

## ESTABLISHMENT OF THE SINGLE-CELL GEL (COMET) ASSAY TO DETECT PRIMARY DNA DAMAGE IN GLIAL CELLS.

Pitanga, B.P.S., Silva-Neto, J.P., Cleide, S.S., Silva, A.R., Costa, M.F.D., El-Bachá, R.S., and Costa, S.L.

Laboratório de Neuroquímica e Biologia Celular, Universidade Federal da Bahia, Salvador-BA-Brazil

The comet test has been utilised on toxicological studies to investigate genotoxic and genoprotector substances. The glial cells are essential for development, homeostasis and detoxification in the central nervous system (CNS). In this work we established a protocol of single-cell gel (SGC)/comet assay in GL-15 glioblastoma cell line to assess DNA integrity and single-strand breaks induced by genotoxic agents. GL-15 cells (100,000 cells/plate) were grown in supplemented DMEM medium and directly exposed to ultraviolet radiation (UV), a well-known genotoxic agent. After alkaline electrophoresis and DNA staining with ethidium bromide, the nucleoids were identified for DNA damage and tail migration at different levels. UV induced DNA damage in the majority of the cells, with a proportion of 66% and 97% after 24h and 72h exposure, respectively. DNA strand breaks observed after UV exposure were especially of level 4 (78.4%). On the other hand the frequency of comets in the negative control (0.5% DMSO) was 13% and 29% after 24h and 72h exposure, respectively, with DNA damages specially distributed at levels 2-4 (6.7-11.2%). DNA damage may trigger genomic instability, a crucial step in carcinogenesis, the present comet assay, may be able to contribute to genotoxicity studies for this important population of CNS cells. Supported by FAPESB and Depto. Patologia UNESP/Botucatu.