Potentiation of Alisertib by Cabozantinib in Glioblastoma Cells

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Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults. With current treatments, GBM has a high rate of recurrence and a five-year mortality rate of 90%. Refractory GBM may require use of a novel combination of antineoplastic drugs. Previous studies have shown that alisertib, a potent selective Aurora A kinase inhibitor, can inhibit the growth of GBM cells in vitro and in vivo. Cabozantinib was well tolerated in clinical trials as a single agent, making it a good candidate to be used in combination therapies for GBM. VEGFR inhibition is currently in clinical trials for GBM. This study assesses the ability of alisertib to potentiate the effects of the VEGFR2 inhibitor cabozantinib in GBM cell lines. We used annexin V binding assays, and proliferation assays and western blotting under normoxic and hypoxic conditions to analyze the effects of alisertib on potentiating the antineoplastic capabilities of cabozantinib in U87 and U251 GBM cells. In annexin V binding assays, alisertib potentiated the apoptotic effect of cabozantinib in U87 cells. Western blotting confirmed the presence of cleaved PARP, a protein indicative of apoptosis, in U251 cells treated with alisertib, cabozantinib, and alisertib-cabozantinib, but not in untreated cells. Under hypoxia, U251 cells treated with cabozantinib and alisertib-cabozantinib expressed the highest levels of cleaved caspase-3, indicating a higher rate of apoptosis. Potentiation of cabozantinib by alisertib, however, was not seen in U251 cells in proliferation assays under normoxic or hypoxic conditions. These preliminary results do not support a synergistic effect between cabozantinib and alisertib in U251 cells. Further studies utilizing other cell lines and tumor models are needed to determine if the drug combination may be useful for treating GBM.

Results

In U87 cell lines, the combination of alisertib and the VEGFR2 inhibitor cabozantinib was found to not be synergistic in a proliferation assay by one-way ANOVA (multiple comparisons). In U87 cells, however, the effects of alisertib appear to be potentiated by cabozantinib, as demonstrated by annexin V binding assay. Additional experiments and a statistical model are required to confirm these findings.

Future Directions

We will continue to study the effects of alisertib and cabozantinib in further GBM cell lines in additional apoptotic and proliferation models. Colony formation assays, caspase detection assays, and TUNEL assays can be used in the future to measure apoptosis of U87 and U251 GBM cells when treated with alisertib and cabozantinib. If future studies conclude if a synergy exists between alisertib and cabozantinib in treating GBM cell lines, we may conduct in vivo experiments with orthotopic xenograft GBM models.

References


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Abstract

Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults. With current treatments, GBM has a high rate of recurrence and a five-year mortality rate of 90%. Refractory GBM may require use of a novel combination of antineoplastic drugs. Previous studies have shown that alisertib, a potent selective Aurora A kinase inhibitor, can inhibit the growth of GBM cells in vitro and in vivo. Cabozantinib was well tolerated in clinical trials as a single agent, making it a good candidate to be used in combination therapies for GBM. VEGFR inhibition is currently in clinical trials for GBM. This study assesses the ability of alisertib to potentiate the effects of the VEGFR2 inhibitor cabozantinib in GBM cell lines. We used annexin V binding assays, and proliferation assays and western blotting under normoxic and hypoxic conditions to analyze the effects of alisertib on potentiating the antineoplastic capabilities of cabozantinib in U87 and U251 GBM cells. In annexin V binding assays, alisertib potentiated the apoptotic effect of cabozantinib in U87 cells. Western blotting confirmed the presence of cleaved PARP, a protein indicative of apoptosis, in U251 cells treated with alisertib, cabozantinib, and alisertib-cabozantinib, but not in untreated cells. Under hypoxia, U251 cells treated with cabozantinib and alisertib-cabozantinib expressed the highest levels of cleaved caspase-3, indicating a higher rate of apoptosis. Potentiation of cabozantinib by alisertib, however, was not seen in U251 cells in proliferation assays under normoxic or hypoxic conditions. These preliminary results do not support a synergistic effect between cabozantinib and alisertib in U251 cells. Further studies utilizing other cell lines and tumor models are needed to determine if the drug combination may be useful for treating GBM.

