STRONG CHILDREN'S RESEARCH CENTER

Summer 2014 Research Scholar

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ABSTRACT

Title: *Identifying the Median Lethal Dosage of Common Chemotherapy Drugs in Primary B-cell Acute Lymphoblastic Leukemia*

Background: B-cell acute lymphoblastic leukemia (B-ALL) is the most prevalent childhood malignancy.[1] While many of patients respond well to current chemotherapies (80-90%), there still remains a subset of patients that are more resistant to common therapies after relapse; these relapse patients generally have a significantly reduced survival outcome.[2-4] Furthermore, this has generated a necessity for current research efforts to develop different types of therapies to treat these resistant patients.

Objective: Our group's focus is on therapies that target the bone marrow microenvironment. Since B-ALL is known to only grow in vitro in the presence of mesenchymal stromal cells, this further suggests the importance of the microenvironment to the malignancy. Our group aims to combine siRNA targeting stromal cell genes with common chemotherapy drugs to determine if the combination leads to synergistic effects. This study focused on determining the median lethal dosage for five commonly used chemotherapy drugs: dexamethasone, vincristine, L-asparaginase, 6-mercaptopurine, and methotrexate.

Methods: Immortalized recombinant human mesenchymal stromal cells, with human TERT and GFP, were plated 20,000 per well in a 96-well plate in RPMI with 10% fetal calf serum and 100 moles of hydrocortisone.[5] After 24 hours of incubation, media was removed and the stromal monolayer was washed with RPMI 1640. Subsequently, primary B-ALL cells were plated 25,000 per well in serum-free media (AIM V) on the stromal monolayer. Each chemotherapy drug was diluted in AIM V and placed in the co-culture to obtain desired concentration. B-ALL cells were harvested by removing media from co-culture. Cell viability of B-ALL was then determined using flow cytometry. Cells were stained for 15 minutes with FITC Mouse Anti-Human CD19, PE Mouse Anti-human CD45, and 7-AAD. Viable B-ALL cells were determined by those that excluded 7-AAD and expressed human CD19 and CD45. Student's t-test was performed to determine the statistical significance between cell viability in the absence and presence of the chemotherapy drug.

Results: The median lethal dosages of a primary B-ALL sample were obtained for the following chemotherapy drugs: dexamethasone (6.25ng/mL), vincristine (3ng/mL), L-asparaginase (1IU/mL), 6-mercaptopurine (250uM), and methotrexate (5uM). These lethal dosages were determined from the co-culture of mesenchymal stromal cells with primary B-ALL. All median lethal dosages were statistically significant from co-culture without drug treatment, as determined by student's t-test.

Conclusion: The median lethal dosages obtained have been established for one primary B-ALL sample *in vitro* for dexamethasone, vincristine, L-asparaginase, 6-mercaptopurine, and methotrexate. Moreover, these median lethal dosages may narrow the range for testing other patient samples. Some limitations to this study include: limited number of patient samples used to analyze the median lethal dosage for each of the five drugs. Additionally, this study has only determined viability using one assay: flow cytometry. In the future, we would like to test these chemotherapy drugs with four other B-ALL patient samples. Subsequently, we would like to

combine the chemotherapy drugs at the median lethal dosage with siRNA targeting stromal cell genes and determine the outcome.

References:

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