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Belgian Research Action through Interdisciplinary Networks

PIONEER PROJECTS

Development and implementation of a new psychoactive substances receptor activation assay

NEW PSYCHOACTIVE SUBSTANCES ACTIVITY ASSAY (NPSSAY)

CONTRACT - BR/165/PI/NPSSAY

FINAL REPORT

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SUMMARY

Context

About 900 new psychoactive drugs (NPS), with the largest group being the synthetic cannabinoids, have appeared on the worldwide drug market the last decade. The major challenge is the continuous chemical development of NPS, making it difficult to obtain up-to-date techniques for monitoring of these substances in bulk form or in biological samples. At the moment, the customs and toxicological laboratories work with high-end equipment such as nuclear magnetic resonance spectroscopy and (time-of-flight) mass spectrometry to structurally elucidate NPS or detect these compounds. However, these techniques are time-consuming, tedious and expensive. Quick and inexpensive, high-throughput-compatible tests, capable of demonstrating the presence of an NPS would increase the ability of the public organizations to respond fast to this global problem. Although at the moment some known NPS can be detected via rapid immunological tests, these tests are quickly outdated as they target a chemical structure and cannot cope with the continuous evolution in NPS structure. In addition, they often lack sensitivity. As a result, the public organisations are always a step behind. Development of high-throughput screening techniques focusing on their activity, rather than their chemical structure is of major importance as Belgium recently published a generic legislation, making a broad range of NPS illegal.

Objectives

Within this project, we aimed at setting up a stable cell system, to be deployed as a novel *in vitro* bio-assay, allowing the detection of 'unknown' NPS in bulk materials or biological matrices (e.g. blood, urine, oral fluid), based on their ability to activate G-protein coupled receptors (GPCRs).
The developed technique, a bio-assay, will help to ensure an up-to-date detection for several public organizations, not only resulting in a better knowledge-database but also allowing a better assessment of potential harm of NPS.

Conclusions

Stable cell systems for a novel and sensitive *in vitro* bio-assay for the detection of synthetic cannabinoid receptor agonists and synthetic opioids were developed. Sample preparation procedures for urine, serum, blood and oral fluid were developed and optimized to make these "cell compatible". Finally, the developed assays were applied on a larger scale to assess the performance of the proposed approach in terms of sensitivity, selectivity and robustness in a laboratory setting. The results were benchmarked against mass-spectrometric instrumentation. For this, we have analysed a large set of serum samples for the presence of SCRAs, acquired from patients with acute drug-related toxicity treated at the Emergency Department of the Guy's and St. Thomas' Hospital in Westminster (London, UK) from April to December 2016. This large scale study resulted in a sensitivity of 100% (52/52) and a specificity of 97.9% (330/337) when applying a sample volume of 300-500 μL.

Keywords

New psychoactive substances, bio-assay, G-protein coupled receptor

SAMENVATTING

Context

De laatste 10 jaar zijn er ongeveer 900 nieuwe psychoactive substanties (NPS) op de wereldwijde drugsmarkt verschenen, waarvan de synthetische cannabinoïden de grootste klasse zijn. De grootste uitdaging voor de opsporing van deze nieuwe drugs is de continue chemische ontwikkeling van nieuwe substanties, wat het moeilijk maakt om up-to-date methoden te ontwikkelen voor de detectie van deze substanties in bulk materialen of biologische stalen. Momenteel werken de douane en toxicologische laboratoria met geavanceerde toestellen, zoals NMR (Nucleaire Magnetische Resonantie) en TOF (time-of flight) massa spectrometrische toestellen om de structuur van deze nieuwe substanties op te helderen of deze te op te sporen. Deze technieken zijn echter tijdrovend en duur. Snelle, goedkope, high-troughput compatibele testen, die in staat zijn om de aanwezigheid van NPS te kunnen aantonen zouden de bevoegde instanties kunnen toelaten om snel te reageren op dit wereldwijd probleem. Hoewel momenteel sommige NPS kunnen gedetecteerd worden via snelle immunologische testen, zijn deze snel verouderd aangezien zij zich op de specifieke chemische structuur richten en zo niet kunnen volgen met de continue chemische evolutie in de NPS structuur. Bovendien missen ze vaak sensitiviteit. Bijgevolg lopen de bevoegde instanties achter de feiten aan. De ontwikkeling van high-troughput opsporingsmethoden, gebaseerd op de NPS activiteit in plaats van hun chemische structuur is van groot belang aangezien België recent een generieke wetgeving implementeerde, wat een hele reeks van NPS illegaal maakt.

