MINIATURIZED CYTOMIC ASSAYS OF OXIDIZED DNA-BASE 8-OXOGUANINE BY FLUORESCENCE BIOIMAGING AND FLOW CYTOMETRY

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96-well plate assays are very common and efficient approaches for screening of in vitro cytotoxicity of xenobiotics. However, those assays provide average data in entire wells and do not resolve heterogeneity in cell sensitivity to toxicants nor detect small percentages of damaged cells per well. Cytomic techniques such as high-content bioimaging assays (HCA) and flow cytometry (FCM) based on single-cell, multiparametric fluorescence measurements of large and heterogeneous cell populations have been recently implemented for miniaturized assays of cytotoxicity in vitro.

Oxidative damage to DNA is observed in vitro during mutagenesis, carcinogenesis and cytotoxicity caused by several different factors including chemicals, radiation, and normal metabolic activity. In vivo it may result from several conditions that involve the generation of oxygen free radicals, including autoimmune, inflammatory and neurodegenerative diseases, as well as atherosclerosis and post-ischemic injury. 8-oxoguanine (8-oxoG) is a major product of oxidative DNA damage and a useful marker of DNA oxidation. 8-oxoG is derived from the attack of hydroxyl radical (•OH) to guanine at the C8-position, resulting in a C8-OH-adduct radical. Previous techniques for the detection of 8-oxoG, such as HPLC and gas chromatography mass-spectrometry, are complex and time-consuming, and do not inform on cell heterogeneity. The OxyDNA Assay (Calbiochem) is a new fluorescent assay for the detection of oxidative damage to DNA in vitro. The assay is based upon the direct binding of a FITC-labelled antibody to 8-oxoG epitopes in the DNA of fixed cells. Unlike other procedures which require extraction or purification of DNA from biological specimens, OxyDNA Assay is performed directly on cells.

We have developed two complementary HCA and FCM assays for the studies of oxidative damage to DNA. The assays are performed on adherent cells growing on 96-well plates. For this particular work human established cell lines of hepatic (HepG2), neuronal (SH-SY5Y) and renal (A704) origin were grown in 96-well plates and acutely exposed to oxidative stress inducers or to a combination of methylene blue plus light (positive control). For HCA, cells were fixed in situ, stained with FITC-labelled anti-8-oxoG antibody and nuclei counterstained with DAPI or propidium iodide. HCA was performed with a InCell Analyzer 1000 (GE Healthcare) with appropriate algorithms for cell segmentation and data calculation. For FCM cells remained attached during exposure to the toxicants and are trypsinized and resuspended in appropriate medium for fixation, fluorescent staining and analysis using a novel flow cytometer interfaced to a 96-well plate loader (Cytomics FC500, Beckman-Coulter). Both assays provided complementary information and allowed for the consistent detection and quantification (EC50 values) of the cytotoxic effects of different compounds that might be mediated by intracellular peroxidation. Sponsored by European Commission (FP 6 Integrated Project A-Cute-Tox, LSHB-CT-2004-512051), Ministerio de Sanidad-Generalitat Valenciana (Programa de Medicina Regenerativa de la Comunidad Valenciana) and GE Healthcare.