Background contamination level of perfluorinated compounds in a belgian general population

C. Pirard1, P. Dufour1, C. Charlier1

1 Laboratory of Clinical, Forensic and Environmental Toxicology, CHU of Liege, B35, 4000 Liege, Belgium

Background:
PFOS and PFOA are well-known perfluorinated compounds (PFCs) included or currently reviewed to be included in the "black list" of the Persistent Organic Pollutants (POPs) according to the Stockholm convention. However in Belgium, the only available data on human exposure are about 10 years old and concerned the Flemish population while no study has been carried out yet in Wallonia. The aims are therefore to assess the current exposure level of the general Walloon population, and to indentify subpopulation highly exposed thus presenting higher risk to develop endocrine disruption related diseases.

Methods:
In 2015 were recruited 252 participants aged from 18 to 76 years old and living in the Province of Liege. They provided a blood sample in clot activator tube and answered to a questionnaire about their food habits, life styles and home environment. Blood samples were centrifuged at 3000 rpm to collect serum which were extracted using mixed mode SPE and analyzed for 11 PFCs (PeFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFBS, PFHxS, PFOS) by LC-MS/MS.

Results:
PFPeA, PFHxA, PFDoA and PFBS were never detected, while the other PFCs were positively measured in 21% to 100% of the samples. PFOS contributed for more than 50% of the global contamination with a median level of 4.30 µg/l, followed by PFOA, PFHxS and PFNA (median ranging from 0.54 to 1.91 µg/l).

Short discussion/conclusions:
100% of the participants were simultaneously contaminated by at least 4 PFCs. The PFOS and PFOA levels were 1.5 to 4 times lower compared to those measured in 2008-2009 within a Flemish study. However a half of the present population showed levels above the HBM-I value (German HBM Commission) meaning that risk to develop adverse health effects is not excluded. Some predictors of exposure such like fish consumption were highlighted but the predictability of the statistical model was poor.

Application of a Fully Automated Dried Blood Spot (DBS) Method for Therapeutic Drug Monitoring of Immunosuppressants: another Step towards Implementation of DBS Analysis

Sigrid Deprez 1 and Christophe P. Stove 1

1Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium

Background: The follow-up of patients under life-long immunosuppressant therapy is pivotal to prevent allograft rejection after transplantation. Part of the difficulties associated with routine monitoring of immunosuppressant concentrations can be alleviated by home sampling using dried blood spots (DBS). In this study, the applicability of a DBS method making use of an automated extraction platform (DBS-MS 500) was evaluated.

Methods: Paired venous DBS and whole blood samples were analyzed in duplicate for tacrolimus (n=162), sirolimus (n=47), everolimus (n=45) and cyclosporin A (n=61) with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), using fully automated extraction for DBS. Both LCMS/MS methods were previously validated based on internationally recognized guidelines (EMA and FDA). During method validation, the relative IS-compensated recovery appeared to be hct-dependent: at lower hct values a higher relative recovery was observed, while at higher hct values a lower relative recovery was present. Therefore, a potential impact of the hematocrit (hct) on DBS quantitation of patient samples was assessed by plotting the % difference between DBS and whole blood concentrations in function of the hct for each analyte. If a significant hct trend would be present, a correction algorithm should be set up for the DBS results, based on the hct value of the liquid whole blood. Secondly, agreement between the automated (for the hct-corrected) DBS and whole blood method was assessed using Bland-Altman comparison. Both an analytical and clinical acceptance limit were pre-defined at more than 67% of all paired samples within 20% of the mean of both samples and more than 80% of all paired samples within 20% of the whole blood concentration, respectively.

Results: An impact of the hct on DBS quantitation was observed for all analytes, which could be alleviated for all analytes using a hct conversion formula based on the tacrolimus dataset: [DBScorrected]=[DBSmeasured]/(1.6305 - 0.0156*hct). After correction, both analytical and clinical acceptance criteria were met for all analytes (tacrolimus, sirolimus, everolimus and cyclosporin A). Although clinical and analytical acceptance limits were met, the applied correction algorithm requires knowledge of the hct. Fortunately, multiple approaches are available to predict the hct from a DBS, such as potassium measurement or non-contact approaches such as near-infrared spectroscopy or reflectance spectroscopy, as described in literature. This would avoid the need for hct determination of whole blood samples based on impedance methodology.