Doelstellingen

Binnen dit project was het de bedoeling om stabiele cel systemen op te zetten en die toe te passen als nieuwe *in vitro* bio-assays voor de detectie van NPS. Dit moet de opsporing van 'ongekende' NPS toelaten in bulk materialen en biologische stalen (bv. bloed, urine, speeksel), via hun vermogen om G-proteïne gekoppelde receptoren (GPCRs) te activeren. De ontwikkelde techniek, een bio-assay, zal resulteren in een up-to-date opsporingsmethode voor verschillende instanties, wat niet alleen zal leiden tot een betere kennisdatabase, maar ook zal toelaten om een betere beoordeling te maken van de potentiële schade die NPS kunnen veroorzaken.

Besluiten

Stabiele celsystemen werden ontwikkeld zodat een nieuwe en gevoelige *in vitro* bio-assay kon ontwikkeld worden voor de detectie van synthetische cannabinoïd receptor agonisten en synthetische opioïden. De staalvoorbereiding voor een urine-, serum-, bloed- of speekselmatrix werd ontwikkeld en verder geoptimaliseerd om ze compatibel te maken met de levende cellen van het testsysteem. Tot slot werd de ontwikkelde test ingezet in een laboratorium omgeving om de gevoeligheid, selectiviteit en robuustheid van de ontwikkelde test te evalueren. De resultaten werden vergeleken met massa-spectrometrische technieken. Serumstalen afkomstig van patiënten die in de spoeddienst van het 'Guy's and St. Thomas' Hospital', Westminster (London, UK) van April tot December 2016 werden behandeld voor druggerelateerde toxiciteit werden gescreend op de aanwezigheid van synthetische cannabinoïd receptor agonisten. Deze studie toonde een sensitiviteit aan van 100% (52/52) en een specificiteit van 97,9% (330/337) wanneer 300-500 μL staal werd gebruikt.

Trefwoorden

Nieuwe psychoactive substanties, bio-assay, G-proteïne gekoppelde receptoren

RESUME

Contexte

Environ 900 nouvelles drogues psychoactives (NPS), la majeure partie étant des cannabinoïdes de synthèse sont apparues sur le marché mondial des drogues cette dernière décennie. Le chalenge principal réside dans le développement continu des NPS, rendant difficile l'obtention de techniques à jour pour le monitoring de ces substances en vrac ou dans des échantillons biologiques. Actuellement, les services douaniers et les laboratoires de toxicologie travaillent avec des équipements sophistiqués tels que la spectrométrie par résonnance magnétique nucléaire et la spectrométrie de masse (time-of-flight) afin d'élucider la structure des NPS ou pour détecter ces derniers. Toutefois, ces techniques nécessitent beaucoup de temps, sont fastidieuses et onéreuses. Des tests compatibles rapides, peu onéreux et à haut rendement capables de révéler la présence de NPS augmenteraient la capacité des organisations publiques pour répondre rapidement à ce problème mondial. Bien qu'actuellement certains NPS connus puissent être détectés via des tests immunologiques rapides, ces derniers sont rapidement dépassés sachant qu'ils ciblent une structure chimique et ne peuvent donc répondre à l'évolution continue de la structure des NPS. De plus ces tests manquent parfois de sensibilité. Il en résulte que les organisations publiques sont toujours dépassées. Le développement de techniques criblages de haut rendement ciblant l'activité des NPS et non leur structure chimique est d'importance majeure vu que la Belgique a publié une législation générique, rendant un grand nombre de NPS illégaux.

Objectifs

L'objectif de ce projet est le développement d'un système cellulaire stable pour le mise en œuvre d'un nouveau bio-essai *in vitro*, permettant la détection de NPS 'inconnus' dans des matériaux en vrac ou dans des matrices biologiques (e.a. le sang, l'urine, la salive). Le projet est basé sur la propriété qu'ont les NPS d'activer les récepteurs couplés aux protéines G (GPCRs).
La technique développée, un bio-essai, aidera à assurer une détection à jour pour plusieurs organisation publiques. Il en résultera non seulement une meilleure banque de données de connaissances mais aussi une meilleure évaluation de la dangerosité potentielle des NPS.

