Policy and Practice

Through this research we identified metabolites suitable as potential markers of use, degradation products, and pyrolysis products that may be left in an ash residue to use for confirmation of the presence of parent compound. The dataset is extensive and is a reliable starting point for forensic laboratories across the United States to develop assays for detection of use, as well as confirmation of the presence of parent compound within residues. New designer drugs are still coming to market, faster than targeted testing can keep up. However, within each class of designer drugs, the elements of chemical structure and design often follow known or rational substitution patterns required to enhance or retain pharmacological activity. By performing a thorough and systematic study looking at families of structurally related compounds, we are able to predict markers for broad classes of compounds, such as aminoalkylindole cannabinoids or phenethylamines, and help practitioners keep up with designer drug manufacturers.

We have shown that depending on the sample preparation technique and route of administration, the compound a person is exposed to may not be the compound originally intended. We have provided the forensic toxicology community with information about known degradation and pyrolysis products and major metabolites that should be included as candidates when searching for markers of use. Armed with this knowledge, these compounds are less likely to go undetected. Knowing conditions under which compounds are likely to degrade will impact sample handling, extraction, and analytical methods employed in sample preparation and forensic analyses, especially for compounds that were found to be particularly labile.