Conclusion: Automated DBS analysis shows great potential for routine therapeutic drug monitoring (TDM) of immunosuppressants, avoiding any manual sample handling.

Near-infrared-based hematocrit prediction of dried blood spots: an in-depth evaluation

Liesl Heughebaert1#, Lisa Delahaye1#, Christoph Lühr2, Stijn Lambrecht3, Christophe P. Stove1

1 Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Belgium
2 BÜCHI Labortechnik, Germany
3 Department of Laboratory Medicine, Ghent University Hospital, Belgium
#Equally contributed

Background: Dried blood spot (DBS) microsampling has received interest in different clinical fields, including therapeutic drug monitoring and pediatrics, owing to its many advantages compared to conventional blood sampling. However, whilst being applied for decades for screening purposes, some challenges, such as the hematocrit (Hct) effect, hinder further widespread use of DBS for quantitative purposes in clinical practice. Amongst the approaches that were developed to cope with this issue, is the Hct prediction of DBS using near-infrared (NIR) spectroscopy.
Objectives: The aim of this study was to extensively evaluate a commercially available NIR set-up for the prediction of the Hct from DBS.

Methods: Using left-over venous EDTA-anticoagulated blood from patients, the accuracy and precision, stability and robustness were assessed. Furthermore, applicability of the method on capillary DBS was evaluated via finger prick samples.

Results: Following actualization of an in-built calibration model, which was needed as an unacceptable negative bias was observed, the method validation resulted in a maximal bias, respectively CV, of 0.013 L/L and 4.5%. The method was robust towards several aspects, including storage (except for storage at 60°C), measurement location, type of filter paper (Whatman 903 vs Ahlström 226) and spotted volume (except for 10 µL spots). Furthermore, the method allowed to discern an altered blood spreading in DBS that had been pressed following collection. In contrast, holding the filter paper at an angle of approximately 45° while collecting the DBS did not relevantly affect the Hct predictions. Finally, the potential to predict the Hct of capillary DBS was demonstrated.

Conclusion: A commercially available NIR set-up was extensively and successfully validated, allowing non-contact Hct prediction of DBS with excellent accuracy and precision. This allows to correct for the Hct-based bias observed in partial-punch DBS analysis and the set-up.

Total Blood Carbon Monoxide (TBCO) as alternative biomarker for CO poisoning diagnosis: application in clinical and forensic settings

Oliverio Stefania 1,2,3, Varlet Vincent 4

1Forensic Toxicology and Chemistry Unit, University Center of Legal Medicine Lausanne-Geneva, Lausanne, Switzerland
2Institute of Environment, Health and Societies, Brunel University London, Uxbridge, United Kingdom
3 Department of Forensic Toxicology, Laboratoire National de Santé, Dudelange, Luxemburg
4Swiss Human Institute of Forensic Taphonomy, University Center of Legal Medicine Lausanne-Geneva, Lausanne, Switzerland

Introduction and Aims

Total Blood Carbon Monoxide (TBCO) was already proposed as alternative biomarker to carboxyhemoglobin (COHb) for CO poisoning diagnosis and showed promising results in improving accuracy of CO determinations in blood in clinical settings. The application in forensic settings requires more specific considerations regarding sample quality and stability during storage, transportation as well as postmortem phenomena. Therefore, the aim of this study was to investigate the effects of storage parameters on TBCO concentrations as opposed to COHb and apply the method to postmortem (PM) samples.

Methods

For the in vitro storage study, we have investigated several parameters, including storage temperature, blood tube preservative, dead volumes, freeze- and thaw as well as reopening cycles, at different initial COHb concentrations by fortifying blank bovine blood with pure CO gas. Observation period was one month. Statistical analysis was performed with R Studio.

For application to PM samples, we have analysed blood samples collected from a cohort of 14 negative PM cases with a short PM interval (≤24h), 7 negative PM cases with a longer PM interval (>24h) and 5 positive PM cases (PMI≤48h) immediately after sampling via gas chromatography-mass spectrometry (GC-MS) as well as co-oximetry. Samples collected included cardiac blood, peripheral blood and spleen blood. Groups were compared by Student t-test.