Conclusions

Des systèmes cellulaires stables ont été développés pour permettre la mise au point d'une nouvelle analyse biologique in vitro sensible, destinée à la détection des agonistes des récepteurs des cannabinoïdes de synthèse et des opiacés synthétiques. Les procédures relatives à la préparation des matrices suivantes, urines, sérum, sang et salive, ont été développées et optimisées pour les rendre compatibles avec les cellules vivantes du système analytique. Finalement, le système analytique développé a été testé en laboratoire pour en évaluer la sensibilité, robustesse et sélectivité. Les résultats obtenus ont été comparés à ceux issus de techniques de spectrométrie de masse. Les échantillons de sérum analysés proviennent de patients du services des urgences du 'the Guy's and St. Thomas' Hospital', Westminster (London, UK) traités pour des intoxications relatives aux drogues sur une période d'avril à décembre 2016. Ces échantillons ont été criblés pour la présence de récepteurs aux cannabinoïdes de synthèse. Cette étude a montré une sensibilité de 100% (52/52) et une spécificité de 97,9% (330/337) lorsque des échantillons de 300-500 µl sont utilisés.

Mots-clés

Nouvelles drogues psychoactives, bio-essai, les récepteurs couplés aux proteïnes G

1. INTRODUCTION

Over 900 new psychoactive drugs (NPS), with the largest groups being the synthetic cannabinoid receptor agonists and the synthetic opioids, have appeared on the worldwide drug market the last decade (1). NPS enlarge the total drug market, as they do not replace classical drugs, but are used by new groups of consumers. Their consumption poses a serious problem not only for public order, but also in terms of public health (1,2). The **major challenge** is the **continuous chemical development of NPS**, making it **difficult to obtain up-to-date techniques for monitoring of these substances in bulk form or in biological samples, and to obtain objective information concerning their traffic, use and effects**. Development of high-throughput screening techniques focusing on their activity, rather than their chemical structure is of major importance as Belgium has recently published a generic legislation, making a broad range of NPS illegal.

At the moment, the customs and toxicological laboratories work with high-end equipment such as nuclear magnetic resonance spectroscopy and (time-of-flight) mass spectrometry to structurally elucidate NPS or detect these compounds. However, these techniques are time-consuming, tedious and expensive. Although known NPS can also be detected via immunological tests, these tests are quickly outdated as they target a chemical structure and cannot cope with the continuous evolution in NPS structure (3). In addition, they often lack sensitivity. As a result, the public organizations are always a step behind.

Within the NPSSAY project, stable cell systems for **novel *in vitro* bioassays** were set up and deployed, allowing the detection of synthetic cannabinoid receptor agonists in bulk materials or biological matrices (e.g. urine, serum, plasma and oral fluid), based on their ability to activate cannabinoid receptors, CB1 and CB2. The principle of activity-based detection was also **extended to another class of NPS**, more specifically the synthetic opioids.

The major objective of the project was the development and deployment of a tool for the fast and general detection of NPS in order to help public federal organizations to ameliorate their public health actions and judicial procedures to counter NPS-related problems. While the tool is certainly developed and applicable, the direct use of the developed assay by the public federal organizations is hampered as the developed technique requires specific trained personal and is not yet ready to be used in different on-site settings. However, the gathered knowledge-database concerning NPS activity is of interest for public health, forensic laboratories and policy makers.

2. METHODOLOGY AND RESULTS

A proof-of-concept for the application of cell-based receptor activation assays for the detection of synthetic cannabinoid receptor agonist (SCRAs) was published in 2016 (4). In the NPSsay project, it was our aim to **create stable cell systems** for our reporter system to allow high throughput screening (WP1) and to evaluate different options to **improve sensitivity** to allow screening of biological matrices (WP2).

WP1: Creating a stable cell system for our reporter system to allow high-throughput Screening (Task 1).

Stable cell systems allow a serious reduction of the workload and do not have the variability in transfection efficiency that can occur with the transient format. Therefore, stable cell lines which can be used for the detection of SCRAs, were developed via viral transduction.

Initially the constructs were cloned in a retroviral vector. Two different retroviral vectors were used with different co-expression markers (e.g. the cannabinoid receptor constructs (CB1/CB2) are co-expressed with the Enhanced Green Fluorescent Protein (EGFP) and the β-arrestine 2 construct (βarr2) with the Nerve growth Factor Receptor (NGFR)). These co-expressed markers allow the sorting (see below) and the evaluation of the expression of the CB- and βarr2- constructs. The retroviral particles were created by transiently transfecting Phoenix cells (viral particle producing cells) with these vectors. After selection and expansion of the transfected Phoenix cells, the viral particles were secreted in the supernatant, harvested and stored at -80°C. Next, the viral particles were used to stably introduce both constructs in the cells. The cells were sorted based on their co-expression of EGFP and NGFR, by using a fluorescence activated cell sorter (FACS). Only the cells positive for both markers contain both constructs and are functionally active. The co-expression permitted us to select cell populations with an adequate expression level and allowed us to check the integrity of the reporter system over time (stability of the expression levels).

