Co-administration of Chemotherapy Agent Bis-chloroethylurea May Enhance 5-Aminolevulinic Acid Photodynamic Therapy in Patient-Derived Glioma Stem Cells

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Disclosures

• The authors have disclosures to report.
Introduction

• 5-aminolevulinic acid-based photodynamic therapy (5-ALA PDT) is a novel laser-based treatment for selective killing of glioblastoma tumors.

• We asked if the co-administration of chemotherapeutic drugs already approved for use in GBM could synergistically enhance 5-ALA PDT-mediated glioma stem cell (GSC) killing.
Methods

• Patient-derived GSC lines were established from intraoperative GBM tumor biopsies.

• Protoporphyrin IX (PPIX) accumulation following 5-ALA exposure was assessed using flow cytometry.

• mRNA expression of enzymes involved in PPIX metabolism or transport was measured with qRT-PCR.
Methods

• For 5-ALA PDT, GSC were incubated in 5-ALA alone or with chemotherapeutic agents bis-chloroethylurea (BCNU) or temozolomide (TMZ) for 6 hours followed by exposure to 635nm laser light.

• GSC killing was assessed using PrestoBlue assay.
Results

• Three patient-derived GSC (GBM 1, 12, 7) were characterized by PPIX fluorescence levels after 5-ALA exposure and enzyme expression.

• Peak fluorescence levels differed significantly in two cell lines, which we termed “high-” and “low-fluorescing” GSC.

• Baseline enzyme expression levels did not differ among cell lines.

Figure 1. Flow cytometry determination of PPIX fluorescence in GSC lines after 5-ALA exposure. Results reported as mean fold increase in PPIX fluorescence (MFI-PPIX) after 5-ALA exposure. Of 3 cell lines tested, the greatest difference in PPIX fluorescence was observed between GBM 1 and GBM 7 (t-test, ***P<0.0001).
Results

- PPIX fluorescence intensity correlated with susceptibility to 5-ALA PDT.

Figure 2. Relationship between PPIX fluorescence and susceptibility to 5-ALA PDT. GSC were incubated in 5-ALA followed by treatment with photodynamic therapy (5-20J). %viability after treatment differed significantly between GBM 1 (“high-fluorescing”) and GBM 7 (“low-fluorescing”) cell lines.
Results

• Combination BCNU and 5-ALA PDT worked synergistically in low-, but not in high-fluorescing, GSC.

• All cell lines were resistant to TMZ, and this drug did not affect 5-ALA PDT killing (not shown).

Figure 3. Evaluation of combination chemotherapy and 5-ALA PDT treatment effect. Representative experiments showing (top) combination BCNU (μM) and 5-ALA PDT (J) treatment. (Top) BCNU and 5-ALA PDT demonstrated an additive effect in “low-fluorescing” GBM 7. (Bottom) This effect was not observed in GBM 1. Representative experiments show mean +/- SEM for 3-4 samples per condition treated. *=P<0.05, **=<0.01, ***=P<0.001, ANOVA with multiple comparisons.
Results

• In high-fluorescing cells, BCNU upregulated expression of heme-oxygenase 1, an enzyme that promotes PPIX catabolism and combats oxidative stress.

Figure 4. Effect of BCNU chemotherapy on enzyme expression. mRNA expression of enzymes involved in PPIX metabolism was compared before and after exposure to 100uM BCNU. Only Heme-oxygenase 1 (HMOX1) expression was significantly upregulated after BCNU exposure in GBM 1 and GBM 7 cell lines. **=P<0.01, ***=P<0.001, ANOVA with multiple comparisons.
Discussion/Summary Points

• We report the first investigation into the effect of combination chemotherapy and 5-ALA PDT on patient-derived glioma stem cells in vitro.

• We demonstrate the potential for synergism between 5-ALA PDT and BCNU.

• Further investigation is needed into the mechanisms underlying treatment synergism.