PROJECT REVIEW

"*Detection of doping with synthetic cannabinoids: metabolite studies and in vitro generated reference material*"

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In late 2008, several synthetic cannabinoids were detected in herbal smoking mixtures, commonly called "spice". Today a great variety of such synthetic cannabinoid receptor agonists is known and it expected that further substances will appear. Many European countries have banned these substances, and the World Anti-Doping Agency (WADA) prohibited synthetic cannabinoids in-competition in 2010. To allow an adequat detection of synthetic cannabinoids in a urine sample, knowledge of metabolism is a key issue. Performing human clinical studies is however difficult, due to the lack of a toxicological profile.

In vitro metabolism studies is an alternative to excretion studies in humans, and human hepatocytes are recognized to be a very close model to the human liver with intact cell membranes and the full complement of enzymes and cofactors. In the proposed project, an in vitro model using cryopreserved human hepatocytes will be used to investigate the metabolism of selected synthetic cannabinoids. The doping control laboratory in Seibersdorf is currently involved in a Wada-funded project testing and optimising the use of commercial available cryopreserved hepatocytes as an in vitro model for doping substances. The optimised model will be applied on the substances in the current study, and the main metabolites will be characterized by various mass spectrometric methods. A verification of the postulated metabolism from the in vitro studies is allowed by the investigation of in vivo urinary metabolic profiles in samples from drug drivers, supplied by the Vienna Police Department.

Finally, the incubation extracts will be evaluated as a potential source of reference material in doping control, as access to such material is of major importance in an unequivocal identification of metabolites in a doping control sample.

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Results and Conclusion

Aim of the project was to use cryopreserved hepatocytes to study the metabolism and characterize metabolites of selected synthetic cannabinoids dominant on the market today. Due to the continuous emergence of new "spice" compounds on the marked, a rapid investigation of the metabolism is of major importance, especially since some synthetic cannabinoids are already reported not to be excreted unchanged in human urine [1].
Four of the most prevalent compounds in positive forensic samples are reported to be JWH-122, JWH-210, AM-2201 and JWH-081 [2]. Thus, these four compounds were selected for the studies on the human metabolism of cannabinoids, in addition to JWH-018 as a model compound.

The results from the study suggest monohydroxylation as a major metabolic pathway for all five compounds investigated. Additionally, a major JWH 018 metabolite is carboxylated, while one of the major JWH-210 metabolites is dihydroxylated. Furthermore, additionally abundant metabolites for AM-2201 included one dihydroxylated, dihydrodiol, one desfluoropentylated, and defluorinated metabolites. Regarding JWH-081, a major dihydrodiol metabolite was identified, in addition to the abundant monohydroxylated compounds. Hence, even though similar metabolic pathways are observed for the five investigated compounds, differences are also present. Although varied conjugation rates were observed, the majority of the main metabolites were excreted as glucuronide conjugates.

Incubations with hepatocytes seem to represent a very useful model for identifying and predicting potential major urinary metabolites. Looking at the model compound JWH-018, the metabolites generated in the incubation mixtures were in good correspondence with reported major metabolites excreted in urine. Furthermore, the JWH-210 hepatocyte extracts resembled the metabolic profile observed in a forensic urine sample. Altogether, taking information from our experiments and available literature into account, it seems that the metabolites generated in human hepatocyte incubation extracts reflect the prevailing human metabolism.

[1] T. Sobolevsky, I. Prasolov, G. Rodchenkov. Detection of JWH-018 metabolites in smoking mixture post-administration urine. Forensic Sci Int. 2010, 200, 141.

[2] V. Auwarter. Freiburg Spice, JWH & Co.: What's the current knowledge? Symposium: "Emerging Drugs of Abuse: From Chemistry to Medicine" Analytica Conference 2012, 18. April 2012 Munich.