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Research Article

Effect of cobalt-60 (γ radiation) on multidrug-resistant multiple myeloma cell lines

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Abstract

Emergence of resistance to chemotherapy and radiotherapy is a major obstacle for the successful treatment of MM (multiple myeloma). Prednisone, vincristine and melphalan are commonly used chemotherapeutic agents for the treatment of MM. In the current study, we examined the presence of possible cross-resistance between these drugs and gamma (γ) radiation. Prednisone, vincristine and melphalan resistant RPMI-8226 and U-266 MM cells were generated by stepwise increasing concentrations of the drugs. The sensitive and resistant cells were exposed to 200- and 800 cGy γ radiation, and proliferation was examined by XTT {2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide} assay. The results showed that Prednisone- and melphalan-resistant RPMI-8226 cells were also cross-resistant to 200 and 800 cGy γ radiation application, while vincristine-resistant cells did not show resistance. On the other hand, Prednisone-, vincristine- and melphalan-resistant U-266 cells showed cross-resistance to 200- and 800 cGy γ radiation application. These results demonstrated that MM cells resistant to anticancer agents respond to radiation in different levels. These findings may be important in the clinical applications of radiation therapy in the treatment of vincristine resistant MM.

Keywords: multiple myeloma; radioresistance; drug resistance; U-266; RPMI-8226

1. Introduction

MM (multiple myeloma) fits in the group of plasma cell disorders characterized by neoplastic proliferation of single clone of plasma cell engaged in the production of a monoclonal immunoglobulin, usually monoclonal IgG or IgA (Gahrton and Durine, 1996). Oral administration of melphalan and Prednisone has remained a standard form of therapy in the treatment of MM (Huang et al., 1999). However, because of the modest success attained using standard chemotherapy, M-2 protocol using multiple chemotherapeutic agents (vincristine, the nitrosourea carmustine, melphalan, cyclophosphamide and Prednisone) have been used (Case et al., 1977). Radiotherapy used in various ways is an important adjunct to chemotherapy in the treatment of MM (Beksaç et al., 2008).

Radiotherapy is necessary when:

- Local radiation therapy at higher doses (with chemotherapy in some cases) is used in the treatment of solitary tumours in bone or soft tissue (plasmacytomas)
- High-dose radiation to a larger part of the body may be used to reduce tumour burden or as salvage therapy
- Local low-dose radiation therapy is sometimes used as palliative treatment to relieve uncontrolled pain and is also used to help prevent or treat bone fractures or spinal cord compression
- Total body irradiation is used in conjunction with high-dose chemotherapy prior to stem cell transplantation in order to help kill myeloma cells in the bone marrow (www.multiple myeloma.org/treatments/3.03.01.php)

Unfortunately, circumvention of drug resistance in MM is a major obstacle to improve clinical outcomes for myeloma patients (Dalton, 2002). The more critical mechanism of drug resistance in MM are considered to reside at the cellular level. Malignant cells may exhibit genetic instability allowing spontaneous generation of variant forms that may result in drug resistance. Altered gene products may result in the development of resistance at the cellular level by causing reduced intracellular drug accumulation, altered drug distribution within the cell, modification of the drug target, enhanced DNA damage repair and decreased apoptosis. Any single one or combination of these alterations may lead to clinically significant cellular drug resistance (Gahrton and Durine, 1996).

Acquired resistance in MM involves a multidrug resistance phenotype that imparts cross-resistance to many classes of chemotherapeutic agents. Even the use of combination chemotherapy, or combination chemotherapy with radiotherapy, can result in the emergence of clinical drug resistance (Dalton and Jove, 1999).

In this study, we examined the possible cross-resistance to radiotherapy in Prednisone-, vincristine- and melphalan-resistant RPMI-8226 and U-266 MM cells.

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Abbreviations: IC_{50} or IC_{20} values, the concentration of any chemical that inhibits cell proliferation 50 and 20%; MM, multiple myeloma; RPMI-8226/500 μM Pred, RPMI-8226 cells resistant to 500 μM Prednisolone; U-266/300 μM Pred, U-266 cells resistant to 300 μM Prednisolone; RPMI-8226/50 nM Vin, RPMI-8226 cells resistant to 50 nM vincristine; U-266/2 nM Vin, U-266 cells resistant to 2 nM vincristine, RPMI-8226/1 μM Melp, RPMI-8226 cells resistant to 1 μM melphalane; U-266/1 μM Melp, U-266 cells resistant to 1 μM melphalane; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide; GSH, glutathione.

