Poster

[P26-10] P26-10: Assay of toxicants

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[P26-10-7] An activity-based screening method for synthetic cannabinoids: from concept to application

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Background

The continuously changing and growing class of synthetic cannabinoids (SCs) poses a real challenge in terms of detection and monitoring. Here, we report on the development and application of a bio-assay that is based on the activity of SCs at the cannabinoid (CB) receptors. This assay may be used for activity profiling of new SCs and their metabolites and as a first-line screening tool to identify positive biological samples.

Methods

CB1/CB2 receptor (fused to one part of luciferase) activation leads to recruitment of β-arrestin 2 (fused to the other part of luciferase). The resulting functional complementation of luciferase can easily be monitored via luminescence (NanoBiT® Technology). The assay was applied in a 96-well format on pure substances, as well as on extracts from 500 l urine and blood. Following deconjugation, urine was extracted with acetonitrile and ammonium formate (10M), whilst for serum a carbonate buffer (pH 10) was added prior to extraction with n-hexane/ethylacetate (99/1). Following evaporation of the supernatant, the residue was redissolved in 100 l of a 50:50 mixture methanol: serum free medium. Ten l of the extract (or pure compound) was used in the bio-assay.

Results

XLR-11, UR-144, AB-CHMINACA, ADB-CHMINACA and their major phase I metabolites were evaluated for their activity at CB1 and CB2, showing that several major metabolites retain activity. Application of the bio-assay on genuine urine samples (n=44) and serum samples (n=45) shows that the bio-assay is capable of detecting positive urine and serum samples. The sensitivity for urine samples from users who consumed either UR-144 or XLR-11 was 94.4% (17/18). For urine samples positive for AB-CHMINACA metabolites and ADB-CHMINACA metabolites, the detection rate was respectively 41.7% (5/12) and 81.8% (9/11). The unknown blank urine sample was scored negative. For the analysis of the serum samples (containing a variety of SCs), we reached a sensitivity of 81.8% (18/22) and a specificity of 100% (21/21).

Conclusions

The developed bio-assay may not only offer better insight into the potential activity of SCs (and their metabolites), it also offers the opportunity to serve as an alternative first-line screening tool for SCs in biological matrices.