**High-dose ascorbate enhances chemo-radio-sensitization in GBM**

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**BACKGROUND**

Ascorbate is a water soluble vitamin that has been previously shown at milli-molar concentrations to be selectively toxic to a variety of cancer cells, including high grade gliomas, while being innocuous to normal cells. In addition, in vitro experiments with human xenografts and clinical trials with pancreatic cancer demonstrated high concentrations of ascorbate (1–20 mM in the circulation) are well tolerated. Because ascorbate crosses the blood brain barrier, we hypothesized high dose ascorbate would enhance chemo-radio-sensitization in glioblastoma multiforme (GBM).

**MATERIALS & METHODS**

**In vitro studies.** U87MG and U1-581 human GBM cells were grown for 24 h and then exposed to 0.5 mM ascorbate and/or temozolomide, 100 μM, for 1 h followed by 2 Gy of concurrent irradiation. Cells were plated at low density for clonogenic survival immediately after radiation treatment and clones were allowed to grow for 10 to 21 days in maintenance media. Cells were then fixed with 70% ethanol and stained with Coomassie blue for analysis of clonogenic survival. Individual assay colony counts were normalized to that of control with at least 3 cloning dishes per condition.

**In vivo studies.** Female 4-6 week old athymic-nu/nu mice were injected subcutaneously into their flanks with Luc U87MG cells and treated with daily intraperitoneal ascorbate (4 mg/kg) and/or temozolomide (75 mg/m2/day) x 5 days for 2 weeks. Mice were treated with combinations of daily ascorbate (4 g/kg), ionizing radiation (12 Gy/2fx), and/or weekly temozolomide (75 mg/m2) for 2 weeks. Mice maintained their weights and activity levels throughout the course of treatment.

**CONCLUSIONS**

- GBM cells are sensitive to ascorbate in clinically achievable concentrations
- Ascorbate toxicity is mediated through the production of hydrogen peroxide and is at least in part dependent upon the presence of metals
- Ascorbate sensitizes GBM cell lines to ionizing radiation
- Ascorbate sensitizes GBM xenografts to ionizing radiation and temozolomide.

**Figure 1.** GBM cells are sensitive to clinically achievable ascorbate concentrations in vitro. U87MG and U1-581 GBM cells were treated with varying concentrations of ascorbate for 1 h followed by clonogenic survival assay. Error bars represent ± 1 SEM.

**Figure 2.** Catalase rescues GBM ascorbate toxicity. U87MG cells were exposed to 0.75 mM catalase for 1 h followed by 0.5 mM ascorbate exposure and 2 Gy of radiation. Clonogenic cell survival assays were set up immediately following ionizing radiation exposure. Catalase completely rescued ascorbate toxicity (red arrows) and partially rescued ascorbate in combination with radiation toxicity (blue arrows).

* p < 0.05 for ascorbate + 2Gy or 2Gy or 0.5 mM ascorbate.
** p < 0.05 for Catalase + 2Gy or ascorbate + 2Gy.

**Figure 3.** Ascorbate in combination with temozolomide and radiation is well-tolerated in a mouse model. Nude mice (5 animals/group) were injected with Luc U87MG cells into the flank. Mice were treated with combinations of daily ascorbate (4 g/kg), ionizing radiation (12 Gy/2fx), and/or weekly temozolomide (75 mg/m2) for 2 weeks. Mice maintained their weights and activity levels throughout the course of treatment.

**Figure 4.** Ascorbate enhances GBM radiation and temozolomide sensitivity. Nude mice (5 animals/group) were injected with Luc U87MG cells into the left flank. Mice were treated with combinations of IP daily ascorbate (4 g/kg), ionizing radiation (12 Gy/2fx), and/or weekly temozolomide (75 mg/m2) for 2 weeks. Tumor sizes were measured daily with calipers. Tumor sizes in mice treated in combination with ascorbate, radiation and temozolomide (red line) were significantly smaller than mice treated in combination with temozolomide alone (*p<0.0005) (panel A). Once maximum tumor diameter exceeded 1.5 cm in size, the mouse was sacrificed. Panel C, E, & G represent pre-treatment tumor burden in control, temozolomide + radiation therapy, and ascorbate + radiation + temozolomide groups respectively. Panels D, F, & H represent tumor burden at the completion of two weeks of therapy in those same groups.

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