Validation of *in vitro* cell viability and apoptosis assays for identifying compounds that affect human multiple myeloma and plasma cell leukemia

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Introduction

Multiple myeloma is a clonal plasma cell malignancy that accounts for 10% of all hematologic cancers. Complex interactions of the cancer cells with the bone marrow microenvironment, including activation of osteoclasts and suppression of osteoblasts, lead to lytic bone disease. Heterogeneity of the myeloma contributes to the rapid emergence of drug resistance in high-risk disease. Despite of recent therapeutic advances, myeloma is widely considered as an incurable disease. The responses to treatments depend on the patient and the type of myeloma, and it is therefore of importance to know the direct response to treatment of cancer cells. Current treatment includes traditional chemotherapy agents, corticosteroids, immuno-modulation agents, proteasome inhibitors, histone deacetylase inhibitors, and their combinations.

Aim of the Study

To optimize *in vitro* cell viability and apoptosis assays for identifying compounds that affect human multiple myeloma and plasma cell leukemia.

Materials and Methods

Cell culture

LP-1, MOLP-8, RPMI-8226 and OPM-2 human multiple myeloma cells and JJN-3 and L-363 human plasma cell leukemia cells (obtained from DSMZ) were used in the study. LP-1, MOLP-8, RPMI-8226, OPM-2 and L-363 were derived from blood and JJN-3 from bone marrow. The anthracycline antibiotic doxorubicin (Sigma-Aldrich) was tested as a reference compound with the range of 1 nM to 10 µM concentrations. MOLP-8, RPMI-8226, OPM-2 and L-363 were cultured in RPMI 1640 supplemented with 10-20% fetal calf serum, 2 mM L-glutamine, penicillin and streptomycin in a humidified atmosphere with 5% CO_2 at 37°C. JJN-3 and LP-1 were cultured in DMEM/IMDM (1:1) and IMDM, respectively, supplemented with 20% fetal calf serum, 2 mM L-glutamine, penicillin and streptomycin.

The cells were cultured for 5 days and the effects of doxorubicin were identified by measuring proliferation of the cells at days 1, 3 and 5 using CellTiter-Glo viability assay (Promega). Effects on apoptosis were assessed at day 1 by measuring caspase 3/7 activity using Caspase-Glo 3/7 assay (Promega).

Statistical analysis

Statistical analysis was performed with SPSS 15.0 using one-way ANOVA and Dunnett's tests. Values of $p<0.05$ were considered significant.

Effects of doxorubicin

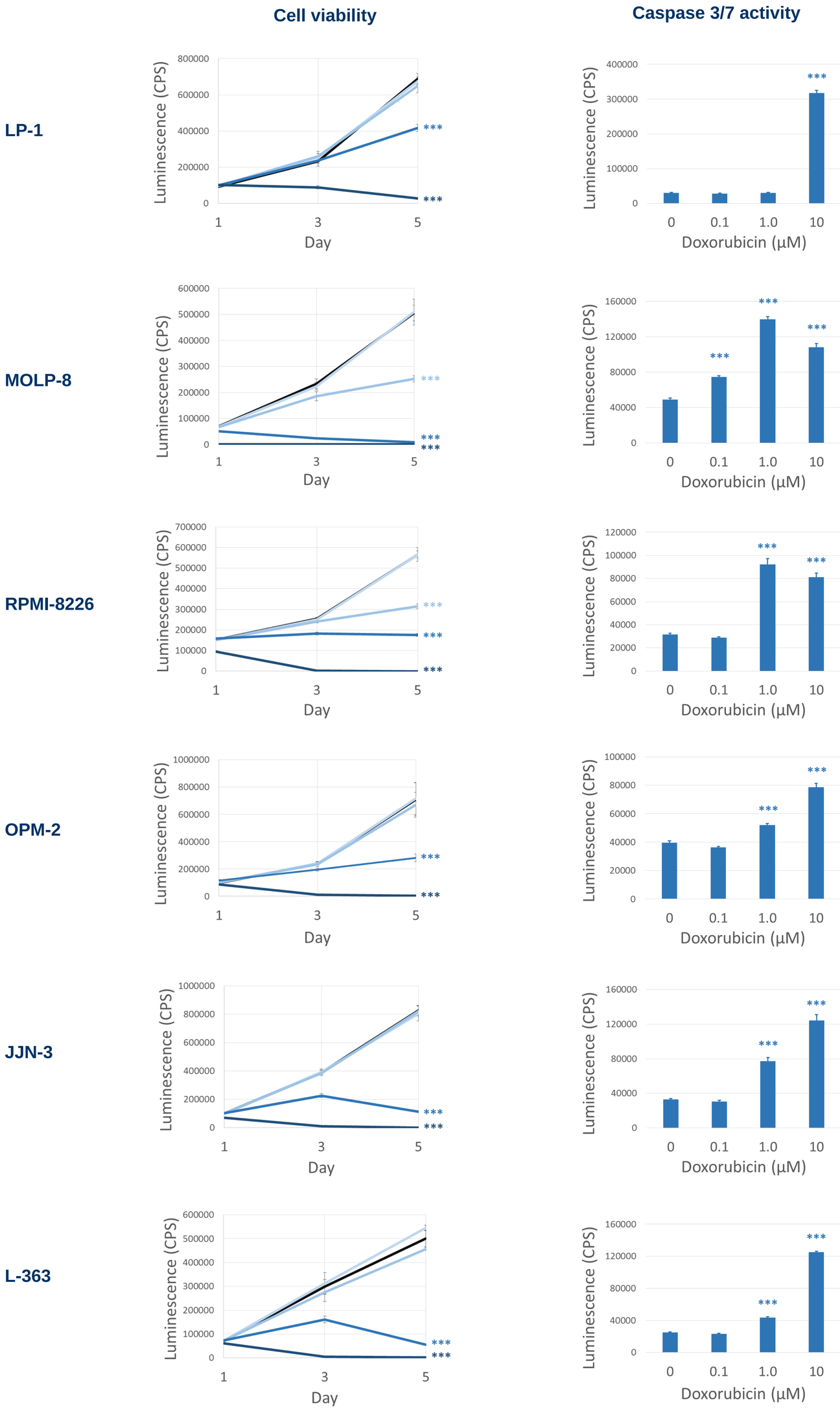

Figure 1: Effects of doxorubicin on the viability and apoptosis of LP-1, MOLP-8, RPMI-8226 and OPM-2 human multiple myeloma cells and JJN-3 and L-363 human plasma cell leukemia cells. *** $p<0.001$ compared to baseline (BL) group. BL 0.001 µM 0.01 µM 0.1 µM 1.0 µM

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Summary

- Doxorubicin showed potent inhibition of cell proliferation at 10 nM concentration in RPMI-8226 and MOLP-8 cells and at 100 nM concentration in all other cell lines tested.
- Doxorubicin induced apoptosis at 100 nM concentration in MOLP-8 cells, at 1000 nM concentration in RPMI-8226, OPM-2, JJN-3 and L-363, and at 10000 nM concentration in LP-1 cells.

Conclusions

- **MOLP-8 and RPMI-8226 cells were more sensitive to doxorubicin induced inhibition of cell proliferation than the other cells tested.**
- **Doxorubicin was more potent to MOLP-8 cells and less potent to LP-1 cells than to the other cells.**
- **We conclude that this culture system can be used as a screening tool for finding new chemotherapy agents on multiple myeloma and plasma cell leukemia.**