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Original Research Article

Evaluation of potential cytotoxic and apoptotic effects of bioymifi on human multiple myeloma cell lines

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Abstract

Purpose: *To investigate the cytotoxic and apoptotic activities of bioymifi (DR5 agonist) on bortezomib-sensitive and bortezomib-resistant cells.*
Methods: *The cytotoxic activities of bioymifi against bortezomib-sensitive (U266) and bortezomib-resistant (U266/BR) cells were evaluated using XTT cell viability test. The cells were exposed to increasing concentrations of bioymifi for 24 h. Cell cycle analysis was performed while apoptosis was examined by flow cytometry.*
Results: *Bioymifi showed significant cytotoxic effects on U266 and U266/BR in a concentration-dependent manner ($p < 0.05$). The IC_{50} values of bioymifi in U266 and U266/BR cells were 10.4 and 20.9 µM, respectively. Moreover, when compared to the untreated cells, bioymifi treatment at IC_{50} concentrations considerably increased the percentage of apoptotic cells. Bioymifi treatment also caused cell cycle arrest at the G2/M phase in both cell types.*
Conclusion: *The results show that bioymifi is a promising candidate for multiple myeloma treatment. However, further studies are required to evaluate the clinical anticancer activity of this agent.*

Keywords: *Bioymifi, Multiple myeloma, Cytotoxicity, Apoptosis, Cell cycle, G2/M phase*

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INTRODUCTION

Multiple myeloma (MM) is a disease qualified by an increase in plasma cells mainly in the bone marrow and rarely in other organs and systems, accompanied by abnormal immunoglobulin production [1]. Although immunomodulatory drugs (IMIDs) such as lenalidomide, pomalidomide, thalidomide, and proteasome inhibitors (PIs) such as bortezomib and carfilzomib are added to the standard treatment of multiple myeloma; it is still an incurable disease, unfortunately [2,3].

Remissions and relapses are typical in the clinical course of MM. The prognosis after treatment with PIs/IMIDs is very poor in refractory or relapsing MM patients. [3]. The major causes of treatment failures and relapses in MM are the development of primary or acquired resistance to treatment regimens and the occurrence of drug-resistant subclones [4]. Therefore, the mechanisms of drug resistance in

MM need to be better understood.

Induction of apoptosis is an important strategy for the management of MM. Apo2L/TRAIL (Apo2L/TNF-related apoptosis-inducing ligand) is a crucial mediator that induces apoptosis in haematologic and non-haematologic cancer cells. Binding to the extracellular domain of the death receptor (DR5), bioymifi induces receptor activation resulting in cell death in several human cancer cell lines independent of TRAIL [5].

The present study screened the cytotoxic, apoptotic, and cell cycle arrest effects of bioymifi against U266 and U266/BR (Bortezomib resistant) MM cells for the first time.

EXPERIMENTAL

Cell culture and reagents

The human MM cell line U266 (TIB-196) was acquired from ATCC (USA). U266 cells were incubated in RPMI-1640including 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. A humidified incubator was used for maintaining the U266 cells at 37°C and 5% CO_2.

Differentiation of U266 cells into BTZ-resistant MM cell lines

Human MM cell line U266 was grown following a routine process in complete medium at 37°C and 5% CO_2. The bortezomib-resistant (BTZ-resistant) U266 subline (U266/BR) was established through stepwise increases in BTZ (Velcade) concentrations in a period of 3 months. The BTZ concentration was fixed at 20 nM in the growth medium for U266/BR.

XTT assay

Cell proliferation was determined by performing the XTT assay (BIOTIUM, Inc) in accordance with the user's guide. In brief; to evaluate the cytotoxic effect of bioymifi on the proliferation of U266 and U266/BR cells, 50 µL of a cell culture containing about 1.5×10^4 cells were added to each well of a 96-well plate and incubated at 0.01, 0.1, 1, 10, and 50 µM concentrations of bioymifi at cell culture conditions. Next, 50 µL XTT labelling reagent was added to each well to identify viable cells. Then, the plates were incubated at 37 °C and 5 % CO_2 for another 4 h. The absorbance of XTT formazan at 450 nm was recorded using a spectrophotometer (Thermo, Germany).

Apoptosis assay

Human Multiple myeloma cell lines U266 and U266/BR were seeded into six-well plates at a density of 5×10^5 cells per well; exposed to bioymifi at IC_{50} concentrations (10 µM for U266 and 20 µM for U266/BR), and incubated for 24 h. After the incubation period, the cells were resuspended in phosphate-buffered saline (PBS) containing 1% FBS and incubated after Annexin V & Dead Cell reagent was added in compliance with the manufacturer's instructions. Finally; viable, dead, and early and late apoptotic cells were identified by using the Muse™ Cell Analyzer (Millipore).

