Metabolism and Toxicological Analysis of Synthetic Cannabinoids in Biological Fluids and Tissues

B. C. Presley[1,2], S. M. R. Gurney[3], K. S. Scott[4], S. L. Kacinko[1], B. K. Logan[1,2,4,5]

[1]NMS Labs
Willow Grove, Pennsylvania
United States of America

[3] Department of Biology, Drexel University
Philadelphia, Pennsylvania
United States of America

[2] Department of Chemistry, Temple University
Philadelphia, Pennsylvania
United States of America

[4] Forensic Science, Arcadia University
Glenside, Pennsylvania
United States of America

[5]Center for Forensic Science Research and Education
Fredric Rieders Family Foundation
Willow Grove, Pennsylvania
United States of America

TABLE OF CONTENTS

* Corresponding author: Dr. Barry K. Logan, NMS Labs, Willow Grove, PA 19090; +1 215 366 1513 (voice); barry.logan@nmslabs.com.

1042-7201/28-2/July 2016/103–169/$100.50 •

Metabolism and Toxicological Analysis of Synthetic Cannabinoids in Biological Fluids and Tissues

REFERENCE: Presley BC, Gurney SMR, Scott KS, Kacinko SL, Logan BK: Metabolism and toxicological analysis of synthetic cannabinoids in biological fluids and tissues; *Forensic Sci Rev* 28:103; 2016.

ABSTRACT: Synthetic cannabinoids, which began proliferating in the United States in 2009, have gone through numerous iterations of modification to their chemical structures. More recent generations of compounds have been associated with significant adverse outcomes following use, including cognitive and psychomotor impairment, seizures, psychosis, tissue injury and death. These effects increase the urgency for forensic and public health laboratories to develop methods for the detection and identification of novel substances, and apply these to the determination of their metabolism and disposition in biological samples. This comprehensive review describes the history of the appearance of the drugs in the United States, discusses the naming conventions emerging to designate new structures, and describes the most prominent new compounds linked to the adverse effects now associated with their use. We review in depth the metabolic pathways that have been elucidated for the major members of each of the prevalent synthetic cannabinoid drug subclasses, the enzyme systems responsible for their metabolism, and the use of *in silico* approaches to assist in predicting and identifying the metabolites of novel compounds and drug subclasses that will continue to appear. Finally, we review and critique analytical methods applied to the detection of the drugs and their metabolites, including immunoassay screening, and liquid chromatography mass spectrometry confirmatory techniques applied to urine, serum, whole blood, oral fluid, hair, and tissues.

KEYWORDS: Designer drugs, drug metabolism and disposition, synthetic cannabinoids, synthetic drug scheduling, toxicological analysis.

Introduction

Initially thought to be a temporary distraction and a novelty, synthetic cannabinoid use in the United States and internationally has grown and diversified over the last seven years to become a discrete and challenging part of the recreational drug use market. The diversity of members of this drug class has expanded rapidly, with over 130 different chemical substances in this class being tracked by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) [39]. In the United States, the National Forensic Laboratory Information System (NFLIS) continues to report increases in the numbers of synthetic cannabinoid cases submitted to crime laboratories for analysis, with almost half the of most common substances reported in mid-2014 having appeared on the list only this year (2015) [167].

The practice in many crime laboratories of not reporting noncontrolled substances means that these data most likely represent a significant underestimate. In addition, the medical and scientific literature is replete with increasing reports of adverse health effects associated with this untested drug class. In early 2014, adverse event reports, including emergency room admissions, impaired driving arrests, psychotic reactions, cardiac events, seizures, and acute kidney injury associated with synthetic cannabinoids were reviewed [55], as well as two sentinel cases of fatalities associated with the drugs. In the last year there have been numerous additional reports of serious adverse events, including more traffic crashes [1,76,98], mass hospitalizations [96,148], psychotic reactions [106,123], cardiac arrest [25,68], and many more deaths [8,127,148,149,172]. This increased reporting reflects the fact that more toxicological testing resources have become available to emergency medical providers, medical examiners, and law enforcement; however, the challenges of matching analytical resources to the needs of public health and public safety agencies have never been greater.

The accelerating pace at which these drugs are reaching the market, their increased potency, and the complexity required for their chemical analysis lead to greater delays in identifying and controlling dangerous substances. In addition, the time lag between new drugs appearing on the street and laboratories developing the capabilities to detect them contributes to an underestimation of the scope of the problem, and thus failure to address the needs of the criminal justice and treatment communities.

In an earlier and complementary review, we considered the pharmacology, receptor binding, toxicological effects, adverse-event profile, human health effects, and forensic implications of use of synthetic cannabinoids on users [55]. That review described the discovery and characterization of the endocannabinoid system, as well as receptor-binding studies of various synthetic cannabinoids from the first wave of naphthoylindoles (e.g., JWH-018) to the emerging indazole carboxamide drugs (e.g., AKB48), and their analogs. The physiological effects of activation of the

ABOUT THE AUTHORS

B. C. Presley; S. M. R. Gurney
K. S. Scott; S. L. Kacinko; B. K. Logan

Brandon C. Presley earned his bachelor's degree in chemistry from Temple University (Philadelphia, PA) in 2010. Mr. Presley is currently a Ph.D. candidate studying analytical chemistry at Temple University and is also employed as a forensic chemist at NMS Labs (Willow Grove, PA). He has worked previously in the clinical and forensic toxicology laboratory at NMS Labs and also participated in an abuse-deterrent formulations study to determine the ease of tampering and abuse of pharmaceutical preparations by drug users.