This has been **published** in *Analytical Chemistry*:
Activity-Based Detection of Consumption of Synthetic Cannabinoids in Authentic Urine Samples Using a Stable Cannabinoid Reporter System. Cannaert A, Franz F, Auwärter V, Stove CP. *Anal Chem*. **2017** Sep 5;89(17):9527-9536.

WP2: Evaluating different options to improve sensitivity to allow screening of biological matrices (Task 2-4)

In this work package, a strategy to improve the bioassays' sensitivity was tested. The process of the recruitment of βarr2 to the receptor was improved. This was achieved by altering the βarr2 protein C-terminally. This was inspired by reports that found that βarr2 deletion mutants lacking amino acids C-terminally can lead to a more efficient βarr2 recruitment to the receptor.

We evaluated a deletion mutant lacking the 28 C-terminal amino acids (βarr2Δ382) as it has been shown to be a constitutively active mutant that exhibits stronger stimulation-dependent βarr2-recruitment to the receptor (Figure 1). Another deletion mutant that was evaluated was βarr2Δ366 (lacking 34 amino acids C-terminally). This mutant leads to an efficient recruitment of βarr2 to the receptor, but blocks the internalization that normally follows. As the βarr2 protein thereby remains associated with the receptor, this could lead to extra sensitivity. Both new deletion mutants were tested in all possible configurations. For both CB1 and CB2, a new configuration was found that was capable of detecting lower concentrations of SCRAs (Task 2).

The sample preparation for urine, serum and blood was optimized, based on what is found in literature for conventional chromatography-mass spectrometry-based methods and was adapted to make these "cell compatible", since in the end the biological extract needs to be brought on the cells (Task 3).

This has been **published** in *Clinical Chemistry*:
Development and application of an improved bioassay for detection of cannabinoid activity in serum and plasma samples. Cannaert A, Storme J, Hess C, Auwärter V, Wille SMR, Stove CP, *Clin. Chem.* **2018**;64(6):918-926.

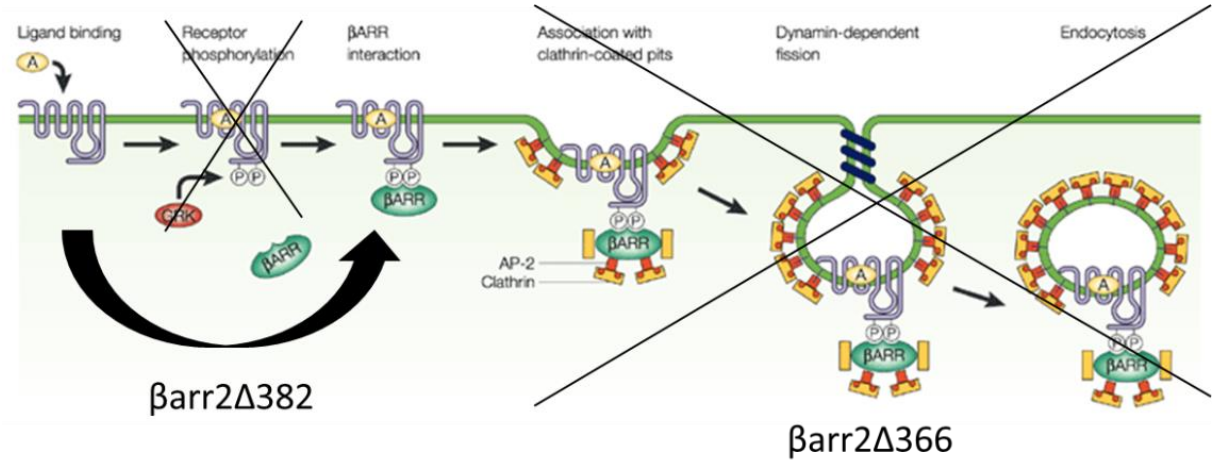

Figure 1. Mechanism on how the βarr2 deletion mutants influence the βarr2 recruitment. βarr2Δ382 is a constitutively active form of βarr2 and does not need the phosphorylation after receptor activation. βarr2Δ366 blocks the internalization of the receptor-βarr2 complex.

