

Effect of Papaverine on Developmental Neurotoxicity: Neurosphere as in vitro Model

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Abstract : Background: Developmental neurotoxicity (DNT) entails the toxic effects imparted by various chemicals on brain during the early childhood when human brains are vulnerable during this period. DNT study in vivo cannot determine the effect of the neurotoxins, as it is not applicable, so using the neurosphere cells of lab animals as an alternative is applicable and time saving. Methods: Cell culture: Rat neural progenitor cells were isolated from rat embryos' brain. The cortices were aseptically dissected out and the tissues were triturated. The dispersed tissues were allowed to settle. The supernatant was then transferred to a fresh tube and centrifuged. The pellet was placed in Hank's balanced salt solution and cultured as free-floating neurospheres in proliferation medium. Differentiation was initiated by growth factor withdrawal in differentiation medium and plating onto a poly-d-lysine/ laminin matrix. Chemical Exposure: Neurospheres were treated for 2 weeks with papaverine in proliferation medium. Proliferation analyses: Spheres were cultured. After 0, 4, 5, 11 and 14 days, sphere size was determined by software analyses (CellProfiler, version 2.1; Broad Institute). Diameter of each neurosphere was measured and exported to excel file further to statistical analysis. Viability test: Trypsin-EDTA solution was added to neurospheres to dissociate neurospheres into single cells suspension, then viability evaluated by the Trypan Blue exclusion test. Result: As regards proliferation analysis and percentage of viable cells of papaverin treated groups: There was no significant change in cells proliferation compared to control at 0, 4, 5, 11 and 14 days with concentrations 1, 5 and 10 μ M of papaverine, but there is a significant change in cell viability compared to control after 1 week and 2 weeks with the same concentrations of papaverine. Conclusion: Papaverine has toxic effect on viability of neural cell, not on their proliferation, so it may produce focal neural lesions not growth morphological changes.

Keywords : developmental neurotoxicity, neurotoxin, papaverine, neuroshperes

Conference Title : ICT 2014 : International Conference on Toxicology

Conference Location : London, United Kingdom

Conference Dates : September 26-27, 2014