E89 Cytochrome P450 and Chemical Oxidation of Synthetic Cannabinoids JWH-015 and Bay 59-3074

Oluseyi A. Vanderpuye, PhD, Forensic Science, 504 College Drive, Rm 118, Hartnett Bldg, Albany, GA 31705; Alexis K. Bailey, 1717 Mazor Drive, Columbus, GA 31907; and Christina Dunn, 10 Ironwood Court, Newnan, GA 30265*

After attending this presentation, attendees will learn the applications of Thin-Layer Chromatography (TLC) and Ultraviolet (UV) -Visible spectroscopy to characterizing chemical oxidation of synthetic cannabinoids, and how *in vitro* systems such as human liver microsomes and recombinant cytochrome P450 enzymes can be used to elucidate metabolism of synthetic cannabinoids.

This presentation will impact the forensic science community by identifying metabolites for detection of the abuse of the synthetic cannabinoids Bay 59-3074 and JWH-015.

It is hypothesized that *in vitro* incubation with human liver microsomes will allow production of metabolites of JWH-015 and Bay 59-3074. It is also postulated that oxidation of synthetic cannabinoids with chemical reagents will produce some of the same products produced by cytochrome p450 enzymes in the course of metabolism and eventually allow production of metabolite standards.

Methods used in this research included TLC and UV-Visible spectroscopy of synthetic cannabinoids exposed to human liver microsomes, recombinant human cytochrome p450 2D6, and chemical oxidants (sodium hypochlorite and hydrogen peroxide). These analyses will be supported by Fourier Transform Infrared (FTIR) spectroscopy and High-Performance Liquid Chromatography (HPLC).

The first set of experiments determined that millimolar concentrations of the synthetic cannabinoids JWH-015 and Bay 59-3074 were detectable by TLC on silica gel 25 under UV light. The JWH-015 and Bay 59-3074 were incubated with Human Liver Microsomes (HLM) or with buffer only as controls and, after centrifugation, the products were analyzed by TLC and UV-Visible spectroscopy. As controls for cytochrome P450 enzymatic activity of the HLM, the substrates coumarin, and eosin were incubated under the same conditions. The synthetic cannabinoids were not detectable by TLC after incubation with the HLM. The UV fluorescence of the main component of eosin was unaffected, but the fluorescence of a faster-moving component was decreased in one incubation with HLM. The coumarin incubation contained a new, faster-migrating (higher Retention factor (Rf) value) blue component after incubation with HLM but not after incubation in the control buffer lacking HLM.

UV-Visible spectroscopy detected increases in absorbance at 340nm, 337nm, and 310nm in the JWH-015 after incubation with HLM compared to the control. For the Bay 59-3074, UV-Visible spectroscopy detected a decrease in absorbance at 337nm and 340nm and an increase at 310nm after incubation with HLM. An issue with the incubations of the synthetic cannabinoids with HLM is the necessity to work with relatively high dilutions of these compounds because of the low solubility in aqueous buffers and the need to minimize concentrations of organic solvents in which they are soluble to avoid denaturation of the cytochrome P450s.

The synthetic cannabinoids JWH-015 and Bay 59-3074 were treated with sodium hypochlorite to see if oxidation could be detected by TLC for future comparison of the changes to those produced by cytochrome p450 or HLM. The Rf value (or mobility) of JWH-015 on TLC was decreased after incubation of the compound with sodium hypochlorite compared to control, but that of Bay 59-3074 was unaffected. Incubations with the oxidizing agent were also performed with two control substances: eosin and coumarin. In the case of eosin, two fluorescent components were detected in untreated controls whose intensity diminished with incubation with increasing concentration of oxidant. In the case of coumarin, two components were observed under UV-light on TLC plates, the slower moving of which decreased in intensity with an increasing concentration of oxidant.

It is concluded that *in vitro* incubation with human liver microsomes reveals some promise for producing metabolites for chemical characterization for the synthetic cannabinoids Bay 59-3074 and JWH-015. This may help in detecting metabolites of these compounds in biological samples. Further work can also be conducted by using FTIR and HPLC, but Liquid Chromatography/Mass Spectrometry (LC/MS) and Gas Chromatography/Mass Spectrometry (GC/MS) approaches are likely to be most suited for characterizing these metabolites. Chemical oxidation may be further explored as a means of generating oxidized forms of synthetic cannabinoids that are similar to those produced by metabolism.

Cannabinoids, Cytochrome P450, Metabolism