Next, the application of the detection of SCRAs in oral fluid (OF) via the assay was assessed (Task 4). The choice of OF collector has an impact on the final result, due to the differences in buffer content and their effect on drug stability, recovery and neat OF dilution (and thus sample concentration). The official selection of the OF collector for the Belgian Drugs and Driving legislation is the Intercept i2 collection device (Orasure Technologies, USA). The detection of SCRAs in this matrix is thus of importance as the Belgian law enforcement evaluates possible driving under the influence of drugs of classical drugs via oral fluid collected in this way. A collaboration with the University of Maastricht was set-up to monitor the detection of SCRAs in OF samples that are taken in the context of a JWH-018 (a SCRA) administration study. The obtained samples were analysed with the developed bioassay and a chromatographic technique (UPLC®-MS/MS).

These **results are currently being written down** in the following manuscript (to be submitted to *Clinical Chemistry*):
Semi-quantitative activity-based detection of JWH-018, a synthetic cannabinoid receptor agonist, in oral fluid after inhalation. Cannaert A, Ramírez Fernández M, Theunissen EL, Ramaekers JG, Wille SMR, Stove CP.

WP3: Expand the application of the current system to other NPS (Task 5).

In WP 3, the project aimed to **expand the application of the current system to other NPS.** The developed methodology that was used for the screening of SCRAs (see above) was adapted to allow screening for synthetic opioids. Synthetic opioids work on the same type of proteins in the human body as the SCRAs, called the opioid receptors. The opioid receptors are a group of inhibitory G-protein coupled receptors (GPCRs) with opioids as ligands. There are several major subtypes of opioid

receptors (δ-, κ-, μ- receptors). The μ-opioid receptor (MOR) was chosen as it is the best potential target, as morphine exerts its euphoric, analgesic, and anxiolytic effects via MOR. The coding sequence of MOR was cloned into the appropriate vectors to generate both MOR-LgBiT and MOR-SmBiT constructs (in a similar way as has been done for the cannabinoid system (4). The optimal combination with the corresponding βarr2-fusion construct was selected (Figure 2).

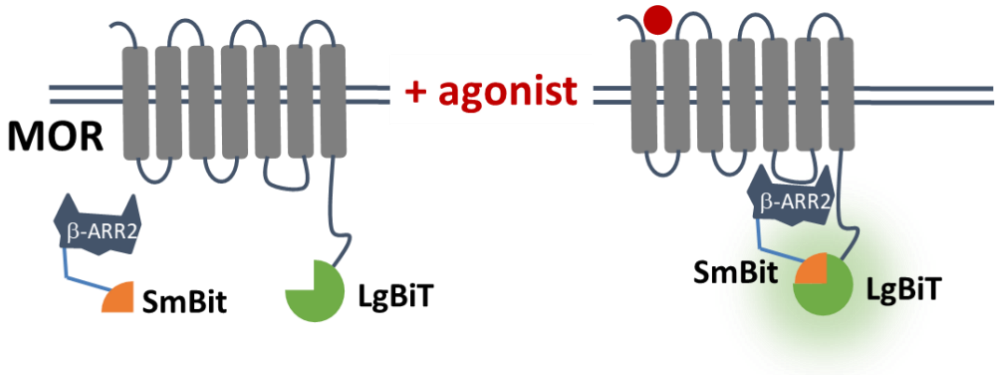

Figure 2. Optimal configuration for the MOR reporter system.

The opioid reporter assay was used for activity profiling of pure compounds, such as new synthetic opioid drugs, but was also evaluated as a tool for screening of biological matrices for opioid activity. The latter was achieved by analysing 107 authentic blood samples, obtained in a collaboration with the Centre for Forensic Science Research and Education (CFSRE; Willow Grove, Pennsylvania).

This has been **published** in *Clinical Chemistry*:
Activity-Based Concept to Screen Biological Matrices for Opiates and (Synthetic) Opioids. Cannaert A, Vasudevan L, Friscia M, Mohr ALA, Wille SMR, Stove CP. *Clin. Chem.* ***2018*** Aug;64(8):1221-1229.

WP4: Experimental tests, small scale field trials and performance assessment

The objective of this work package was to apply the developed assays on a larger scale to assess the performance of the proposed approach in terms of sensitivity, selectivity and robustness in a laboratory setting. The results were benchmarked against mass-spectrometric instrumentation. Sensitivity was determined using as quantitative criterion the rate of true positives (positives identified as such by the assay) and false negatives (positives identified as negatives). Selectivity was determined using as quantitative criterion the rate of false positives (negatives identified as positives) and true negatives (negatives identified as negatives).

