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), an alkylating agent that interrupts DNA synthesis. However, tumors expressing O6-alkylguanine DNA alkyltransferase (MGMT) tend to respond poorly, as this enzyme repairs DNA damage. Previous research has shown that combinations of alisertib, a selective AURKA inhibitor, and the platinum-based drug carboplatin are effective in inducing growth inhibition in MGMT-expressing GBM cells (Sak et al., 2019).

The aim of this project was to investigate if combinations of alisertib and carboplatin are more effective than alisertib and TMZ in inhibiting GBM cell growth and inducing apoptosis *in vitro*. We examined the effects of combinations of alisertib and TMZ, carboplatin, or cisplatin on apoptosis and cell survival by using colony formation assays and active caspase detection assays. The Bliss and Chou-Talalay models were used to statistically test for synergy in colony formation assays. In colony formation assays, we observed that the combination of alisertib and TMZ exhibited only mild synergy at high concentrations in MGMT-expressing U1242 cells while alisertib and cisplatin exhibited synergy at a much wider range of concentrations in the same cell line with both Bliss and Chou-Talalay analyses. In active caspase detection assays in U1242 cells treated for 3 days, the combination of alisertib and TMZ exhibited 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 did not exhibit an increase in apoptosis. Additionally, we observed that when combined with alisertib, concentrations of carboplatin that induced 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.

Introduction

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. Despite treatment advances in recent years, average survival remains 15 months (Lau et al., 2014). Combination treatment may be the most promising way to extend patient survival. The current first line treatment for GBM is temozolomide (TMZ), a drug that inhibits replication by adding an alkyl group to DNA. However, tumors expressing O6-alkylguanine DNA alkyltransferase (MGMT), an enzyme that repairs this type of DNA damage, tend to respond poorly to TMZ. The platinum-based drug carboplatin adds a platinum group to DNA, which results in DNA cross-linking and inhibits replication. Aurora A (AURKA), a kinase that regulates cell cycle progression and is essential in mitosis, is commonly overexpressed in GBM. For this reason, the AURKA inhibitor alisertib is of particular interest for use in combination therapies in GBM. Previous work has shown that carboplatin and alisertib are effective in MGMT-expressing GBM cells (Sak et al., 2019). This project aimed to compare the effects of combination treatments using alisertib with TMZ, carboplatin, or another platinum-based drug, cisplatin. Using colony formation assays and active caspase detection assays, we have shown that despite similar mechanisms of action, carboplatin is more effective than TMZ when paired with alisertib, likely due to carboplatin's platinum-based structure.

Methods

Colony Formation Assay

Cells were seeded in ninety 60-mm^2 plates at a density of 600 cells per plate and were treated the following day with varying concentrations of single drugs, or a combination of two 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 cells or more.

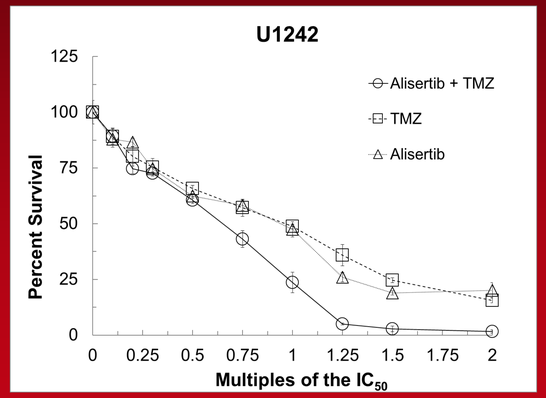
Active Caspase Detection Assay

Cells were seeded in 6-well plates and were treated the following day with alisertib, TMZ, carboplatin, cisplatin, 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-Talalay and Bliss independent models (Chou-Talalay, 1984; Bliss, 1939) were used to statistically test for synergy, which is observed when the effect of two drugs is greater when combined than the additive effects of the single agents.

