K27 Metabolic Profiling of the Synthetic Cannabinoid AB-FUBINACA Using an Electrochemical Cell, Human Liver Microsomes, Cryopreserved Hepatocytes, and Liquid Chromatography/High Resolution Mass Spectrometry (LC/HRMS)

Eduardo M. Cardoso, MSc, Federal Police of Brazil, Minas Gerais 30441-170, BRAZIL*

Learning Overview: After attending this presentation, attendees will better understand the challenges associated with Synthetic Cannabinoids (SCs) analyses and the different approaches to tackle the problem. The goal of this presentation is to assess electrochemical cell forced oxidation as a suitable tool for metabolite investigation and to identify major and specific biomarkers to unequivocally confirm intake of the synthetic cannabinoid AB-FUBINACA.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by presenting an unusual tool as a complementary technique for the characterization of metabolic pathways of new chemical entities.

In the present research, the goal is to characterize the phase I metabolism of the SC AB-FUBINACA, chosen due to recent prevalence studies.[1] *In vitro* metabolism was investigated using Human Liver Microsomes (HLM), Cryopreserved Human Hepatocytes (CHH) and forced electrochemical oxidation assays, and the metabolic entities separated and identified using LC/HRMS.

One of the main issues of SC analysis is the emergence of new compounds with unknown metabolic profiles, which means no urinary marker metabolites are known. Since the clandestine chemists' strategy is to make minor alterations on the pharmacophore core of the molecule, it is not uncommon to have the same metabolites coming from different parent SCs. For instance, 5'-OH-JWH-018 can derive from hydroxylation of the JWH-018 pentyl chain, but also from oxidative defluorination of AM-2201.[2] This is even more problematic when isomeric compounds are present with similar metabolic pathways, with one drug scheduled while its pair is not, such as the case with BIM-2201 (FUBIMINA) and THJ-2201 (each scheduled in the United States on different dates).[3]

With the data gathered in this research, a metabolic pathway was proposed. The incubation of AB-FUBINACA with HLMs in the presence of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) resulted in the formation of six metabolites, including two mono-hydroxylations (M1.1 and M1.2), the amide hydrolysis (M2), a dehydrogenation (M3), and two mono-hydroxylations of the amide hydrolysis (M4.1 and M4.2). The hepatocytes yielded similar results, but the prevalence of the metabolites changed, being mainly dominated by the amide hydrolysis and some related by-products. The Electrochemical Cell (EC) -forced oxidation displayed a range of multiple hydroxylations (mono, di, and tri), carbonylations/ epoxylations, and carboxylations, although no amide hydrolysis could be detected. Authentic urine samples confirmed that the hepatocytes displayed the closest correlation between *in vivo* and *in vitro* investigations. The electrochemical cell-forced oxidation proved to be a fast and cost-effective tool for the prediction of new chemical entities. However, it was not able to predict the main *in vivo* metabolic pathway, probably due to the conditions used in the experiment (low buffer strength, room temperature, and duration of the assay). Optimization of the EC assay conditions may lead to better results.

It seems that the amide hydrolysis (M2) and the glucuronidation of the amide hydrolysis (M5) are suitable urinary markers, but these are not specific markers for intake of AB-FUBINACA, since they can be formed by ester hydrolysis from AMB-FUBINACA or EMB-FUBINACA. A better biomarker would be a metabolite with intact terminal carboxamide groups, like M1.2, but it was only found in the *in vitro* experiments and not in the urine samples. Further *in vivo* investigations are suggested to confirm or refute M1.2 as a suitable marker and evaluate the influence of inter-individual variability.

Reference(s):

1. Advisory Council on the Misuse of Drugs. *Third Generation' Synthetic Cannabinoids 2014*. (Addendums March 2015, January 2016, July 2016, and November 2016).
2. Diao X., Huestis M.A. Approaches, Challenges, and Advances in Metabolism of New Synthetic Cannabinoids and Identification of Optimal Urinary Marker Metabolites. *Clinical Pharmacology & Therapeutics* 2017; 101(2): 239-253.
3. Diao X., Scheidweiler K.B., Wohlfarth A., Zhu M., Pang S., Huestis M.A. Strategies to Distinguish New Synthetic Cannabinoid FUBIMINA (BIM-2201) Intake From Its Isomer THJ-2201: Metabolism of FUBIMINA in Human Hepatocytes. *Forensic Toxicology* 2016; 34:256-267.

***In Vitro* Metabolism, Electrochemical Cell, Synthetic Cannabinoids**