2. Materials and methods

2.1. Reagents

Prednisone and vincristine were kindly provided by Gülhane Medical School, Department of Hematology, and melphalan was obtained from Sigma. The XTT {2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide} cell proliferation kit was obtained from Biological Industries (Israel).

2.2. Cell lines and culture conditions

RPMI-8226 and U-266 MM cells were donated kindly by Gülhane Medical School. The cells were maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum and 100 μg/ml gentamycin sulfate in a 37°C incubator with humidified air and 5% CO_2 atmosphere. The sublines resistant to Prednisone [RPMI-8226/500 μM Pred (RPMI-8226 cells resistant to 500 μM Prednisolone), U-266/300 μM Pred (U-266 cells resistant to 300 μM Prednisolone)], vincristine [RPMI-8226/50 nM Vin (RPMI-8226 cells resistant to 50 nM vincristine), U-266/2 nM Vin (U-266 cells resistant to 2 nM vincristine)] and melphalan [RPMI-8226/1 μM Melp (RPMI-8226 cells resistant to 1 μM melphalane), U-266/1 μM Melp (U-266 cells resistant to 1 μM melphalane)] were generated by incubation of the cells by stepwise increasing concentrations of each drug.

2.3. XTT cell proliferation assay

In order to quantify the resistance gained by RPMI-8226, and U-266 cells after drug application, XTT assay was performed both for sensitive and resistant sublines. The XTT assay was performed as described previously (Baran et al., 2007). The cells (2×10^4 cells/well) were placed into 96-well plates and incubated for 24 h at 37°C. After 72 h treatment for the stated incubation doses of Prednisone, vincristine and melphalan, 50 μl XTT solution was added to each well. Plates were incubated for four more hours, and absorbance values were read at 490 nm using a 96-well plate reader. Finally, IC_{50} or IC_{20} values (the concentration of any chemical that inhibits cell proliferation 50 and 20%) of the drugs were determined.

2.4. Co-60 γ radiation application

Sensitive and drug-resistant sublines of RPMI-8226 and U-266 cells were placed into 96-well plates (2×10^4 cells/well) and incubated in fresh medium at 37°C for 24 h. Then, plates containing the cells were irradiated with 200 and 800 cGy with a Theratron 780 Cobalt-60 Teletherapy Unit (AECL Medical). The doses were selected from the literature (Cameron and Sun, 2005) on the fact that 180 to 250 cGy/day is the commonly used acute dose for radiotherapy of humans, and 800 cGy is the cumulative maximum dose. Plates were incubated for 24 h, and 50 μl of XTT reagent was applied onto the cells. Finally, the plates were read at 492 nm with 96-well plate reader.

2.5. Statistical analysis

The data is reported as the means ± S.D. Statistical analysis was carried out using SPSS software (version 11; SPSS Inc.). The two-tailed Student *t* test was used to determine statistical significance of detected differences. $P<0.05$ was considered statistically significant.

3. Results

3.1. Antiproliferative effects of chemotherapeutic agents on drug-resistant MM cells

Sensitive RPMI-8226 and U-266 cell lines were exposed to gradually increasing concentrations of Prednisone, vincristine and melphalan. By this way, drug-resistant sublines of RPMI-8226 and U-266 cells were generated and named as RPMI-8226/500 μM Pred, RPMI-8226/50 nM Vin, RPMI-8226/1 μM Melp and U-266/300 μM Pred, U-266/2 nM Vin and U-266/1 μM Melp. In order to make it sure if these cells are resistant to chemotherapeutic agents, we conducted XTT cell proliferation assay.

IC_{50} or IC_{20} values were calculated from cell proliferation plots to quantitatively compare the resistant and sensitive cells. IC_{50} values of Prednisone on sensitive and RPMI-8226/500 μM Pred cells were 1308 and 2207 μM (Figure 1A), while IC_{20} values were 43.4 and 1624 μM for U-266 and U-266/300μM Pred, respectively (Figure 1B). These results also show that RPMI-8226/500 μM Pred

