Using 3D Neural Spheroid Cultures to assess Harmane Neurotoxicity

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Introduction:

β-Carbolines alkaloids (βCAs) are indole alkaloids, such as harmane, that naturally occur in plants and food and are also endogenously produced in mammals and humans. Some βCAs are potent tremor inductors and exhibit neurotoxicological activities. They have been incriminated in the pathogenesis of Essential tremor (ET) as blood Harmane levels have been found to be abnormally increased in ET patients. Harmane has also been detected in post-mortem brain samples. Essential tremor (ET) is one of the most prevalent neurological diseases but, despite a high prevalence, its physiopathology remains poorly understood. There is converging evidence that both genetic and environmental factors play a role in ET pathogenesis and so the identification of involved environmental factors is a critical step towards risk reduction through the implementation of prevention strategies or interventional studies. 2D cell cultures do not represent the real cell environment where cells spatially and chemically interact; their lack in predictivity increases the cost and failure rate of clinical trials, especially in neurosciences. Recently, 3D cell cultures have received much attention, as these are closer to tissues models, although their main challenges reside in end-point measurements.

Methods:

We propose the use of a 3D neural spheroid cultures (*"mini-brains"*) *in vitro* model to assess the neurotoxic effects of harmane. We have generated a model of 3D spheroids from embryonic mouse cortical neurons using micro-molded agarose wells. After exposure to harmane at increasing concentrations of 50, 100, 250 µM, a resazurin assay was performed to measure cell viability and a highly sensitive fluorometric method, based on the oxidation of di-chlorodihydrofluorescein DCFH, was used to measure eventually induced reactive oxygen species (ROS).

Results:
Hydrogel microwells facilitated the assembly of spheroids containing neurons and glia into a complex 3D structures and prevented the agglomeration of spheroids.

Exposure to harmane induced a cytotoxicity in 3D neural spheroids that was correlated with harmane concentrations, with a viability down to 24 % at 250 µM. On

the other hand, lower levels of oxidative stress were detected as the harmane concentration increased, although the effect was limited in time.

Conclusions:

We demonstrated our ability to produce a 3D culture using simple materials and routine laboratory equipment. Despite high challenges related to 3D cultures and data acquisition, this 3D neural spheroid model mimics a neuronal microenvironment, allowing a fine study of neurodegenerative disorder pathologies and the effect of chemicals on the brain. The viability and oxidative stress data support so far a dual role of harmane, as both neurotoxic and neuroprotective agent. Further studies are required to elucidate its biological activities and their eventual relationship with the ET disorder.