For this, we have analysed a large set of serum samples (n = 471) for the presence of SCRAs, acquired from patients with acute drug-related toxicity treated at the Emergency Department of the Guy's and St. Thomas' Hospital in Westminster (London, UK) from April to December 2016. For 300-500 μl samples this resulted in a sensitivity of 100% (52/52) and a sensitivity of 97.9% (330/337). The reduction of the sample volume from to 100 μl resulted in a slight decrease in sensitivity and specificity (88.9% (16/18) and 96.6% (56/58) respectively).

These results were **published** in *Clinical Chemistry*.
Validation of Activity-Based Screening for Synthetic Cannabinoid Receptor Agonists in a Large Set of Serum Samples. Cannaert A, Vandeputte M, Hudson S, Wood DM, Dargan PI, Stove CP. *Clin. Chem.* ***2019*** Feb;65(2):347-349.

3. DISSEMINATION AND VALORISATION

The exploitation and dissemination of the results (**WP5**) was carried out via different activities.

(1) The project achievements were published in leading scientific journals and presented at conferences (see publications section + annex).
(2) A broader public was informed via national coverage of this project in National newspapers such as 'De Morgen', 'De Standaard', 'Het Nieuwsblad', 'Het Laatste Nieuws', 'Gazet van Antwerpen', 'Het belang van Limburg', 'Metro', etc.
(3) Collaborations with the Belgian Public Health department, drug rehabilitation and psychiatric centra (*Crisisinterventiecentrum De Sleutel*, Botestraat 102 9032 Wondelgem and the *Openbaar Psychiatrisch Zorgcentrum Rekem*, Daalbroekstraat 106, 3621 Lanaken)

Additionally, a valorisation project, NPSaction was set-up and sent in for evaluation for the Belspo Valorization projects. However, the proposed project NPSaction was not selected and due to a lack of finances, this could not be achieved (yet). The objective of this valorisation project, NPSaction, was to address a major judicial and societal challenge: a better detection, risk estimation and harm reduction of NPS: by ensuring an up-to-date detection for several public organizations; by distributing the obtained scientific knowledge to various stakeholders, not only resulting in a better knowledge-database but also leading to a better epidemiology and thus a better assessment of potential harm of NPS on the Belgian territory.

The **NPSaction network** (NICC, UGent, HoGent) was created to valorize the current Belspo NPSSAY project. NPSaction had two concrete actions to ensure an optimal impact on the obtained knowledge, the mindset and the activities of diverse stakeholders:

(1) the transfer of the activity-based detection system to other toxicological laboratories;
(2) the distribution of NPS information to a broader public of policy makers, prevention organisations and judicial authorities. Given its objectives, NPSaction targets stakeholders such as forensic science institutes, judicial authorities, law enforcement agencies and policy makers.

The main added-value lies in the fact that the newly developed technology and the NPS knowledge would be brought closer to its beneficiaries. Bringing together scientists, judicial authorities, prevention workers, health care workers and policy makers will increase the awareness on the situation of local drug markets and tendencies, and will result in sharing best practices and leading to evidence based policy.

4. PERSPECTIVES

On a scientific level

In the next step, we are evaluating if this screening approach can be further expanded to other classes of (synthetic) drugs of abuse. One option that we are currently working on is the class of the (hallucinogenic/serotonergic) NPS. Also the option to multiplex the different bioassays for the different types of drugs in one combined system is currently being explored.

We are also still optimizing the practical approach of the bioassays. Currently the outcome of the bioassay is still of a subjective nature, making the widespread application of the bioassays less straight-forward. Therefore, we are working with the research unit KERMIT (Knowledge-based Systems) from Ghent University to generate a computer-assisted algorithm that will be able to perform the scoring process (positive or negative).

Concerning valorization

The obtained scientific results were already published in leading scientific publications for the scientific research community and via press releases to the general public (with coverage via the radio and national newspapers). However, the impact of the obtained results of the NPSsay BRAIN project, in view of the results of other projects such as NPS-care, to the judicial authorities, enforcement agencies, prevention workers, health care workers and policy makers has not yet been deployed. Therefore, an interactive bi-lingual symposium could be organized in the future, addressing the novel Belgian legislation concerning NPS (NICC), the epidemiology of NPS (NPS-Care), the ways of NPS detection (NPSsay UGent and NICC), in addition to a roundtable including all actors. The aim is to increase the knowledge of NPS in Belgium and to create an awareness of the policy makers, judicial authorities and prevention workers of the current risks and solutions.