Introduction

Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults. With current treatments, GBM has a high rate of recurrence and a five-year mortality rate of 90%. Refractory GBM may require use of a novel combination of antineoplastic drugs. Previous studies have shown that alisertib, a potent selective Aurora A kinase inhibitor, can inhibit the growth of GBM cells in vitro and in vivo. Cabozantinib was well tolerated in clinical trials as a single agent, making it a good candidate to be used in combination therapies for GBM. VEGFR inhibition is currently in clinical trials for GBM. This study assesses the ability of alisertib to potentiate the effects of the VEGFR2 inhibitor cabozantinib in GBM cell lines.

Methods

Anxenin V Binding Assay
Cells were seeded at 1.25×10^5 cells in 96-well plates for 24 hours. Cells were then treated with 500 nM alisertib, 10 μM cabozantinib, or 500 nM alisertib+10 μM cabozantinib for 24 hours. An annexin V binding assay was conducted using Alexa Fluor 488 annexin V conjugate (Invitrogen A13303) according to the manufacturer's instructions. To measure cell apoptosis, we used a Four-FL Cell Couter (Amersham, UK) with a Texas Red light channel that was used to measure the percent of annexin V positive cells. Cells were seeding in triplicate for each drug treatment, and two readings were taken for each sample.

Proliferation Assay under Normoxic and Hypoxic Conditions
Cells were seeded at 1.25×10^5 cells in 6-well plates and treated with 500 nM alisertib, 10 μM cabozantinib, or 500 nM alisertib+10 μM cabozantinib. The samples were then incubated in normoxia (standard conditions: 5% CO2 or hypoxia (2% CO2 and 5% CO2) for 6 hours. Cell survival was then measured with a Countess II FL cell counter.

Western Blotting
10 μl of protein was loaded into a 10% acrylamide gels. Gel electrophoresis was conducted for 90 minutes at 100 volts constant. Transfer took place over night. Primary antibodies were incubated for 90 minutes at room temperature (HF-8-u, Nova BioScience, NB100-109, 1:500; cleaved PARP, Cell Signaling Technology, 9441, 1:1000; VEGF, Abcam, ab1316, 1:1000; Bcl-x; Sigma-Aldrich, sc228, 1:1000; VEGFR2, Cell Signaling Technology, DS11, 1:1000; cleaved caspase-3, Cell Signaling Technology, 9661, 1:1000), while HRP-conjugated secondary antibodies were incubated for 60 minutes (anti-rabbit IgG, Cell Signaling Technology, 7074, 1:200; anti-mouse IgG, Cell Signaling Technology, 7076, 1:200).

Statistical Analysis
The one-way ANOVA multiple comparisons statistical tests was used to test for potentiation and synergy of alisertib by cabozantinib in the proliferation assay under normoxic and hypoxic conditions.

Figure 1: Anxenin V binding assays were conducted in U87 cells to test for potentiation and synergy between alisertib and cabozantinib. U87 cells were treated for one day with 500 nM alisertib, 10 μM cabozantinib, and 500 nM alisertib+10 μM cabozantinib. Percent apoptosis was recorded with respect to untreated controls. n=2.

Figure 2: Western blot evaluation of cleaved PARP and VEGF expression in U251 cells after treatment. U251 cells were treated with 250 nM alisertib, 10 μM cabozantinib, and 200 nM alisertib+15 μM cabozantinib. Administration of a drug is noted by a ‘+’. The combination of alisertib and the VEGFR2 inhibitor cabozantinib was found to not be synergistic in a proliferation assay by one-way ANOVA (multiple comparisons). In U87 cells, however, the effects of alisertib appear to be potentiated by cabozantinib, as demonstrated by annexin V binding assay. Additional experiments and a statistical model are required to confirm these findings.

Future Directions

We will continue to study the effects of alisertib and cabozantinib in further GBM cell lines in additional apoptotic and proliferation models. Colony formation assays, caspase detection assays, and TUNEL assays can be used in the future to measure apoptosis of U87 and U251 GBM cells when treated with alisertib and cabozantinib. If future studies conclude if a synergy exists between alisertib and cabozantinib in treating GBM cell lines, we may conduct in vivo experiments with orthotopic xenograft GBM models.