

## **Hyperbaric oxygen as the way to overcome the problem of hypoxia-induced protection of glioblastoma cells against modified isothiourea derivative (ZKK-3)**

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**BACKGROUND:** High-grade gliomas are the most malignant primary brain tumours with poor prognosis for patients survival and therapy response. Transport of oxygen within solid tumour mass is significantly limited leading to hypoxic conditions. Lack of oxygen promotes the formation of mechanisms associated with higher resistance to standard chemo- and radiotherapy of neoplastic cells. It gives ground to consider if increasing oxygen partial pressure could give a positive therapeutic effect in the treatment of malignant gliomas.

Pentabromobenzylisothioureas are the group of novel S-substituted isothiourea derivatives. It was documented that some of that compounds show proapoptotic activity and substantial cytotoxicity toward various kinds of tumour cells in vitro, including human glioblastoma.

**HYPOTHESIS:** Hyperbaric oxygenation can exert enhancement of anti-tumour potential of selected pentabromobenzylisothiourea against malignant glioma cells.

**METHODS:** Experiments were conducted on human glioblastoma T98G cell line (WHO grade IV). Cells were cultured in medium supplemented with N,N'-dimethyl-S-(2,3,4,5,6-pentabromobenzyl)-isothiourenium bromide (ZKK-3). Three gas mixtures with various oxygen contents were tested: 1/ normoxic gas (21% O<sub>2</sub>/5% CO<sub>2</sub>/78% N<sub>2</sub>, 24 hours), 2/ hypoxic gas (1% O<sub>2</sub>/5% CO<sub>2</sub>/94% N<sub>2</sub>, 24 hours), 3/ hyperbaric oxygen (HBO, 97,5% O<sub>2</sub>/2,5% CO<sub>2</sub>, pressure of 3 ATA, 1 hour followed by 23 hours of normoxia). Proliferation and viability of neoplastic cells subjected to hypoxia or hyperbaric oxygenation were compared with those sustained in standard normoxic conditions. Direct comparisons between hypoxia and HBO results were also performed.

Proliferation of glioma cells was assessed 24 hours after ZKK-3 treatment using Multisizer 3 Beckman Coulter. The viability of T98G cell line was evaluated 24 and 48 hours post ZKK-3 administration by CellTiter 96@Aqueous One Solution Cell Proliferation Assay (Promega).

**RESULTS:** Anti-proliferative properties of ZKK-3 seemed to be slightly inhibited after hypoxic gas administration in comparison to the normoxic conditions. On the other hand, the hyperbaric oxygenation slowed down cells' growth. Those differences were observed for higher concentrations of tested agent, whether compared to normoxia or directly between hypoxia and HBO.

Long-term low oxygen conditions affected also viability of cells, which were more resistant to selected compound than cells maintained in normoxia. In contrast, higher oxygen partial pressure increased cytotoxicity of ZKK-3, especially after higher doses or prolonged exposure to the agent. Parallel juxtaposition of hypoxic and hyperbaric oxygen conditions additionally confirmed that conjectures. In conclusion, hypoxic conditions impair the therapeutic effect of ZKK-3, while HBO appears to enhance the anti-proliferative and cytotoxic efficacy of tested isothiourea derivative compound against malignant glioma cells in vitro. The effect of HBO is more pronounced with higher dose of tested agent and longer duration of treatment.

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