5. PUBLICATIONS

a. Validation of Activity-Based Screening for Synthetic Cannabinoid Receptor Agonists in a Large Set of Serum Samples. Cannaert A, Vandeputte M, Hudson S, Wood DM, Dargan PI, Stove CP. *Clin. Chem.* ***2019***;65(2):347-349.
b. Activity-based reporter assays for the screening of abused substances in biological matrices. Cannaert A, Vandeputte M, Wille SMR, Stove CP. *Crit Rev. Toxicol*. **2019**; 49(2): 95-109.
c. Activity-Based Concept to Screen Biological Matrices for Opiates and (Synthetic) Opioids. Cannaert A, Vasudevan L, Friscia M, Mohr ALA, Wille SMR, Stove CP. *Clin. Chem*. **2018**;64(8):1221-1229
d. Activity-Based Detection of Cannabinoids in Serum and Plasma Samples. Cannaert A, Storme J, Hess C, Auwärter V, Wille SMR, Stove CP, *Clin Chem*. **2018**;64(6):918-926.
e. Activity-Based Detection and Bioanalytical Confirmation of a Fatal Carfentanil Intoxication. Cannaert A, Ambach L, Blanckaert P, Stove CP. *Front Pharmacol*. **2018**;9:486
f. Activity-Based Detection of Consumption of Synthetic Cannabinoids in Authentic Urine Samples Using a Stable Cannabinoid Reporter System. Cannaert A, Franz F, Auwärter V, Stove CP. *Anal Chem*. **2017**;89(17):9527-9536.

6. ACKNOWLEDGEMENTS

We would like to acknowledge the research groups that collaborated with us to make this project work such as the Centre of Forensic Science Research and Education (CFSRE; Willow Grove, Pennsylvania, US), the University of Maastricht (The Netherlands), the University of Bonn (Germany), Freiburg University (Germany) and Guy's and St Thomas' Hospital (London, UK).

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2. European Monitoring Center of Durgs and Drug Addiction. European Drug Report: Trends and developments (2019). EMCDDA, 2019
3. Franz F, Angerer V, Jechle H, Pegoro M, et al. Immunoassay screening in urine for synthetic cannabinoids — an evaluation of the diagnostic efficiency. Clin Chem Lab Med 2017;55:1375 – 84.
4. Cannaert A, Storme J, Franz F, Auwarter V, Stove CP. Detection and activity profiling of synthetic cannabinoids and their metabolites with a newly developed bioassay. Anal Chem 2016;88:11476 – 85.

ANNEXES

Presentations at national and international conferences

Oral presentations

1. Scientific session of The Toxicological society of Belgium and Luxembourg (BLT), Antwerp, Belgium, March 30th 2017. An activity-based screening method for synthetic cannabinoids: from concept to application. (Cannaert A, , Franz F, Hess C, Auwärter V, Stove C).

2. Research Day 2017, Ghent, Belgium, April 4th 2017. Application of a new activity-based assay that allows activity profiling of synthetic cannabinoids (and metabolites) and their detection in urine. (Cannaert A, Storme J, Franz F, Auwärter V, Stove C).

3. Knowledge for Growth, Ghent, Belgium, May 21st 2017. New bioassay for detection and activity profiling of synthetic cannabinoids & metabolites. (Cannaert A, Storme J, Franz F, Auwärter V, Stove C).

4. 15th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), Kyoto, Japan, September 24-27th 2017. New bioassay for detection and activity profiling of synthetic opioids. (Cannaert A, Vasudevan L, Wilde M, Auwärter V, Van Craenenbroeck K, Wille S, Stove C)

5. 6e Journées Internationales de Toxicologie, Liège, Belgium, October 19-20th 2017. An activity-based screening method for synthetic cannabinoids: From concept to application. (Cannaert A, Franz F, Hess C, Wille S, Auwärter V, Stove C)

6. Lisbon Addictions, 2nd European Conference on Addictive Behaviors and Dependencies, Lisbon, Portugal, October 24-26th 2017. Development and Activity Profiling of Synthetic Cannabinoids and Their Metabolites with a Newly Developed Bioassay. (Cannaert A, Storme J, Franz F, Auwärter V, Stove C).