Results and Discussion

Statistical analyses show that there is no significant difference for freeze- and thaw-cycles as well as multiple reopening of the tubes. For COHb concentrations, all other parameters have an impact except EDTA as preservative, storage at -20°C and a HS volume of >50%. For TBCO, no significant impact is shown except for storage at room temperature and preservatives other than EDTA.

For the different blood types in PM cases, TBCO results show a good agreement between cardiac, peripheral and also spleen blood, which shows that there seems to be no significant PM redistribution occurring and introduces the potential use of spleen blood as an alternative in absence of cardiac or peripheral blood. Averages of the negative low PMI group show no significant differences compared to higher PMI group, suggesting that PM CO formation or degradation at initial PMI is low.

Conclusions

TBCO is confirmed to be a more stable biomarker for CO poisoning diagnosis as opposed to COHb, especially in non-optimal storage conditions, but also in postmortem cases, where blood quality affects the routinely used biomarker COHb. Despite the rather promising results, it must be noted that the number of samples in each group is not very high, thus an increased number of samples can increase the confidence in these results.

Overdose of Genvoya® in two self-intoxications

Celine Gys1, Olivier Mortelé2, Babette Van Rafelghem3, Eleanor Berry1, Alexander L. N. van Nuijs1, Willy Lambert4, Werner Jacobs3, Sofie Schouwers2, Anissa Meskal2, Hugo Neels1, Adrian Covaci1

1 Toxicological Center, University of Antwerp, Universiteitsplein 1, Antwerp, Belgium
2 Laboratorium for Clinical Biology, GZA Sint-Augustinus, Oosterveldlaan 24, Antwerp, Belgium
3 Department of Forensic Medicine, Antwerp University Hospital, University of Antwerp, Edegem, Belgium
4 Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, Ghent, Belgium

Highly active antiretroviral therapy is employed in Human Immunodeficiency Virus (HIV) seropositive patients to suppress HIV replication, resulting in restored immune function, reduced morbidity and limited risk of transmission. The combination treatment Genvoya® consists of emtricitabine (200 mg), elvitegravir (150 mg), tenofovir alafenamide (10 mg), and cobicistat (150 mg). Toxicological information on overdose with HIV-inhibitors is scarce and reports including analytical data of these compounds under overdose conditions are, to our best knowledge, inexistent. This case report presents two independent suicide attempts by self-administered overdose with Genvoya® HIV-medication. Levels of emtricitabine were quantified in various samples from both cases using liquid-liquid extraction followed by LC-MS/MS.

Case 1 involves a 40-year-old HIV seropositive male staying at a psychiatric care-unit and stated to have voluntarily taken 60 tablets of Genvoya® and 10 g of paracetamol. Upon admission to the hospital and at 6 points in time until 79 h after, blood samples were taken from the patient. After discontinuation of Genvoya® and supportive measures during 7 days, this patient was discharged from the hospital.
Case 2 concerns an 18-year-old male who stated in the early morning to have voluntarily ingested during the night 30 tablets of the same Genvoya® formulation and collapsed subsequently. The medication was likely prescribed to the partner of the patient and HIV-infection in the patient was not confirmed because of the lack of further medical history. Despite advanced supportive measures and transfer to a tertiary care hospital, the patient died. During the post-mortem examination and autopsy, peripheral and cardiac blood and urine were collected.

The measured emtricitabine levels in the serum samples from both cases confirmed the self-declared overdosages. Based on the time profile of the measured emtricitabine levels in the different serum samples of Case 1 (maximum concentration: 17.6 mg/L; therapeutic concentrations: < 3 mg/L), it was concluded that this patient was admitted to the hospital within 3 h post-ingestion of the Genvoya® tablets. The higher concentration of emtricitabine in the cardiac serum (21.1 mg/L) of Case 2 as compared to the peripheral serum (14.4 mg/L), suggests post-mortem redistribution to some extent. This is the first time emtricitabine concentrations following overdose are reported. Both patients presented cardiogenic shock, renal insufficiency and lactic acidosis. The reason for the differing outcomes of the two self-intoxications remains unconfirmed and data regarding overdosing of Genvoya® is scarce. Potential explanations could be the inter-individual variability in physiological status and susceptibility to adverse effects, whether or not combined with a different interval between ingestion and admission to the hospital. The worse outcome of Case 2 may also be explained by the more pronounced metabolic acidosis (arterial lactate concentration 6.30 mmol/L for Case 2 as opposed to 3.80 mmol/L for Case 1), possibly linked to a longer interval between overdose and medical care. A short interval between an intoxication with Genvoya® and the start of symptomatic and supportive measures clearly is a relevant parameter for a favourable outcome.

