Resveratrol Enhances the Chronic Citotoxic Effect of Temozolomide in Glioma Cells: Role of Senescence and DNA Damage

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Gliomas are the most common and malignant tumors of the central nervous system. Temozolomide (TMZ), the chemotherapeutic of choice to gliomas, improves the survival of the patients in only 4 months. Recently we demonstrated that the polyphenol Resveratrol (Rsv) exerts a cytotoxic effect on human glioma cells through induction of autophagy and modulation of cell cycle. Moreover, we observed that Rsv potentiates the acute effect of TMZ through modulation of cell cycle and induction of mitotic catastrophe. The aim of this study is to evaluate the long-term effects of cotreatment with TMZ and Rsv in glioma cells in vitro. For this, U87 human glioma cell line was treated with Rsv 30 µM (R30), TMZ 100 µM (T100) or cotreatment (T100R30) for 48h, followed by replating on drug-free medium (DFM). After 7 and 15 days, respectively, the induction of senescence and the proliferative potential were evaluated by beta-galactosidase staining and clonogenic assay. TMZ induced senescence after 7 days in DFM. Addition of Rsv enhanced TMZ-induced senescence from 60±4% to 92±6%. This effect was accompanied by a reduction in the clonogenic potential of the U87 cells: while Rsv and TMZ reduced the colony formation to 56±% and 10±% in relation to control, on the co-treatment there were no colony formation. Co-treatment did not alter intracellular reactive oxygen species levels, measured by DCFH assay through flow cytometry, suggesting that oxidative stress was not involved in the cotreatment-induced senescence. On the other hand, despite cotreatment induced similar levels of DNA damage, measured by alkaline comet essay, levels of histone H2AX phosphorylated (a marker of double-strand DNA breaks), phospho-ATM and phospho-Chk2 were increased in T100R30-treated cells when compared to treatments isolated. In conclusion, Rsv potentiated the chronic cytotoxic effect of TMZ by induction of senescence, probably mediated by an increase of DNA damage.

Key-words: Glioma, Resveratrol, Temozolomide