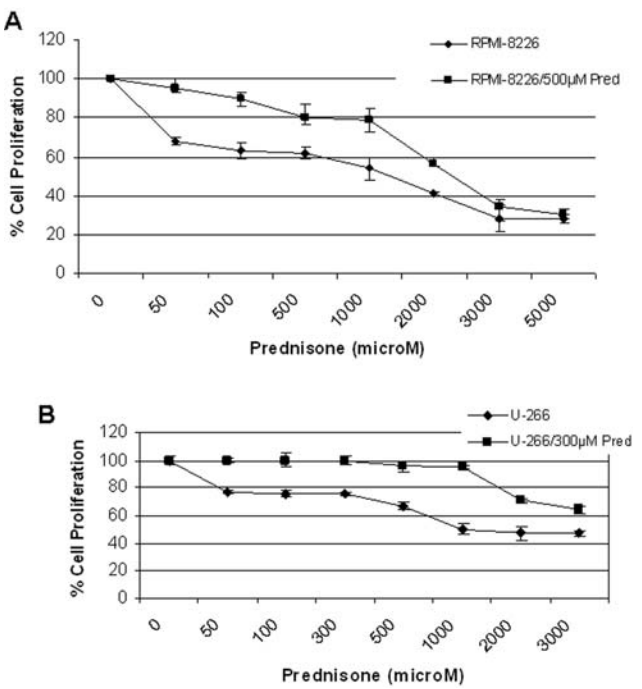

Figure 1 Effect of Prednisone on proliferation of sensitive and Prednisone-resistant RPMI-8226 (A) and U-266 (B) cells

IC_{50} and IC_{20} values of Prednisone for RPMI-8226 and U-266 cells were calculated from cell proliferation plots. The XTT assays were performed using triplicate samples in at least two independent experiments. The error bars represent the S.D.

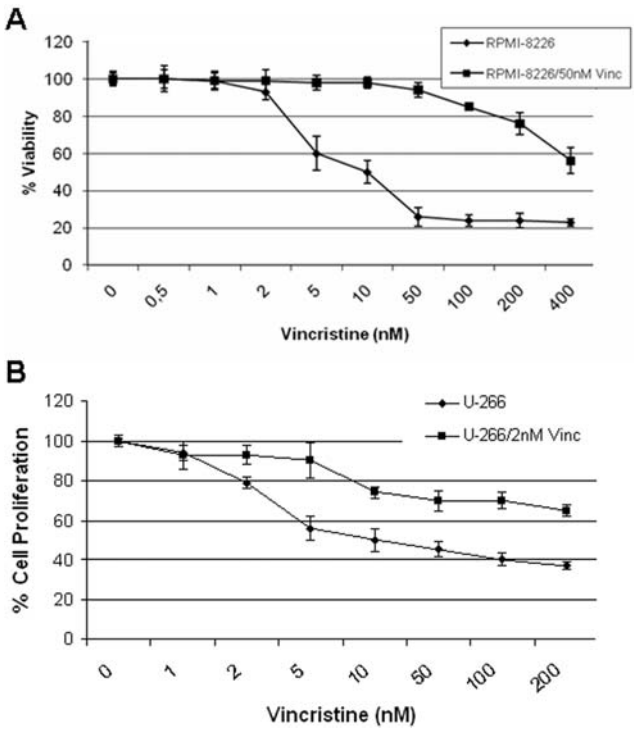

Figure 2 Effect of vincristine on proliferation of sensitive and vincristine-resistant RPMI-8226 (A) and U-266 cells (B)

IC_{50} values of vincristine was calculated from cell proliferation plots. The XTT assays were performed using triplicate samples in at least two independent experiments. The error bars represent the S.D.

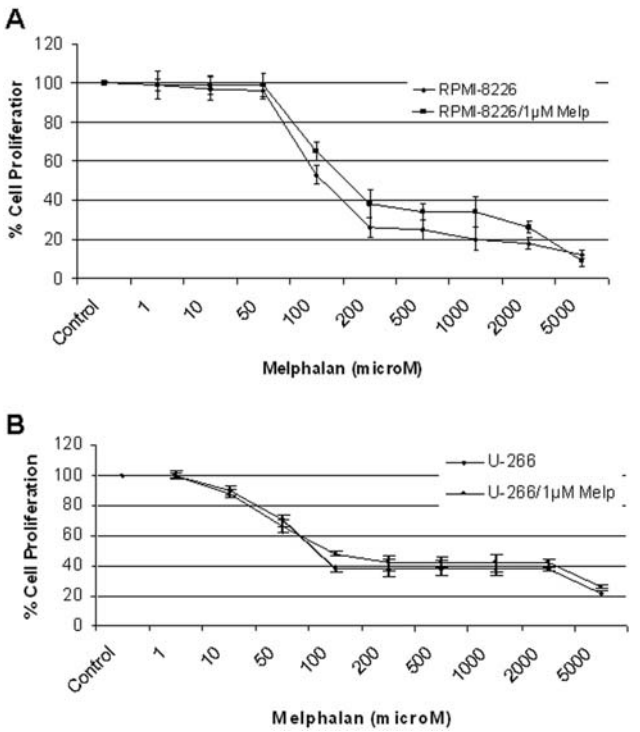

Figure 3 Effect of melphalan on proliferation of sensitive and vincristine-resistant RPMI-8226 (A) and U-266 cells (B)

