"In vitro metabolism studies of selected synthetic cannabinoids"

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Project Summary

Synthetic cannabinoids mimic the psychoactive effects of tetrahydrocannabinol (THC) and are found in herbal smoking mixtures, commonly sold as "spice". Like THC, these compounds are cannabinoid receptor agonists. Today, there is an immense variety of synthetic cannabinoids and spice blends available on the market and new substances constantly appear. Slight chemical modifications of existing compounds make many new cannabimimetics possible. For doping analysis, a fast elucidation of the metabolic pathways of new synthetic cannabinoids is of high importance in order to be able to implement them in doping control procedures. Due to a lack of a toxicological profile, in vivo excretion studies are however difficult to perform. Hence, the current study will employ in vitro metabolism studies on selected cannabimimetics using human cryopreserved hepatocytes. Human cryopreserved hepatocytes are recognized to be a close model to the human liver, showing excellent metabolizing and transporting activities. After hepatocyte incubation and a clean-up procedure, analysis will be performed with liquid chromatography and gas chromatography, combined with mass spectrometry. The selection of the target substances for the in vitro experiments will be based on the frequency of their detection in forensic samples. This is due to a rapidly changing market. The proposed metabolism is verified through the analysis of samples from traffic controls.

Results and Conclusions:

For doping analysis, the elucidation of the metabolic pathways of new synthetic cannabinoids is of high importance in order to be able to implement them in doping control procedures. Because of the high variability of synthetic cannabinoids and new herbal mixtures in a rapidly changing marked, a fast characterisation of metabolites is required. In terms of non-approved substances like this, though, human excretion studies are difficult to perform due to ethical considerations. In vitro studies, however, can identify human metabolites, and human primary hepatocytes are recognized to be a very close model to the human liver. Furthermore, the improvement of cryopreservation techniques has facilitated the use of cryopreserved hepatocytes which are now commercial available.
The aim of the current project was to use human cryopreserved hepatocytes to study the metabolism and characterize metabolites of selected synthetic cannabinoids predominating on the marked today. Two frequently reported cannabinoids in herbal smoking mixtures are JWH-307 and AM-694. Thus, these two compounds were selected for the study.
The incubation mixtures were analysed for substrate and metabolites using liquid chromatography-mass spectrometry (LC/MS/MS) with electrospray

ionisation (ESI). Free, glucuronide and sulphate products were separated during sample preparation. Although varied conjugation rates were observed, the majority of the main metabolites were to a high extent excreted as glucuronide conjugates. For all metabolites, the structures are proposed, not confirmed.

After incubation of AM-694 with human hepatocytes, 10 metabolites were recovered in the extracted culture medium. The most abundant generated products were a defluoroniated monohydroxylated metabolite and a carboxylated metabolite. Furthermore, the results from the hepatocyte assay were compared to analytical results from a forensic AM-694 urine sample. The in vitro data correlated well with observed metabolites in the urine sample.

After incubation of JWH-307 with human hepatocytes, 22 metabolites were identified. A monohydroxylated compound, a dihydroxylated compound, and three dihydrodiol metabolites were the prevailing metabolites generated in the incubation mixture.

In conclusion, incubations with human hepatocytes seems to represent a useful model for identifying and predicting major urinary metabolites.