NPS detected in the clinical lab of Ghent University Hospital

Nick Verougstraete & Alain G. Verstraete

Department of laboratory medicine. Ghent University Hospital, Ghent, Belgium

Introduction

Although the number of new NPS seems to be decreasing, they continue to appear on the (online) drug market. Up until the end of 2020, 830 NPS had been reported to the EMCDDA, 46 of which were first reported in Europe in 2020, and 1047 to the UNODC. In 2020, 3/4-MMC and etizolam were the most frequently notified NPS to the Belgian Early Warning System.

Material & methods

Biological patient samples (urine and serum) and drug collections, obtained from our hospital (mostly emergency department) and from external labs, were analyzed with an in-house LC-HRMS NPS screening method. Samples were precipitated/diluted with a methanol/acetonitrile mixture and the resulting supernatant was analyzed on an Ultimate 3000 UHPLC system coupled to a Q Exactive HRMS (Thermo Fisher Scientific). The HRMS database was derived from HighResNPS.com. Additionally, obtained MS^2 spectra were compared to the mzCloud database. Prior to HRMS analysis, samples were tested with (meth)amphetamine and benzodiazepine CEDIA (Indiko, Thermo Fisher Scientific) immunoassays for serum or EMIT (Architect c8000, Abbott Diagnostics) for urine.

Results

Since 2019, 3-/4-MMC (urine n=40, serum n=12; powder n=2) was the most frequently detected NPS. Other stimulants alpha-PHP (urine=1; serum=1), alpha-PVP (urine=2), MDPHP (serum=1), N-ethylhexedrone (urine=1), x-MEC (powder=1), x-fluorophenmetrazine (serum=1), x-fluoroamphetamine (urine=2) and x-fluoromethamphetamine (urine=1; serum=1) were less frequently found. The following hallucinogens were identified: 2C-B (urine=1; serum=1; tablet=1), x-Me-PCP (urine=1), x-MeO-PCP (urine=1; powder=1), x-HO-PCP (powder=1), N-ethyldeschloroketamine (powder=1), x-fluoro-deschloroketamine (powder=1) and methoxpropamine (liquid=1). Flualprazolam (urine=9; serum=1; pills=2) was the most frequently detected designer benzodiazepine, followed by etizolam (urine=6; serum=1; powder=1), bromazolam (urine=2), flubromazolam (urine=1) and clonazolam (urine=1). All these designer benzodiazepines gave positive results in the benzodiazepine immunoassay. Only one synthetic cannabinoid (MDMB-4en-PINACA in an e-liquid), one NPS opioid (brorphine in powder and corresponding serum samples) and one plant extract NPS (mitragynine in urine) were found.

Conclusions

Since the implementation of an LC-HRMS NPS screening method, NPS detections have markedly increased. 3-/4-MMC clearly stood out as the most frequently detected NPS over a 2-year timespan, followed by flualprazolam and etizolam. Various other stimulants and hallucinogens with similar structures were found. For the detection of designer benzodiazepines, the use of an immunoassay is a helpful (screening)tool.

Getting to know the new opioids on the block: functional characterization of emerging cinnamylpiperazines and closed-ring 2-benzylbenzimidazole 'nitazene' opioids

Marthe M. Vandeputte1, Melissa Fogarty2, Donna Papsun3, Sara Walton2, Alex J. Krotulski2, Michael H. Baumann4, Barry Logan2,3, Christophe P. Stove1

1 Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium; 2 Center for Forensic Science Research and Education, Fredric Rieders Family Foundation, Willow Grove, Pennsylvania, USA; 3 NMS Labs, Horsham,
Pennsylvania, USA; 4 Designer Drug Research Unit, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, USA

Background & aims: The last years have seen a rapid growth in the number of new synthetic opioids appearing on the recreational drug market. Combined with the substantial risk of fatal overdoses due to respiratory depression, this rapid growth is coupled with significant public health risks. Adding an extra layer of complexity, the nature of opioids entering the market has largely diversified in recent years, as fentanyl derivatives increasingly made room for structurally diverse non-fentanyl opioids.
Here, we discuss the in vitro μ-opioid receptor (MOR) activation potential of two classes of nonfentanyl opioids that have recently gained momentum. The first class is that of the cinnamylpiperazines ('AP'-opioids), of which a total of four analogues have emerged since 2019. Secondly, we focus on two 'closed-ring' analogues of the 2-benzylbenzimidazole or 'nitazene' class of opioids. To date, knowledge concerning the toxicological effects and harm profile of both classes of opioids is scarce.