IC_{50} values of melphalan were calculated from cell proliferation plots. The XTT assays were performed using triplicate samples in at least two independent experiments. The error bars represent the S.D.

and U-266/300 μM Pred cells became resistant to Prednisone. IC_{50} values of vincristine on sensitive and RPMI-8226/50 nM Vin cells were 10 and 400 nM, respectively (Figure 2A). On the other hand, IC_{20} values of vincristine on sensitive and U-266/2 nM Vin cells were calculated to be 2 and 8.1 nM, respectively (Figure 2B). These results shows a 40- and 4-fold resistance to vincristine in RPMI-8226/50 nM and U-266/2 nM Vin cells with respect to their sensitive controls. On the other hand, results for melphalan resistance showed that both of the cell lines became resistant to melphalane, since IC_{50} values were calculated to be 111 and 156 μM for RPMI-8226 and RPMI-8226/1 μM Melp, respectively (Figure 3A). On the other hand, IC_{50} values of melphalane in U-266 and U-266/1 μM Melp were found to be 81 and 188 μM, respectively (Figure 3B).

3.2. Effects of radiation on Prednisone-, vincristine- and melphalan-resistant MM cells

Sensitive and drug-resistant RPMI-8226 and U-266 cells were exposed to 200 and 800 cGy cobalt-60 γ radiation. After 24 h incubation, XTT assay was performed to examine the changes in cell proliferation. Cell lines exposed to radiation were normalized with their own non-radiated controls. The radiation effect was compared for sensitive and drug-resistant sublines of RPMI-8226 and U-266 cell lines (Figures 4 and 5).

There were 2-fold increases in cell proliferation of RPMI-8226/500 μM Pred ($P<0.05$) to both 200 and 800 cGy γ radiation with respect to its sensitive controls, whereas RPMI-8226/50 nM Vin subline was sensitive ($P>0.05$) to radiation. On the other hand, RPMI-8226/1 μM Melp-resistant cells showed 2- ($P<0.05$) and 2.5-fold ($P<0.05$) increases in cellular proliferation in 200 and 800 cGy γ radiation, respectively (Figure 4).

The effect of radiation was different in U-266/1 μM Melp subline than RPMI-8226/1 μM Melp cells. Melphalan-resistant cells showed 1.3-fold ($P<0.05$) increase in proliferation in response to 800 cGy γ radiation. However, the results are similar for vincristine- and Prednisone-resistant sublines. U-266/300 μM Pred and U-266/2 nM Vin cells proliferated 1.7- ($P<0.05$) and 1.4-fold ($P<0.05$) in response to 200 cGy radiation, respectively, compared with their sensitive controls (Figure 5). On the other hand, they both were quite sensitive to 800 cGy ($P>0.05$).

4. Discussion

MM remains a difficult disease to treat because of its marked resistance to chemotherapy. With conventional treatment, not all patients respond, and even in responding patients, complete remissions are rare and with the extremely rare exception death is inevitable (Bergsagel et al., 1979). Radiotherapy is also used in the treatment of MM if plasmacytomas exists in bone or soft tissue or as palliative treatment to relieve uncontrolled pain. MM is a very radiosensitive tumour, as shown by the ability of radiotherapy

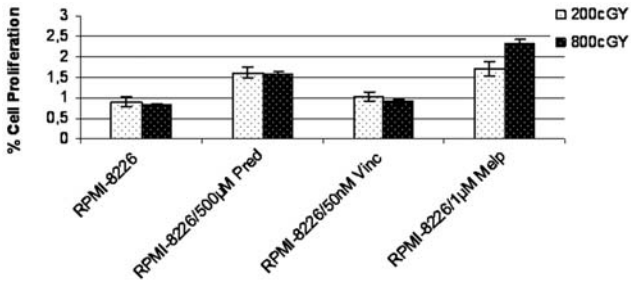

Figure 4 Antiproliferative effects of 200 and 800 cGy γ radiation on sensitive and drug-resistant RPMI-8226 cells

The XTT assays were performed using triplicate samples in at least two independent experiments. The error bars represent the S.D.

