Oral

[O25-3] O25-3: Drug abuse

Chairs: Eric J.F. Franssen, The Netherlands / Ryuji Kato, Japan
Mon. Sep 25, 2017 1:30 PM - 2:30 PM Room E (1F)

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[O25-3-3] A new bioassay for detection and activity profiling of synthetic opioids

Christophe Stove[1], Annelies Cannaert[2], Lakshmi Vasudevan[3], Maurice Wilde[4], Volker Auwaerter[5], Kathleen Van Craenenbroeck[6], Sarah Wille[7], Christophe Stove[8] (1.Ghent University, 2.Ghent University, 3.Ghent University, 4.University of Freiburg, 5.University of Freiburg, 6.Ghent University, 7.Federal Public Service Justice, National Institute of Criminalistics and Criminology, 8.Ghent University)
Keywords: bio-assay, synthetic opioids, new psychoactive substances, screening

Background

Following series of synthetic cannabinoid and synthetic cathinone derivatives, the illicit drug market shows an increased incidence of synthetic opioids, including fentanyl and its derivatives, and other chemically unrelated opioids, including U-47700. These synthetic opioids, together with the natural (e.g., morphine, codeine) and semi-synthetic opioids (e.g., hydromorphone, desomorphine) are common toxicological findings in death investigation cases. Here, we report on the development and application of a bio-assay that is based on the activity of (synthetic) opioids at the -opioid receptors (MOR). This assay may be used for activity profiling of new synthetic opioids and as a first-line screening tool to identify opioid-positive biological samples.

Methods

The bio-assay utilizes transiently transfected HEK293T cells, in which the NanoBiT® technology (Promega) was applied. Activation of MOR (fused to one part of luciferase) leads to recruitment of β-arrestin 2 (fused to the other part of luciferase). The resulting functional complementation of luciferase can be easily monitored via luminescence. The assay was applied in a 96-well format on pure substances and blood extracts. The latter were prepared by subjecting 250 l of blood to SPE (Waters Oasis® MCX), drying of the eluate and reconstitution in 100 l of serum free medium. Ten l of the extract (or pure compound) was used in the bio-assay.

Results

Several semi-synthetic opioids (hydromorphone and desomorphine) and synthetic opioids (fentanyl and derivatives such as 4-chloro-isobutyrfentanyl, 4-methoxybutyrfentanyl, acryloylfentanyl, alfentanil, benzodioxole-fentanyl, cyclopentylfentanyl, methoxyacetylfentanyl, ocfentanil, tetrahydrofuranfentanyl and other unrelated opioids (U-49900 and methene-U-47700 (U-54754)) were tested in our bio-assay, showing a variety in level of MOR activation. Application of the developed bio-assay on blind-coded authentic blood samples from morphine users and non-users shows that the bio-assay is capable of detection opioid activity in blood samples (sensitivity 100% (5/5), specificity 100% (7/7)).

Conclusions

The developed bio-assay may not only offer better insight into the potential activity of new synthetic opioids, it also offers the opportunity to serve as a first-line screening tool for opioids in biological matrices in an alternative way. Applicability of this assay will be further demonstrated using biological samples from authentic users of newly emerging synthetic opioids.