K562: An In-Vitro AML Model for Studying Synergistic Effects of Doxorubicin & MIA-602

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Introduction

Acute Myeloid Leukemia (AML) is a malignant neoplastic disease arising from myeloid cell lines. AML is characterized by the proliferation of immature, nonfunctional cells in the bone marrow. Excessive proliferation of immature blasts in the bone marrow impairs all other cell lines, resulting in anemia, clotting disorders, and increased susceptibility to infections. Standard of care for treatment involves cytarabine and anthracycline-based regimens. A major concern with such regimens is the acquisition of drug-induced toxicities and subsequent development of drug-resistance. This study aimed to investigate the role of MIA-602, a growth hormone-releasing hormone receptor (GHRH-R) antagonist in the treatment of AML cell line K-562.

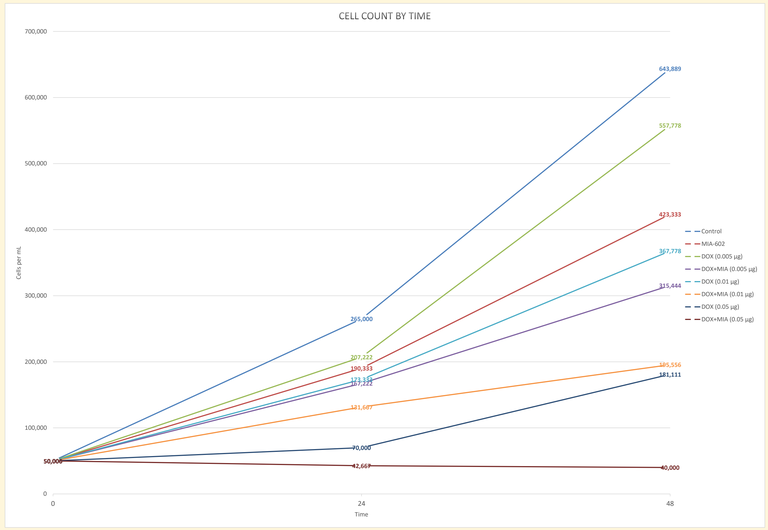


Figure 1: Cell Count Averages of Control, MIA-602, DOX and DOX+MIA at concentrations of 0.005, 0.01 and 0.05 µg of DOX at 24/48 hours.

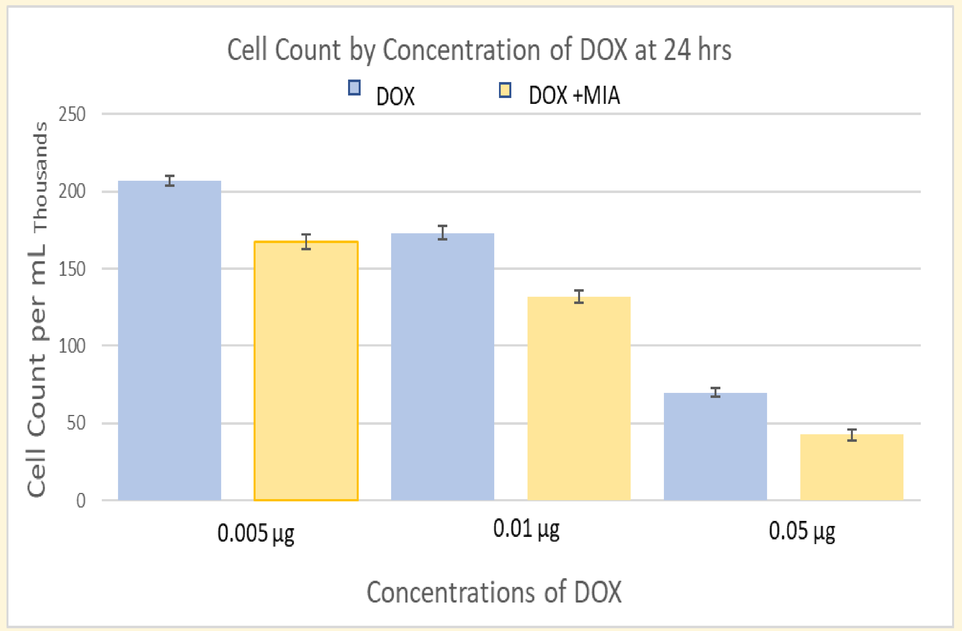


Figure 2: Cell Count Averages of DOX and DOX+MIA at 24 hours by concentration: 0.005, 0.01 and 0.05 µg of DOX. Cell counts decrease as concentration of DOX increases with an additive effect after the addition of MIA-602 in all concentrations of doxycycline.

Design

GHRH-R antagonist MIA-602 was synthesized by solid phase method and purified by reversed-phase high performance liquid chromatography (HPLC). The peptide was dissolved in dimethyl sulfoxide (DMSO) and diluted with incubation media. Doxorubicin was purchased from Sigma-Aldrich.

K562 cells were maintained as a cell suspension in the commercially defined RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS) and gentamicin.

K562 cells were seeded onto six well microplates containing 10% FBS at a density of 5.0 x 10^4 cells/ml. Doxorubicin treatment concentrations were 0.005, 0.01, and 0.05 µg/µl. Combination therapy concentrations 0.005, 0.01, and 0.05 µg/µl of doxorubicin and 5 µmol/L of MIA-602. Proliferation was observed and recorded at 24 hours and 48 hours.

Results

Incubation of cells with doxorubicin showed a decrease in proliferation in a dose-dependent manner. Co-incubation of K562 cells with MIA-602 and doxorubicin showed a significantly greater reduction in proliferation ($p<0.05$). Co-administration of doxorubicin and MIA-602 was shown to have a synergistic effect, when compared to doxorubicin alone at all concentrations at 24 and 48 hours. The 0.05 µg DOX + MIA was shown to be the most effective dose in reducing proliferation.

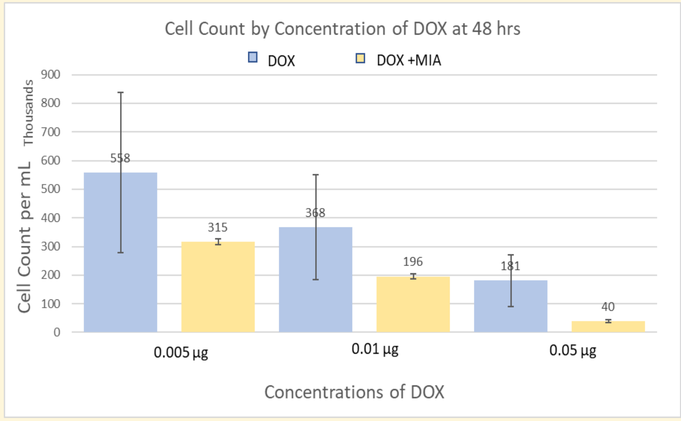


Figure 3: Cell Count Averages of DOX and DOX+MIA at 48 hours by concentration: 0.005, 0.01 and 0.05 µg of DOX. Cell counts decrease as concentration of DOX increases with an additive effect after the addition of MIA-602 in all concentrations of doxycycline.

Discussion:

Co-administration of doxorubicin and the GHRH-R antagonist MIA-602 was shown to have a synergistic effect in reducing proliferation of the K-562 AML cell line. Use of MIA-602 in combination with chemotherapy-based regimens may help prevent the acquisition of resistance in AML. Further investigation into molecular mechanisms behind the synergistic effect of MIA-602 could provide insight into improving clinical outcomes AML treatment.