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Effect of high-energy radiation on the formation of 8hydroxy-deoxyguanosine

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Reactive oxygen species (ROS) cause mutation of the DNA bases, which is implicated in carcinogenesis and variety of age-related disorders. 8-hydroxy 2'-deoxyguanine (8- OHdG) is a modified form of the guanine base that forms due to cellular exposure to ROS and is used as a biomarker for oxidative stress. Clinical radiation treatments expose cells to high levels of ROS, leading to an accelerated accumulation of 8-OHdG mutation. The objective of this study is to investigate the effect of high energy radiation on the rate of 8-OHdG formation in a human astrocytes. An enzyme-linked immunosorbent assay (ELISA) was performed in plate reader equipped with absorbance detection was used to quantify the level of 8-OHdG accumulation in cells treated with 0.5 Gy and 3 Gy proton and photon radiation to compare with normal, untreated cells. Reverse transcription quantitative PCR (RT-qPCR) analysis was applied to assess the mRNA expression levels of 8- oxoguanine glycosylase (OGG1) in both the treated and non-treated cells. OGG1 is an enzyme of the base excision repair pathway that main function is to remove 8-OHdG mutation. Decreased levels of OGG1 expression correlates with increased accumulation of 8-OHdG which leads to an increased level of cellular damage. In this study, human astrocytes were cultured and transported to Willis-Knighton Cancer Center in Shreveport for radiation treatment with 0.5 Gy and 3 Gy proton and photon radiation. The cells were incubated for 14 hours in humidity incubator and the analyzed. Cell media was collected and the concentration of oxidative damage by-product was analyzed using ELISA, with the results showing a steady increase of 8-OHdG accumulation as with the increased radiation dosage. The cells were lysed and RNA was purified using Qiagen total RNA purification kit. The results of this analysis reveal a strong trend of decreased levels of OGG1 expression as radiation dosage increased.