Comparing Effects of Combination Treatments Containing Alisertib and DNA Damage-Inducing Agents in Glioblastoma Cells

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Abstract

Glioblastoma (GBM) is a malignant primary brain tumor with a poor prognosis and an average survival of only 15 months (Lau et al., 2014). Combination therapies are promising in prolonging patient survival. Currently, the first-line treatment is temozolomide (TMZ), a DNA alkylating agent that disrupts DNA synthesis. However, tumors expressing O6-alkylguanine DNA alkyltransferase (MGMT) tend to repair DNA damage. Previous research has shown that combinations of alisertib, a selective Aurora A inhibitor, with the platinum-based drug carboplatin are effective in inducing growth inhibition in MGMT-expressing GBM cells (Sak et al., 2019). We aimed to compare the effects of combination treatments using alisertib with TMZ, carboplatin, and cisplatin in glioblastoma cells, and future studies should aim to further elucidate its mechanism of action.

Methods

Cells were seeded in 6-well plates and were treated the following day with alisertib, TMZ, carboplatin, or a combination of 2 drugs. After treating for 3 days, media was changed, and cells were fixed and stained with crystal violet. A dissecting microscope was used to count colonies with 20 or more cells.

Active Caspase Detection Assay

Cells were seeded in 6-well plates and were treated the following day with alisertib, TMZ, carboplatin, or a combination of 2 drugs. After 3 or 5 days, cells were collected, and active caspase detection assays were performed using CellEvent Caspase Green Detection Reagent (Invitrogen, C10423). Results were obtained using a Countess II FL cell counter according to the manufacturer’s instructions.

Statistical Analysis

The Chou-Talay and Bliss independent models (Chou-Talay, 1984; Bliss, 1939) were used to perform statistical tests for synergism, which is observed when the effect of two drugs is greater when combined than the additive effects of the single agents.

Results

U1242 cells treated for three days showed increased apoptosis when combined with alisertib in both cell lines tested regardless of MGMT status. In U1242 cells treated for three days, the combination of alisertib and TMZ resulted in a decrease in apoptosis in comparison to alisertib alone. In U87 cells, which do not express high levels of MGMT, the combination of alisertib and TMZ exhibited levels of apoptosis similar to alisertib alone. Additionally, in both cell lines, levels of carboplatin that induced little to no apoptosis on their own showed increased apoptosis when combined with alisertib in both cell lines. Despite similar targets, carboplatin is more effective at inducing apoptosis in combination with alisertib in GBM cells than the combination of alisertib and TMZ. In both cell lines tested regardless of MGMT status, alisertib potentiated the effects of carboplatin more than TMZ. Carboplatin's platinum-based structure is likely important to its cytotoxic properties, and future studies should aim to further elucidate its mechanism of action.

Conclusion

Despite being the current treatment standard, TMZ is not completely effective at inducing GBM cell death, particularly in patients with tumors expressing high levels of MGMT. Considering these results, the combination of alisertib and carboplatin is more promising than the combination of alisertib and TMZ, particularly in cells expressing high levels of MGMT. Since both alisertib and carboplatin have been used previously in clinical trials, these agents are promising for future combined use in a clinical trial. Carboplatin’s platinum-based structure likely contributes to its increased efficacy, and pharmacodynamic studies are needed to elucidate the hypothesis. Since tumor MGMT status is routinely collected, this work can be further developed to help guide clinicians in their treatment decisions.

Future Directions

Collecting data using a larger panel of GBM cell lines with varying levels of MGMT expression will further validate these results. We hope to translate this work in vivo using an in vivo model. Additionally, we plan to further examine the role of alisertib and TMZ's combination in glioblastoma's biochemcial mechanism of action to better understand why it is effective in combination therapy with alisertib in MGMT-expressing cells. We also hope to conduct studies using CRISPR-Cas9 to develop knock-out MGMT cell lines to better understand the role of MGMT status in GBM treatment with these agents.

References


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Figure 1: Colony formation assays were conducted in U1242 cells to test for synergy between alisertib and TMZ, and alisertib and carboplatin. Concentrations are expressed as multiples of the IC50, and percent survival is recorded with respect to untreated controls.

Figure 2: Active caspase detection assays were conducted in U1242 and U87 cell lines. U1242 cells treated for 3 days were treated with 250 nM alisertib, 500 nM TMZ, 10 uM carboplatin, and 500 nM cisplatin, and U1242 cells treated for 5 days were treated with 125 nM alisertib, 25 uM TMZ, 5 uM carboplatin, and 500 nM cisplatin, and U87 cells treated for 3 days were treated with 125 nM alisertib, 25 uM TMZ, 5 uM carboplatin, and 250 nM cisplatin.

Figure 2: Active caspase detection assays were conducted in U1242 and U87 cell lines. U1242 cells treated for 3 days were treated with 250 nM alisertib, 500 nM TMZ, 10 uM carboplatin, and 500 nM cisplatin, and U1242 cells treated for 5 days were treated with 125 nM alisertib, 25 uM TMZ, 5 uM carboplatin, and 500 nM cisplatin, and U87 cells treated for 3 days were treated with 125 nM alisertib, 25 uM TMZ, 5 uM carboplatin, and 250 nM cisplatin.