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Using Cryopreserved Neuronal Cells for Assessment of Chemically Induced Oxidative Cell Stress in Primary Cell Culture. Anthony Krantis, William Staines, Susan VanderHoek, Tony Durst. Centre for Research in Biopharmaceutics and Biotechnology, University of Ottawa, Ontario Canada. K1Y 0Z3

Cryopreserved primary neuronal cells from the rat brain represent an ideal and sensitive cell culture assay for screening drug neuroactivity. When thawed and grown in cell culture, these dissociated neuronal cells display normal morphology, neurochemistry and physiological responsiveness within 10 days. We have obtained similar results with neuronal cells dissociated from the mouse brain. However, the susceptibility of cryopreserved mouse brain neuronal cells to neurotoxic stress in primary culture is unknown. Such information would be useful in determining their suitability for employment in assay screens for chemically induced neurotoxicity.

On this basis, we examined cryopreserved neuronal cells prepared from mouse and rat cortex for the effects of exposure to a classic oxidative stress, hydrogen peroxide (H_2O_2), and to a series of novel compounds for their putative oxidative vs protective properties. Neuronal cells dissociated from brain cortex and cryopreserved by QBM Cell Science, were thawed and cultured in 96 well plates (100,000 cells/well) for 10days. At this stage they displayed typical neuronal neurite networks and morphology. Wells were then treated for 24hrs with H_2O_2 (50-100μM), or with novel compounds over a range of concentrations (0.1-50μg/ml). The wells were then assayed using the OxyDNA (Biotrin) fluorescence-based assay for detection of DNA damage. Typical cell damage and disruption of neurite networks were evident with H_2O_2 50μM as monitored by phase contrast microscopy. A concentration dependent increase in fluorescence associated with 8-oxoguanine fragments, typical of DNA damage due to free radical action was also evident. The novel compounds could be classified as either protective or deleterious. These results show the sensitivity of cryopreserved rodent brain neuronal cells for plate based cell culture assay of oxidation induced neurotoxicity.