Methods: The in vitro MOR activation potential of four cinnamylpiperazines (AP-237, 2-methyl AP-237, para-methyl AP-237, AP-238) and two closed-ring 2-benzylbenzimidazoles (N-pyrrolidino etonitazene or 'etonitazepyne'; N-piperidinyl etonitazene or 'etonitazepipne') was studied by means of a cell-based β-arrestin 2 (βarr2) recruitment assay. In short, activation of human MOR, fused to one subunit of a nanoluciferase enzyme, leads to recruitment of βarr2, fused to the complementing subunit. This results in the functional complementation of the enzyme, restoring its luciferase activity. Upon
addition of a substrate, a bright bioluminescent signal is generated (NanoBiT®, Promega). From this, in vitro potency and efficacy values were calculated (the latter relative to hydromorphone).

Results & discussion: Of the different tested cinnamylpiperazines, AP-238 was the most potent compound (EC50 = 248 nM; Emax = 91.3%), whereas 2-methyl AP-237 was found to be the most
efficacious (EC50 = 749 nM; Emax = 125%). For AP-237 and para-methyl AP-237, maximum receptor activation could not be reached. Despite the relatively low MOR activation potential of these analogues (compared to e.g. fentanyl and hydromorphone), these compounds appear to be circulating among drug users, as indicated by discussions on user fora and as confirmed by recent in vivo (postmortem) findings. N-pyrrolidino and N-piperidinyl etonitazene are the newest members of the highly potent class of 'nitazene' opioids. With a potency comparable to that of etonitazene, the former (EC50 = 0.348 nM; Emax = 298%) is among the most potent (non-fentanyl) opioids described to date. Interestingly, findings for N-pyrrolidino etonitazene confirm that the employed in vitro assay is a better predictor of in vivo opioid activity than traditional binding assays measuring MOR affinity. N-piperidinyl etonitazene (EC50 = 1.60 nM; Emax = 252%) was around 5 times less potent than the pyrrolidino-analogue.
Conclusion: Cinnamylpiperazines and 2-benzylbenzimidazole opioids are increasingly emerging on the recreational drug market, each new member carrying a substantial risk of toxicity. In vitro research on MOR activation offers a first realistic estimation of the potential danger these compounds may evoke in vivo.

Large-scale activity-based SCRA screening on patient plasma samples: CB1 bioassay supported by machine learning

Liesl Janssens, Dimitri Boeckaerts, Simon Hudson, Daria Morozova, Annelies Cannaert, David M. Wood, Caitlin Wolfe, Bernard de Baets, Michiel Stock, Paul Dargan, Christophe P. Stove*

Introduction: Synthetic cannabinoid receptor agonists (SCRAs) are a prominent danger to public health. Emerging SCRAs are most often highly active at the CB1 cannabinoid receptor. This high activity imposes serious health threats, illustrated by intoxications with SCRAs presenting at emergency departments (ED's). The rapid emergence of novel analogs makes the detection of these new derivatives challenging. However, there is a strong need for continuous monitoring of these compounds to adapt legislations and ensure public health. The ED's of some hospitals, located in relevant areas regarding drug abuse, serve as some kind of 'sentinels', allowing to keep guard on circulating, potentially highly dangerous SCRAs. An example is the ED of Guy's and St-Thomas' Hospital in central London, which is ideally positioned to keep track of the latest changes on the illicit drug market.

Methods: In the context of screening ED patient samples, the ideal assay is easy-to-perform and easily implementable. To speed up the work process and reduce the workload in the case of activity-based screening, we assessed whether artificial intelligence and machine learning could be of any help to the expert in deciding the eventual outcome of the screening assay. Following up on the success of a prior large-scale screening of serum samples for the presence of SCRA activity, we set out to screen a new large set (942 samples), with the following aims 1) assess the performance of the assay to using biological samples potentially containing newer circulating SCRAs, 2) exploration of a more structured way of manual scoring, and 3) exploration of computer-based scoring.

