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Molecular dosimetry of alkylating agents: Quantification of O6-methylguanine and correlation with DNA double-strand breaks, apoptosis and senescence

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Alkylating agents are potent environmental mutagens that are also used in cancer chemotherapy. One of the methylating drugs applied 1st line in brain cancer therapy is temozolomide (TMZ). Similar to other SN1 alkylating agents it induces N- and O-base adducts, including O6-methylguanine (O6MeG). Although being a minor lesion, O6MeG is responsible for almost all genotoxic, cytotoxic and cytostatic effects caused by the so-called O6-methylating agents. How many O6MeG adducts are required to induce specific cell responses was not precisely known. Therefore, we determined the dose-dependent formation of O6MeG in glioblastoma cells by mass spectroscopy and set it in relation to DSBs, p53Ser15 phosphorylation, apoptosis/necrosis and cellular senescence. Our results indicate a linear increase of O6MeG, along with DSBs and p53Ser15 phosphorylation. Apoptosis/necrosis and senescence also increased linearly, with senescence being the main response induced. A possible threshold for the induction of apoptosis in A172 was indicated by the Hockey-stick model at a concentration of 2.5 µM TMZ, while no threshold was detected in LN229 cells. In all cell lines no threshold was detected for the endpoint senescence. In A172 cells, treatment with 20 µM TMZ induced 14.000 O6MeG adducts, which gave rise to 32 DSBs (measured by yH2AX and 53BP1 foci), 12 % cell death and 35 % senescence. In LN229 cells, 20 µM of TMZ induced 20.600 O6MeG adducts, 66 DSBs, 24 % cell death and 52 % senescence.

Since this dose of TMZ approximates the serum concentration that can be achieved in cancer therapy, the data can be translated to the therapeutic situation, indicating that the intra-tumoral concentrations of TMZ trigger a significant amount of cytotoxic and cytostatic responses such as apoptosis and senescence.