7. 55th International conference of The International Association of Forensic Toxicology (TIAFT), joint meeting with the Society of Forensic Toxicologists (SOFT), Boca Raton, Florida, US, January 7-11th 2018. An activity-based screening method for synthetic cannabinoids: From concept to application. (Cannaert A, Franz F, Hess C, Wille S, Auwärter V, Stove C)

8. 55th International conference of The International Association of Forensic Toxicology (TIAFT), joint meeting with the Society of Forensic Toxicologists (SOFT), Boca Raton, Florida, US, January 7-11th 2018. New bioassay for detection and activity profiling of synthetic opioids. (Cannaert A, Vasudevan L, Wilde M, Auwärter V, Van Craenenbroeck K, Wille S, Stove C)

9. 55th International conference of The International Association of Forensic Toxicology (TIAFT), joint meeting with the Society of Forensic Toxicologists (SOFT), Boca Raton, Florida, US, January 7-11th 2018. Bioassay-based screening of Synthetic cannabinoids: adding a new spice to the toxicologist's palette. (Cannaert A) Part of a workshop: Strategies for the Detection of Synthetic Cannabinoids in Biological Specimens.

10. Young Scientist Symposium at the 55th International conference of The International Association of Forensic Toxicology (TIAFT), joint meeting with the Society of Forensic Toxicologists (SOFT), Boca Raton, Florida, US, January 7-11th 2018. An alternative detection strategy for alternative drugs: the potential of bioassays to screen for new psychoactive substances. (Cannaert A)

13. 56th International conference of The International Association of Forensic Toxicology (TIAFT), Ghent, Belgium, August 26-30th 2018. A novel activity-based concept to screen biological matrices for the presence of opiates and (synthetic) opioids. (Cannaert A, Vasudevan L, Friscia M, Mohr A, Wille S, Stove C)

14. 56th International conference of The International Association of Forensic Toxicology (TIAFT), Ghent, Belgium, August 26-30th 2018. It doesn't matter what you look like, it's what you do that counts: Activity-based bioassays for the detection of synthetic cannabinoid receptor agonists in serum. (Vandeputte M, Cannaert A, Hudson S, White J, Bundell M, Archer J, Wood D, Dargan P, Stove C)

15. 26th Conference of SFTA (Société Française de Toxicologie Analytique), Marseille, France, June 6-8th 2018. Challenges and considerations for the detection of NPS in biological matrices. (Wille S, Richeval C, Nachon-Phanithavong, Cannaert A, Di Fazio V, Gaulier JM, Allorge D, Stove C, Samyn N)

16. 16th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), Brisbane, Australia, September 16-19th 2018. A novel activity-based concept to screen biological matrices for the presence of opiates and (synthetic) opioids. (Cannaert A, Vasudevan L, Friscia M, Mohr A, Wille S, Stove C)

17. Koninklijke Belgische Genootschap voor Gerechtelijke Geneeskunde, Leuven, Belgium, January 14th 2019. Prevalence and detection of New Psychoactive Substances. (Wille S).

18. 5th Young Scientist Symposium of European Bioanalysis Forum (EBF), Bologna, Italy, March 21-22th 2019. Alternative screening tools for the detection of novel psychoactive substances using an activity-based approach. (Cannaert A, Wille S, Stove CP)

19. Research Day & Student Research Symposium 2019, Ghent, Belgium, April 4th, 2019. Alternative screening tools for the detection of novel psychoactive substances using an activity-based approach. (Cannaert A, Wille S, Stove CP)

20. Flanders 2019 meeting, Lille, France, May 21-24th 2019. Alternative screening tools for the detection of novel psychoactive substances using an activity-based approach. (Cannaert A, Wille S, Stove CP)

Poster presentations

1. 15th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), Kyoto, Japan, September 24-27th 2017. An activity-based screening method for synthetic cannabinoids: From concept to application. (Cannaert A, Franz F, Hess C, Wille S, Auwärter V, Stove C)

2. Research Day & Student Research Symposium, Ghent, Belgium, April 19th, 2018. It doesn't matter what you look like, it's what you do that counts: Activity-based Bioassays for the Detection of Synthetic Cannabinoid Receptor Agonists in Serum. (Vandeputte M, Cannaert A, Stove C)

3. 16th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), Brisbane, Australia, September 16-19th 2018. It doesn't matter what you look like, it's what you do that counts: Activity-based Bioassays for the Detection of Synthetic Cannabinoid Receptor Agonists in Serum. (Cannaert A, Vandeputte M, Hudson S, White J, Bundell M, Archer J, Wood D, Dargan P, Stove C)