Results: Plasma samples, collected at the ED of Guy's and St-Thomas' Hospital in central London, were subjected to activity-based SCRA screening using a cell-based bioluminescence assay and to High Resolution Mass Spectrometry (HRMS)-based analysis for confirmation and identification. Both strategies were run independently and were performed blind-coded. Screening results from the bioassay (obtained through an improved scoring system by the expert) were compared with analytical (HRMS) results (considered as the 'gold standard'). The bioassay yielded a sensitivity of 94% and a specificity of 98%. The sensitivity obtained for a plasma sample volume of 250µL is in line with our earlier data, obtained on another sample set containing other SCRAs, where starting volumes of 500 or 100 µL were used. A positive correlation between sample volume and sensitivity was confirmed. The concluding specificity of 98% is in concordance with previously published results, which is very high for a broad screening assay. Sample volume does not seem to have a (pronounced) influence on this assay characteristic. The panel of identified SCRAs is largely distinct from the panel identified from April to December 2016, exemplifying the well-known phenomenon of market dynamics and, importantly, also underscoring the universal nature of activity-based screening.

Conclusions: A machine learning model was designed in order to automatically discriminate positive from negative samples. The model was trained on both the analytical outcome and the expert scoring to determine whether expert knowledge can be of added-value for training the predictive model. Two cross-validation settings were employed to evaluate the performance of the trained models and to assess the robustness of the machine learning approach. Depending on the desired sensitivity/specificity and the corresponding threshold applied within the model, we can conclude that machine learning is an adequate alternative for manual scoring by the expert (e.g. sensitivity of 94.6% and specificity of 94.6% at a 0.063 threshold). Automation of this scoring process results in significant time saving and reduction of the workload.

NNL-3: a synthetic intermediate or a new class of hydroxybenzotriazole (HOBt) esters with cannabinoid receptor activity?

Deventer Marie

Introduction: SCRAs containing ester linkers are intermediates en route to typical carboxamide SCRAs, but have been identified and marketed as NPS, exemplified by the suspected synthetic impurity NNL-3, a SCRA containing a hydroxybenzotriazole (HOBt) ester moiety. Similar HOBt esters can be formed and isolated during carboxamide SCRA synthesis. To explore this class of putative HOBt ester SCRAs, NNL-3 analogues were synthesized incorporating common SCRA scaffolds (indole, indazole, azaindole) and their binding affinity and functional activity at CB1 and CB2 was evaluated.
Methods: The biological activity of NNL-3 and 6 synthesized analogous HOBt ester SCRAs was evaluated at both CB1 and CB2, using earlier reported cell-based bio-assays developed to monitor β-arrestin2 recruitment to the CB1 and CB2 receptor. The assay relies on the NanoLuc Binary Technology® (Promega) which exploits functional complementation of a split nanoluciferase enzyme, of which the inactive subunits are either fused to the receptor (CB1 or CB2) and the intracellular protein βarr2. Receptor activation results in βarr2 recruitment, causing the 2 subunits to come in close proximity, resulting in restoration of the nanoluciferase activity and, following addition of substrate, luminescence. Activity of the compounds was also assessed using a fluorescence-based membrane potential assay which monitors Gβγ activation of inwardly-rectifying potassium channels, while binding affinity was evaluated using a competition [3H]CP55,940 radioligand binding assay.
Results: The NanoBiT® assay highlighted 2 highly efficacious "super-agonist" compounds. Methylindole HOBt-2-Me-5F-PIC and its unsubstituted analog HOBt-5F-PIC exhibited efficacies at CB1 of 724% and 831% respectively, in comparison to the reference compound CP-55,940, with potencies of 131 nM and 1.26 μM respectively. None of the compounds were scored as full agonists at CB2, with HOBt-2-Me-5F-PIC being over 60-fold more potent than the other compounds at this receptor. These findings were confirmed by the radioligand binding assay, which revealed overall low affinity for both CB1 and CB2, except for HOBt-2-Me-5F-PIC and HOBt-5F-PIC. In line with the binding and NanoBiT® data, the membrane potential assay scored HOBt-2-Me-5F-PIC and HOBt-5F-PIC as full agonists, while even at micromolar concentrations all other compounds showed negligible activity. Interestingly, addition of a methyl at the 2-position resulted in both increased potency at and affinity for both receptors.
Conclusion: NNL-3 as well as other 7-azaindole-bearing HOBt ester SCRAs showed only weak activity at CB1 and CB2. Methylindole and indole analogs, by contrast, were shown to be potent and efficacious, with substitution at the 2-position resulting in higher potency. This demonstrates that, while the marketed NNL-3 is unlikely to cause serious cannabinoid receptor-related toxicity, this cannot be concluded for the 2-methylindole HOBt ester (and its potential future analogues), with a potency and efficacy similar to that of SCRAs that have been involved in intoxications. However, one does have to keep in mind that the used assays can only determine in vitro activity, and cannot always accurately predict the in vivo effect as it will be subject to metabolic stability since esters may hydrolyze rapidly in the human body.