Mr. Presley is currently a Future Faculty Fellow at Temple University and has also served as a graduate teaching assistant for an undergraduate research course. He is a member of the American Chemical Society (ACS) and the American Academy of Forensic Sciences (AAFS) and is a Project Task Group member of the International Union of Pure and Applied Chemistry (IUPAC) for a project on novel psychoactive substances. Mr. Presley's research interests include the metabolism and analysis of drug molecules in biological matrices as well as determining quantitative structure-activity relationships (QSAR) and retention relationships (QSRR) of various drug classes.

Research that Mr. Presley has conducted includes the analysis of prostaglandins and thromboxanes as inflammatory biomarkers for rheumatoid arthritis by HPLC; synthesis of heterocyclic indole derivatives for use as inhibitors in disease modeling; synthesis and characterization of transition-metal complexes using benzenedithiol for use in scintillators. He has given a number of research presentations at various locations including the IUPAC Chemistry World Congress; Southwestern Association of Forensic Scientists and Northeastern Association of Forensic Scientists regional meetings; Drexel University, Eastern Analytical Symposium; ACS national meetings, and the Pennsylvania Senate and House of Representatives.

Susan M. R. Gurney earned a bachelor's degree (2000) in cell biology and pathology from the University of St. Andrews (St. Andrews, UK), an M.Sc. in forensic science (2007) from Anglia Ruskin University (Cambridge, UK), an M.Sc. in molecular biology (2011) from the University of Cambridge (Cambridge, UK), and a Ph.D. in genetics (2012) from the University of Münster (Münster, Germany). Dr. Gurney is an assistant teaching professor in the Department of Biology, Drexel University (Philadelphia, PA). Courses taught at Drexel University include forensic toxicology, forensic biology, and advanced immunology.

Dr. Gurney's doctoral research involved examining human and equid mitochondrial DNA for forensic and evolutionary applications, and she continues to pursue new research developments on these topics. In 2011–12, Dr. Gurney carried out an internship in forensic toxicology in the Department of Forensic Medicine and Science at Glasgow University (Glasgow, UK), performing analyses on biological samples provided as forensic casework and specializing in LC-MS/MS. Since 2012, Dr. Gurney has worked as a co-director for Roots for Real (Fluxus Technology: Cambridge, UK), a DNA ancestry service that performs mitochondrial and Y-chromosomal profiling.

Karen S. Scott has a bachelor of science (honors) degree (1994) in forensic and analytical chemistry from the University of Strathclyde (Glasgow, UK) and a Ph.D. (1998) in forensic toxicology from the University of Glasgow (Glasgow, UK). Dr. Scott has been director of the Forensic Science Program and an associate professor at Arcadia University (Glenside, PA) since October 2012. On completion of her degrees, Dr. Scott carried out postdoctoral research in Tokyo, Japan, investigating incorporation rates and detection of drugs in hair. Prior to joining Arcadia University, Dr. Scott held the position of senior lecturer and consultant forensic toxicologist at Forensic Medicine and Science, University of Glasgow.

She has over 18 years of experience in the fields of forensic and clinical toxicology. Dr. Scott has published in the areas of postmortem toxicology and hair and alternative matrix testing and is a reviewer for three of the main forensic toxicology journals. She is a chartered scientist, a chartered chemist, and an authorized analyst for the purposes of Section 16 of the Road Traffic Offenders Act (UK).

Sherri L. Kacinko earned her bachelor of science degree in chemistry at the University of Pittsburgh (Johnstown, PA) and took graduate classes in forensic science at George Washington University (Washington, DC). She received her Ph.D. in toxicology at the University of Maryland (Baltimore, MD). Dr. Kacinko is now a toxicologist at NMS Labs and an adjunct faculty member in the chemistry department at Arcadia University.

Upon finishing her undergraduate education, Dr. Kacinko performed pharmaceutical quality control at Lancaster Laboratories (Lancaster, PA), then worked for three years as a crime laboratory analyst in the chemistry section of the Florida Department of Law Enforcement's Orlando Regional Operations Center (Orlando, FL). Dr. Kacinko is board certified by the American Board of Forensic Toxicologists (ABFT). In recognition of her work and contributions, Dr. Kacinko has received the AAFS Irving Sunshine Award.

Barry K. Logan earned his bachelor's degree (1982) in chemistry and Ph.D. degree (1986) in forensic toxicology from the University of Glasgow (Glasgow, UK). Dr. Logan is vice president of forensic sciences and chief of forensic toxicology at NMS Labs in Willow Grove, PA, where his responsibilities include management of toxicology resources, new test design and development, and expert testimony in forensic toxicology and chemistry. He is frequently consulted as an expert in death investigation and impaired driving and vehicular crimes cases.

He is a Fellow of the ABFT and has over 100 publications in toxicology and analytical chemistry, including work on the effects of drugs and driving impairment, and cause and manner of death for a wide range of drugs and toxins. His recent work has focused on the analytical and interpretive toxicology of emerging recreational and designer drugs.

Dr. Logan's other appointments include executive director of the Robert F. Borkenstein course at Indiana University (Bloomington, IN), and executive director at the Center for Forensic Science Research and Education at the Fredric Rieders Family Foundation in suburban Philadelphia. He also holds academic appointments at Indiana University, Arcadia University, Thomas Jefferson University (Philadelphia, PA), and Temple University, and he oversees a variety of research initiatives with academic institutions and medical examiners' offices.

In recognition of his work and contributions, Dr. Logan has received numerous national and international awards including the AAFS Rolla N. Harger Award, the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) Widmark Award, the National Safety Council's Robert F. Borkenstein Award; in 2013–14 he served as president of the AAFS.