Toxichem Krimtech 2021;88(3):150

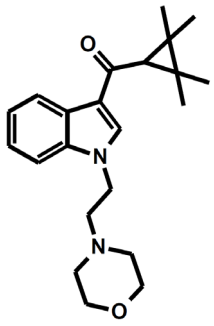
Bester Vortrag auf dem GTFCh-Symposium 2021

Optimization of a high content screening assay for studying the cytotoxicity of new psychoactive substances – First results for the synthetic cannabinoid A-796260

Tanja M. Gampfer[1], Lea Wagmann[1], Anouar Belkacemi[2], Veit Flockerzi[2], Markus R. Meyer[1]

[1]Department of Experimental and Clinical Toxicology, Saarland University, Homburg
[2]Department of Experimental and Clinical Pharmacology, Saarland University, Homburg

Introduction and Aims: New psychoactive substances (NPS) are usually brought onto the drugs of abuse market without any safety testing, hence knowledge about their potential cytotoxicity is sparce or even unknown. Therefore, a health risk for consumers could not be excluded. Also, some case reports associate their ingestion with an acute organ toxicity [1,2]. There exist different in vitro approaches to measure cytotoxicity parameters such as cell viability, leakage of lactate dehydrogenase, mitochondrial membrane potential, mitochondrial redox activity, or apoptosis. However, many previous studies only investigated isolated parameters as single endpoints [3,4], which could lead to false negative or positive results. By using a high content screening assay (HCSA), this major drawback could be avoided, since several parameters are monitored simultaneously within the same experiment [5]. Therefore, the present study aimed to improve an existing HCSA, to study the cytotoxic potential of NPS using the hepatoma cell line HepG2 [6]. The model compound fluvastatin was used for method optimization to achieve a simplified use, increased sample throughput, and improved reproducibility. Afterwards, the applicability of the method was exemplified for the synthetic cannabinoid (SC) A-796260 (structure see Figure).



A-796260

Methods: HepG2 cells were seeded in self-coated imaging plates in a density of 1500 or 1750 cells/well and incubated for 24 h in RPMI medium. Afterwards, cells were treated with fluvastatin (0.14, 1.23, 3.7, and 11.12 µM) or A-796260 (1.95, 3.91, 7.81, 15.6, 31.3, 62.5, and 125 µM) for 48 h or 72 h. Thereafter, the cells were incubated for 1h with a fluorescent dyes cocktail (0.8 µM Hoechst33342, 20 nM TMRM, 1 µM CAL-520, 1 µM TOTO-3). Cell plates were analyzed using a full automated epifluorescence microscope (BioTek Lionheart FX Automated Microscope) by means of a 20x/0.45 objective. The following six parameters were monitored: cell count, nuclear size, nuclear intensity, mitochondrial membrane potential, cytosolic calcium levels, and plasma membrane integrity. Hoechst33342 was used for identification of number and area of regions to define cell count and nuclear size. Nuclear intensity, mitochondrial membrane potential, cytosolic calcium levels, and plasma membrane integrity were measured by the total fluorescence intensity of Hoechst33342, TMRM, CAL-520, and TOTO-3, respectively. Six images/well were collected and analyzed by comparison of blank incubation (untreated) to treated incubations.

Results and Discussion: Fluvastatin was used to improve the former HCSA method, such as a reduced drug exposure from 72 h to 48 h to prevent cells from multilayer formation. As a consequence, the number of seeded cells was adjusted from 1500 cells/well to 1750 cells/well.

Also, a higher level of automation was achieved by using a fully automated epifluorescence microscope instead of a half-automated one. This optimized HCSA method was used to assess the cytotoxic potential of the SC A-796260, which significantly impaired the mitochondrial membrane potential, cytosolic calcium levels, and nuclear intensity of HepG2 cells in concentrations lower than 10 µM. As maximum plasma concentrations of NPS are usually unknown, cytotoxicity assessment criteria were based on a previous study [6]. Thus, the cytotoxic potential of A-796260 was ranked as high, as already observed for other SC such as 5F-PB-22 [6].

Conclusion: A-796260 significantly affected three parameters in HepG2 cells below 10 µM. The most sensitive parameters turned out to be the mitochondrial membrane potential and cytosolic calcium levels. Thus, a cytotoxicity after consumption of A-796260 could not be excluded. The optimized HCSA should now be used to systematically study the cytotoxic potential of further NPS.

References

[1] Frohlich S, Lambe E, O'Dea J (2011) Acute liver failure following recreational use of psychotropic "head shop" compounds. Ir J Med Sci 180 (1):263-264. doi:10.1007/s11845-010-0636-6

[2] Solimini R, Busardo FP, Rotolo MC, Ricci S, Mastrobattista L, Mortali C, Graziano S, Pellegrini M, di Luca NM, Palmi I (2017) Hepatotoxicity associated to synthetic cannabinoids use. Eur Rev Med Pharmacol Sci 21 (1 Suppl):1-6

[3] Araujo AM, Valente MJ, Carvalho M, Dias da Silva D, Gaspar H, Carvalho F, de Lourdes Bastos M, Guedes de Pinho P (2015) Raising awareness of new psychoactive substances: chemical analysis and in vitro toxicity screening of 'legal high' packages containing synthetic cathinones. Arch Toxicol 89 (5):757-771. doi:10.1007/s00204-014-1278-7

[4] Grafinger KE, Mandhair HK, Broillet A, Gertsch J, Weinmann W (2019) Cytotoxicity of the synthetic cannabinoids 5C-AKB48, 5F-MDMB-PINACA, ADB-CHMINACA, MDMB-CHMICA and NM-2201 in A549 and TR146 cell lines. Forensic Toxicology 37 (2):398-411. doi:10.1007/s11419-019-00474-1

[5] O'Brien PJ, Edvardsson A (2017) Validation of a Multiparametric, High-Content-Screening Assay for Predictive/Investigative Cytotoxicity: Evidence from Technology Transfer Studies and Literature Review. Chem Res Toxicol 30 (3):804-829. doi:10.1021/acs.chemrestox.6b00403

[6] Richter LHJ, Beck A, Flockerzi V, Maurer HH, Meyer MR (2019) Cytotoxicity of new psychoactive substances and other drugs of abuse studied in human HepG2 cells using an adopted high content screening assay. Toxicol Lett 301:79-89. doi:10.1016/j.toxlet.2018.11.007

Kurzvita der Preisträgerin Tanja M. Gampfer. Briefly, I was born in Homburg (Saar), but spent my childhood in a small village in the Palatinate. With twenty, I started my study of pharmacy at the Saarland University in Saarbrücken and graduated in 2013. Afterwards, I worked a few years in public pharmacies. In 2018, I returned to the Saarland University and started my PhD thesis at the Experimental and Clinical Toxicology Lab under the supervision of Prof. Markus R. Meyer, whom I am very grateful to for the opportunity offered.

Danksagung. First, I would like to thank the GTFCh Committee for the great honor to receive the Award for the Best Oral Presentation at the GTFCh-Symposium 2021. Also, I would like to express my gratitude to all of my colleagues for their support.