Severe baclofen intoxication in a 16 years old woman: a case report

A. NIZET, R. DENOOZ, C. CHARLIER
Laboratory of Clinical, Forensic and Environmental Toxicology, Center for Interdisciplinary Research on Medicines (CIRM), University Hospital of Liege, Belgium.

Introduction: Baclofen, a selective agonist of the receptor GABA(B), is originally used as treatment for central spasticity. In recent years, the drug has also been used at higher doses to treat symptoms of alcohol withdrawal syndrome. Intoxication is not uncommon, especially as recreational use as a hallucination drug is emerging among adolescents. In case of overdose, its depressant action on the automatic and nervous system can cause delirium, autonomic disturbances, seizures, severe neuromuscular and respiratory depression, and sometimes a coma requiring intensive supportive care. These nonspecific symptoms make diagnosis complicated in the absence of anamnesis, and few laboratories have analytical methods for rapid detection of the product.
Case presentation: We reported the case of a 16-year-old girl who was found unresponsive by her father at home. On admission to the emergency room, clinical examination showed respiratory acidosis (pH 7.17) and hypotension (101/53 mmHg). The Glasgow Coma Score was 3, pupils were fixedly dilated with no response to light, and she had no spontaneous movements. For optimal care, patient was sedated, intubated and ventilated. Rapid screening analysis performed by peripheral hospital was negative (alcohol, benzodiazepines, barbiturates, tricyclic antidepressants and conventional illicit drugs). As a result of the symptoms and drugs present at home, baclofen intoxication was considered. The admission sample was sent to our toxicology lab for further investigation. Results confirmed trazodone (4500 µg/L), quetiapine (339 µg/L) and baclofen (12500 µg/L) intoxication. A severe renal failure occurred within 48 hours (RGF: 22 ml/min), explaining the low elimination of baclofen (1360 µg/L at +48 hours, 437 µg/L at +96 hours). She left the intensive care unit after 9 days without sequelae.
Discussion: Baclofen blood concentration was defined as therapeutic or “normal” between 80 to 400 µg/L. Invasive supportive cares with intubation and mechanical ventilation may occur at doses equivalent to 2-4 usual therapeutic range[1]. Admission baclofen concentration was 12500 µg/L, more than 30 time higher than reference values. Indeed, the elimination of parent drugs and inactive metabolites, normally quick by glomerular filtration (t1/2: 2 at 4 hours), could be, in case of severe intoxication, strongly slowed down (t1/2: 12-36 hours)[2]. As no specific treatment exits, the therapy should include purifying mechanisms. In the present case, hemofiltration wasn’t established due to persistent diuresis.
Conclusion: Baclofen intoxication can severely disturb the automatic and central nervous system, even at low concentrations. Admissions in intensive care unit are frequently prolonged by induced renal failure. Without specifical treatment, heamodialysis should frequently be considered to avoid worsening of the condition and reduce the length of hospitalization.

References: [1] G. Drevin, M. Briet, S. Ghamrawi et al. Baclofen overdose following recreational use in adolescents and young adults: A case report and review of the literature. Forensic Sci. Int. 316 (2020) 110541 - [2] N. Franchitto, B. Rolland, F. Pelissier et al. How to manage self-poisoning with baclofen inalcohol use disorder? Current Updates. Front Psychiatry. 9 (2018) 417.

