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A Study of Multiple Drug Resistance Mechanisms Improved Against Bortezomib on Multiple Myeloma Cell Lines *In Vitro*.

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Abstract

The most important problem in the treatment of Multiple Myeloma (MM) is the multi drug resistance (MDR) observed before and after the treatment. For this reason in MM cases an early resistance to treatment can be developed or the disease can relapsed in early period. Yet, there has been no improved drug resistance against proteazom inhibitor Bortezomib (Bor), which is used alone or with other chemotherapeutic agents in resistant or relapsed MM cases. In this study, bortezomib resistant human MM cell lines; RPMI-8226, secreting lambda light chain, and ARH-77, secreting IgG, were developed and responsible resistance mechanisms were investigated. For this purpose, by exposing to the cells to sequentially gradual doses of Bor *in vitro* conditions, resistant cell lines were acquired throughout one year. The IC_{50} values for Bor were determined after 48 hour incubation by MTT cytotoxicity assay (IC_{50}:1,16nM for RPMI-8226 and IC_{80}:0,6nM for ARH-77) against wild type cells. Throughout one year some cell lines resistant to 1,3nM Bor were acquired by performing Bor to both cell lines in gradual doses. In resistant cell lines IC_{50}:18,07 for RPMI-8226 and IC_{50}:97,56 nM for ARH-77 were determined by MTT assay. In parallel of the gradual increase in drug concentration; the expression changes of the genes of ATP binding cassette protein; MDR1 (Multi Drug Resistance Protein), MRP1 (Multi Drug Resistance Associated Protein), BCRP (Breast Cancer Resistance Protein); and LRP (Lung Resistant Protein) which is responsible for accumulation of the drug in cytoplasm with the aid of nuclear membrane were determined with Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and densitometric

analysis. In resistant cells, high expression of MDR1, MRP1, BRCP and LRP genes showed that; pumping the drug out of the cell membrane and decrease in accumulation of the drug in the cytoplasm had effects on the resistant mechanisms against Bor. Furthermore, expression changes of an important sing of apoptosis ‘caspase-3’, pro-apoptotic ‘bax’ and an anti-apoptotic ‘bcl-2’ genes were examined by RT-PCR and we could come to a point that when compared the sensitive cells to resistant cells, expression of caspase-3 gene and pro-apoptotic bax protein decreased but bcl-2 gene expression increased in resistant cell lines. Finally, we concluded that resistant cell lines acquired resistance against apoptosis by means of mitochondria. By means of this project, the genes which are responsible for secondary drug resistance in ARH-77 and RPMI-8226 MM cell lines in vitro conditions against Bor were determined. Also resistance mechanisms against apoptosis were demonstrated. Cross resistance to different chemotherapeutic agents mechanisms are still continuing.

Author notes

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