Results



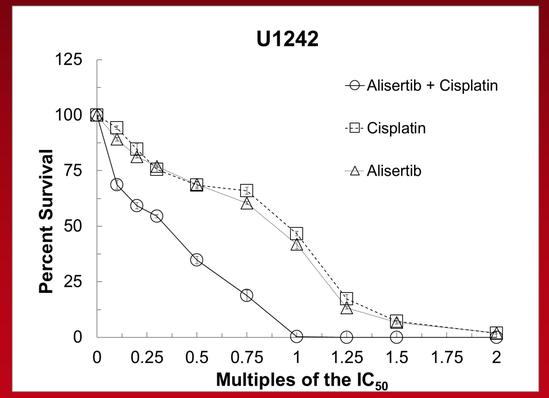
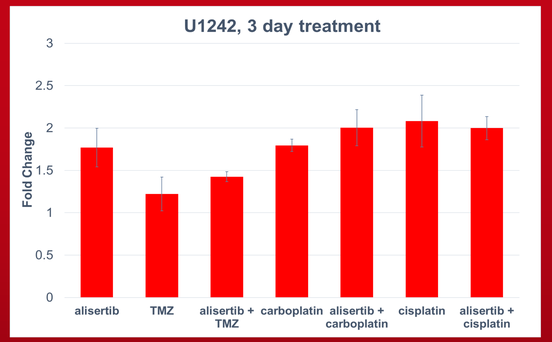
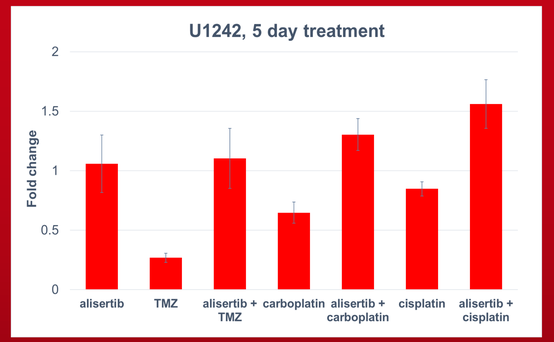
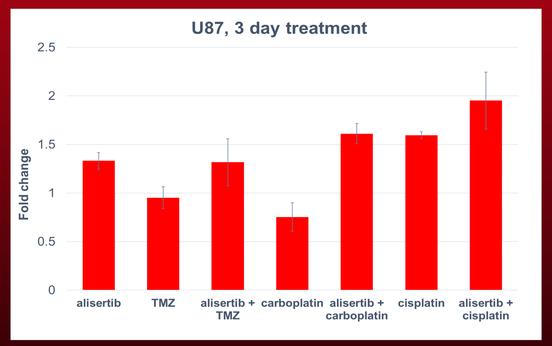


Figure 1: Colony formation assays were conducted in U1242 cells to test for synergy between alisertib and TMZ, and alisertib and cisplatin. Concentrations are expressed as multiples of the IC_{50}, and percent survival is recorded with respect to untreated controls.







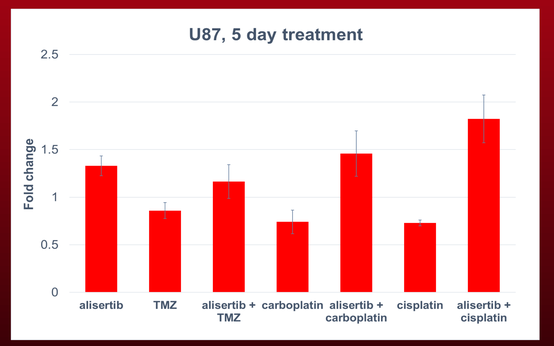


Figure 2: Active caspase detection assays were conducted in U1242 and U87 cell lines. U1242 cells treated for 3 days were treated with 250 nM alisertib, 500 uM TMZ, 10 uM carboplatin, and 500 nM cisplatin, and U1242 cells treated for 5 days were treated with 125 nM alisertib, 250 uM TMZ, 5 uM carboplatin, and 250 nM cisplatin. U87 cells treated for 3 days were treated with 100 nM alisertib, 25 uM TMZ, 5 uM carboplatin, and 500 nM cisplatin, and U87 cells treated for 5 days were treated with 125 nM alisertib, 25 uM TMZ, 5 uM carboplatin, and 250 nM cisplatin.

Results

Colony formation data (Figure 1) showed that in U1242, a cell line expressing high levels of MGMT, alisertib and TMZ only demonstrated mild synergy at high concentrations (≥1.25 times the IC_{50}). However, alisertib and cisplatin, a platinum-based drug similar to carboplatin, exhibited synergy over a greater range of concentrations in U1242 cells. Previous work has shown that alisertib and carboplatin also exhibit strong synergy in U1242 cells (Sak et al., 2019). This suggests that platinum-based drugs are more promising than TMZ for combination therapies with alisertib, particularly for tumors expressing high levels of MGMT.

Active caspase detection assays (Figure 2) showed that the combination of alisertib and TMZ resulted in lower levels of apoptosis in comparison to alisertib and carboplatin 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 U87 and U1242 cells treated at five days, levels of carboplatin that induced little to no apoptosis on their own showed increased apoptosis when combined with alisertib, suggesting that carboplatin may potentiate alisertib's effects. Unlike the three days of drug treatment, TMZ did potentiate alisertib's effects in U1242 cells treated for five days as measured by the active caspase detection assay. However, in colony formation assays, the difference between cisplatin and TMZ in U1242 cells was more pronounced than in the apoptosis assays, and these results were confirmed with rigorous statistical analyses. It is important to note that colony formation assays take more factors affecting cell proliferation into account while the active caspase detection assays only screened for drug-induced apoptosis.

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 further elucidate this 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 orthotopic xenograft mouse model of GBM. Additionally, we would like to further examine carboplatin's biochemical mechanism of action to better understand why it is effective in combination 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.

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