The Belgian Poison Centre: update anno 2020 and the impact of COVID-19

D. Vandijck1,2, E. Bekaert1, E. De Smet1, J. Moens1, P. Selway1, J. Van Baelen1, G. Verstegen1, P. Wallemacq1, AM. Descamps1,2

1 Belgian Poison Centre, Brussels, Belgium
2 Ghent University, Faculty of Medicine and Health Sciences, Ghent, Belgium

Introduction

The COVID-19 pandemic puts a significant burden, not only on social life, but also on healthcare services such as Poison Centres. Hygienic measures (e.g. frequent use of soaps, detergents, alcohol-based hand sanitizers (ABHS), etc) in order to prevent COVID-19 infection, can potentially cause problems especially in case of paediatric exposures and if used inappropriately.

The present study aims to provide an overview of the total number and type of calls to the Belgian Poison Centre (BPC), and to assess the impact of COVID-19.1

Methods

Data of all calls to the BPC (period January 1 – December 31, 2020) were collected in a database and analysed using appropriate statistics (SAS).

Results

Being the expertise and reference centre for (acute) toxicological incidents for both Belgium and the Grand Duchy of Luxembourg, the BPC received 65,308 calls in 2020 (60,668 in 2019, $p<0.05$), of which 56,106 (86%) (involving 57,523 victims) due to an exposure, and 9,202 (14%) due to an information request.

Despite a minor decrease of 2.3% (21,151 in 2019 vs. 20,666 in 2020, $p>0.05$), the vast majority (35.9%) of exposures were drug-related. Drugs within the category 'nervous system' (e.g. antipsychotics, antidepressants, etc.) were most frequently involved (39.6%), and paracetamol represented 8.2% of drug-related exposures. The number of exposures according to incidents following the use of chemical household products was 11,836 in 2019 vs. 12,247 in 2020 ($p>0.05$). A 12.3% increase of the number of cosmetic- and food-related exposures was noted (8,291 in 2019 vs. 9,308 in 2020, $p<0.05$). Within this group, a stable number of exposures (877 in 2019 vs. 876 in 2020, $p>0.05$) due to essential oil exposures were observed.

Partly due to the impact of the COVID-192 pandemic, exposures to biocides doubled (104.9%) from 1,964 in 2019 to 4,024 in 2020 ($p<0.05$). Exposures to type 1 biocides (i.e. human hygiene, which include ABHS) significantly increased from 322 in 2019 to 1,676 in 2020 ($p<0.05$), and exposures to type 2 biocides (i.e. disinfectants and algaecides not intended for direct application to humans or animals) from 406 to 902 ($p<0.05$). In 2020 the BPC received a fivefold of the number of calls for ABHS incidents (both, liquid and gel-based, as well as ethanol and isopropanol products) as compared to 2019 (1,676 vs. 323 in 2019 vs 1,676 in 2020 calls, $p<0.05$), accounting for 2.6% of all calls in 2020. In 71% of exposures, ingestion was the primary route (1,195/1,676), followed by 28.6% accidental ocular exposures (480/1,676) of which more than half of the incidents among children (257/480, $p<0.05$), and primarily among young children aged between 1 and 4 years (136/257, $p<0.05$). Finally, as people went into the garden and nature to relax during the lockdowns, a 28.2% increase in exposures related to the group 'plants, mushrooms and animals' was found, with 3,256 exposures in 2019 and 4,175 in 2020 ($p<0.05$).

Conclusion

In its history, the BPC never received as much calls as in 2020. It seems that, in case of (potential) intoxication, both the general public and healthcare professionals increasingly appeal to the unique expertise, knowledge, experience and data of the BPC within the broad field of toxicology. The COVID-19 pandemic contributed to a significant number of additional exposures, and consequently requests for toxicologic advice.

References

1 Bekaert E, De Cock P, De Smet E, Moens J, Pire R, Stammen L, Van Baelen J, Verstegen G, Wallemacq P, Vandijck D, Descamps AM. Jaarrapoort 2020. Antigifcentrum 2021.

2 Vandijck D., Roels D., van Baelen J., De Paepe P., Descamps A. Accidental ocular chemical injury following alcohol-based hand sanitizer exposure: incidence and management. Clin Toxicol. 2021. doi: 10.1080/15563650.2021.1916518