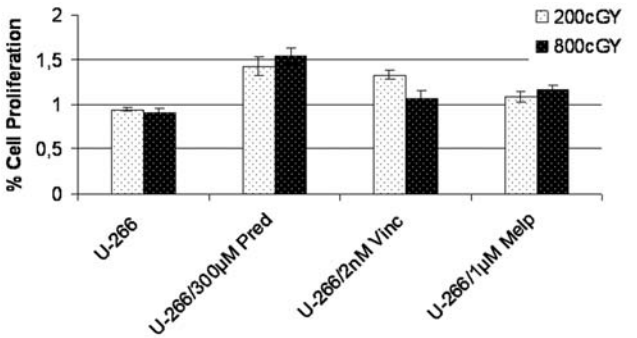

Figure 5 Antiproliferative effects of 200 and 800 cGy γ radiation on sensitive and drug-resistant U-266 cells

The XTT assays were performed using triplicate samples in at least two independent experiments. The error bars represent the S.D.

alone to cure a significant proportion of patients with solitary myeloma of bone (Gahrton and Durine, 1996). In this study, we assessed the radiation effect on Prednisone-, vincristine- and melphalan-resistant RPMI-8226 and U-266 MM cells.

Prednisone-, vincristine- and melphalan-resistant RPMI-8226 and U-266 cells were irradiated with 200 and 800 cGy γ radiation. The results demonstrated that vincristine-resistant RPMI-8226/50 nM Vin cells do not show any cross-resistance to radiation, while U-266/2 nM Vin cells showed slight resistant to 200 cGy radiation, whereas it is sensitive ($P>0.05$) to 800 cGy. These results are correlated with the other studies in the literature using paclitaxel, which has the similar effect of vincristine (Choy et al., 1993; Liebmann et al., 1994; Milas et al., 1994; Jaakkola et al., 1996; Gupta et al., 1997; Raitanen et al., 2002; Klappe et al., 2004). Their results showed that paclitaxel enhanced the tumour radioresponse, since it is a chemotherapeutic agent with potent microtubule-stabilizing activity that arrests cells in G_2-M-phase. Because G_2 and M are the most radiosensitive phases of the cell cycle, paclitaxel has potential as a cell cycle-specific radiosensitizer (Klappe et al., 2004). Vincristine is also a chemotherapeutic agent that stabilizes microtubule activity like paclitaxel. In our study, vincristine-resistant MM cells do not show any cross-resistance when exposed to γ radiation, and vincristine-resistant sublines are radiosensitive.

The mechanisms responsible for the cross-resistance between radiation and melphalan have been examined in human ovarian cancer cell lines (Milross et al., 1997). Cell lines with resistance induced *in vitro* to melphalan have increased cellular levels of GSH (glutathione) compared with drug-sensitive cell line and were cross-resistant to radiation. In addition, depletion of GSH levels in cell lines with acquired resistance to melphalan led to a marked sensitization of these cells to irradiation (Milross et al., 1997). The results from our experiment show that RPMI-8226/1 μM Melp-resistant cells showed significant resistance to 200 and 800 cGy γ radiation. However, these results were not similar with that of U-266/1 μM Melp subline, since it showed low levels of resistance to 800 cGy γ radiation. This can be due to different GSH levels of these two cell lines, since they were isolated from two different patients.

In the literature, there are not many studies about the effect of radiation and glucocorticoids (Ozols et al., 1988). However, in this study, we found that both Prednisone-resistant RPMI-8226 and U-266 MM cells are cross-resistant to applied 800 cGy γ radiation.

As a conclusion, in this study, we evaluated the interaction between Prednisone, vincristine, melphalan and radiation therapy in combination against MM cells *in vitro.* Our results showed that the combination of Prednisone/γ radiation and melphalan/γ radiation resulted in cross-resistance; however, vincristine/γ radiation did not. These results may suggest that clinical application of combinations of vincristine and γ radiation may increase the therapeutic efficiency in contrast with prednisone/γ radiation and melphalan/γ radiation combinations. The results of this *in vitro* study will be a guide to the clinicians in the treatment of drug-resistant MM.

Author contribution

Ufuk Gunduz was the director of the study. Pelin Mutlu participated in all of the experimental procedures and writing of the paper. Yusuf Baran helped in writing the paper and contributed in the discussion. Ali Ural gave the clinical feedback for the study. Ferit Avcu made the statistical analysis. Bahar Dirican and Murat Beyzadeoglu helped in the experimental procedure that took place in Gülhane Military Medical School, Department of Radiation Oncology.

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