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Late Abstracts

P-Late-01

Synthetic cannabinoids enhance neuronal differentiation in neuroblastoma cells at in vivo relevant concentrations

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Synthetic cannabinoids (SCs) comprise a group of new psychoactive substances that bind and activate at least one cannabinoid receptor, with stronger potency than tetrahydrocannabinol (THC), the main psychoactive substance of Cannabis sativa L. SC consumption by pregnant women or women in reproductive age is of major concern due to the potential onset of neurodevelopment disorders in the offspring. This work aimed at investigating how SCs affect neuronal differentiation in vitro.

Briefly, neuroblastoma (NG108-15) cells were exposed to commonly reported SCs, including THJ-2201, JWH-122, 5F-PB-22, MDMB-CHIMCA and ADB-FUBINACA (the latter two kindly provided by TicTac Communications Ltd, UK) at a concentration range of 1pM – 100μM. Differentiation ratios were calculated as the number of newly formed neurites per total cell number, following differentiation with forskolin and retinoic acid, with or without SC. These were either added once (at day 0) or every 24h for 3 days (3 additions), at in vivo relevant concentrations (< 1μM). In part of the experiments, 500 nM SR141718, a specific CB1 receptor (CB1R) antagonist, was added 20 min prior to SC exposure. Different toxicological parameters, including cell viability, mitochondrial membrane potential and cellular energy levels, were also analyzed.

Increased differentiation of NG108-15 cells was observed following a single addition of 1pM THJ-2201 (2-fold increase), as well as 3 additions of 1pM and 1nM of 5F-PB-22. Preliminary assays in the presence of SR141716 suggest these effects may be modulated by CB1R. None of the other tested SCs altered neurodifferentiation. None of the SCs reduced cell viability up to a concentration of 10 μM, as indicated by MTT reduction, Neutral Red inclusion and LDH release assays. On the other hand, a statistically significant increase in mitochondrial membrane potential (TMRE staining) was observed following 24h incubation with all the SCs, except ADB-FUBINACA, particularly at lower concentrations (<1nM). Interestingly, no alterations were detected on intracellular ATP levels.

Overall, these results indicate the enhancement of neuronal cells' differentiation by SCs, involving the regulation of mitochondrial function and possibly CB1R-mediated. However, clarification of the pathways involved demands further investigation.

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P-Late-02

Metabolomic analysis of the toxicity pathways elicited by subtoxic concentrations of methylone in primary mouse hepatocytes

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Metabolomics is the comprehensive study of the metabolome, which encompasses the repertoire of low-molecular weight molecules required for the maintenance, growth and normal cellular function. One of the major goals of metabolomics is the discovery of specific metabolic alterations associated with a disease or other external stimuli. The aim of the present study was to identify metabolic changes caused by subtoxic concentrations of methylone (the ß-keto analogue of 3,4-methylenedioxymethamphetamine (MDMA)) in primary mouse hepatocytes (PMH), in order to investigate the base of its hepatotoxic mechanisms, since its use has been associated with several cases of toxicity (including hepatotoxicity) and deaths. For this purpose, a non-targeted metabolomic approach, based on gas chromatography/mass spectrometry (GC-MS), was performed after exposing PMH to two subtoxic concentrations (corresponding to LC01 and LC10 levels assessed by the MTT assay) of methylone for 24 h. Both the intracellular (metabolites within the cell) and extracellular (metabolites excreted into or consumed from the surrounding extracellular medium) metabolome were analyzed in order to obtain a more complete mechanistic view. Obtained results showed that metabolic patterns of methylone exposed cells are separated from control in a concentration-dependent manner. Most of the discriminant metabolites belong to the class of amino acids, fatty acids, organic acids (intracellular metabolome), alkanes, alcohols, aldehydes and ketones (extracellular metabolome) that are involved in energy and antioxidant metabolism.

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