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Novel non-nucleoside MGMT inhibitors: potential in combined alkylating chemotherapy

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Aim. O^6-methylguanine-DNA methyltransferase (MGMT) is an inducible repair enzyme that removes alkyl residues from DNA, thereby reducing the effectiveness of alkylating chemotherapy. Therefore, the MGMT inhibitors are used in medical practice. However, the most common of them, O^6-benzylguanine and its analogs, have proven to be toxic to hematopoietic cells. That is why the search for new alternative inhibitors is essential. **Methods.** New non-nucleoside potential MGMT inhibitors were developed using semi-flexible docking. From an initial pool of 98 inhibitors, the most cytotoxic ones were screened out using the MTT-test and the clonogenic assay. Subsequently, a selected number of the inhibitors were tested alone and in combination with alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) for investigation of the efficacy. This phase included clonogenic assay, Western blot analysis, and studies on their effects on autophagy and cell mortality in cancer cells *in vitro* using Monodansylcadaverine dying for autophagosomes and dying by Live-Dead Imaging Cell Kit respectively. In the final stage of the research, the efficacy of the combination therapy was evaluated using an *in vivo* ICR mice model. The tumor growth dynamics and changes in MGMT and other proteins level were investigated via Western blot. **Results.** Based on the data obtained, a number of new MGMT inhibitors exhibited low cytotoxicity and high efficacy at a concentration of 10 μM *in vitro* compared to O^6-benzylguanine. Western blot analysis indicated that the inhibitors 41, 41B, 72, and 89 reduced MGMT level in the HEp-2 laryngeal carcinoma cells and T98G glioma cells compared to the controls. The combined treatment with the inhibitors and MNNG induced a high level of autophagy in the T98G cell line (glioma cells with high *MGMT* expression). However, a low level of autophagy was observed under the same conditions in U251MG cells (glioma cells lacking *MGMT* expression). The inhibitors did not increase the level of dead cells in either glioma cell line. Nevertheless, the combined treatment resulted in a high death rate exclusively in T98G cells. *In vivo*, the combined therapy with inhibitors 41, 41B, and 89 led to a significant reduction in tumor growth or remission, with inhibitor 89 more frequently achieving complete remission. Western blot analysis of treated tumors revealed decreased MGMT levels and increased cleaved caspase 3, suggesting apoptosis induction. **Conclusions.** The new low molecular weight non-nucleoside MGMT inhibitors effectively reduce MGMT protein levels and enhance the cytotoxic effects of MNNG across different cancer cell lines. In T98G cells, the combination treatment increases autophagy and makes cells more sensitive to MNNG. The *in vivo* study confirms the therapeutic potential of these inhibitors, with significant tumor reduction or remission observed. Inhibitor 41B demonstrated the greatest overall efficacy, making it a promising candidate for further development in the combined alkylating chemotherapy.

Keywords: MGMT, non-nucleoside inhibitors, alkylating chemotherapy, HEp-2, T98G, U251MG, ICR mice, tumor reduction.