Cell cycle analysis

Cell cycle distribution in MM cells following the treatment of the U266 and U266/BR cell lines with 10 µM and 20 µM concentrations of bioymifi was examined by flow cytometry. After an incubation period for 24 h; the cells were resuspended with PBS, and fixed in 1 mL cold 70% (v/v) ethanol. The fixed cells were washed with PBS once and incubated in the darkroom for half an hour with the cell cycle reagent according to the manufacturer's instructions. Finally, rates of the cells at different phases of the cell cycle were recorded by flow cytometry.

Statistical analysis

The GraphPad 7 software was used for performing statistical analyses and developing graphical presentations. Data are described as mean ± SD. One-way ANOVA was performed for analyzing data across multiple groups. Statistical significance was considered at a p-value of ≤ 0.01.

RESULTS

Bioymifi diminished the proliferation of multiple myeloma cells

As shown in Figure 1, the proliferation of MM cells treated with bioymifi for 24 h diminished significantly in a dose-dependent manner compared to the untreated cells. The IC_{50} values of bioymifi for U266 and U266/BR cells were 10.4 µM and 20.9 µM, respectively.

Effects of bioymifi on apoptosis of multiple myeloma cells

Apoptosis was assessed in U266 and U266/BR cells following bioymifi treatment. As seen in Figure 2, 10 µM of bioymifi for U266 and 20 µM of bioymifi for U266/BR cells activated a

significant apoptotic effect compared to the untreated cells ($p < 0.01$). The total apoptotic cell population percentages were 38.94 % in U266 cells and 15.54 % in U266/BR cells.

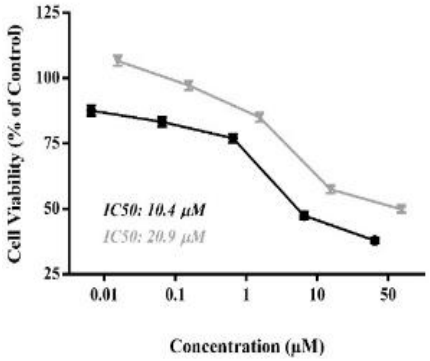

Figure 1: Cytotoxic effect of bioymifi on U266 and U266/BR cells. Results are mean ± SD; (n = 3, $p \leq 0.01$); U266 (■); U266/BR (▼)

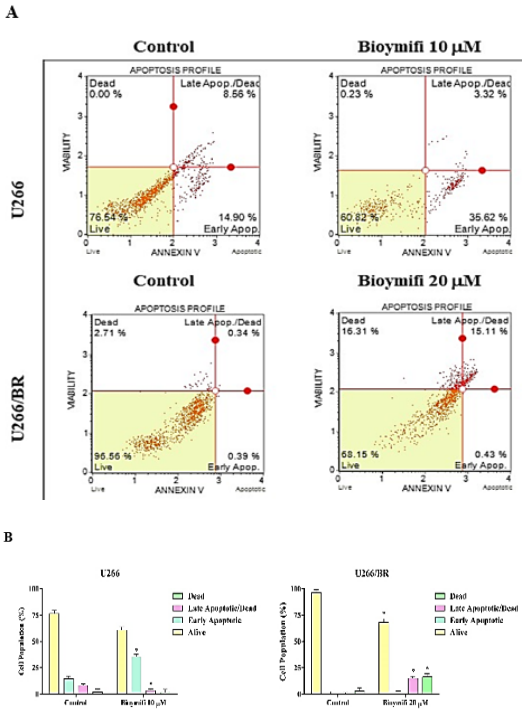

Figure 2: Apoptotic effects of bioymifi on MM cells treated with IC_{50} values (10 µM for U266; 20 µM for U266/BR) for 24 h. (A): Results of the flow cytometric analysis of MM cells. (B): Percentage of apoptotic/non-apoptotic cells in the bar graphs; $p \leq 0.01$ compared to the control cells

Bioymifi treatment caused cell cycle arrest of multiple myeloma cells at G2/M mitotic phase

In order to determine if the anti-proliferative role of bioymifi against the U266 and U266/BR multiple myeloma cells occurred because of the induction of cell cycle arrest, the myeloma cells were incubated at 10 µM (for U266) and 20 µM (for U266/BR) concentrations of bioymifi and, then, cultured for 24 h at 37 °C. Next, the effect of the bioymifi on cell cycle progression was determined by using the cell cycle kit. The flow cytometry results indicated that treatment with bioymifi caused an increase in the population of MM cells at the G2/M phase (Figure 3 A and B).

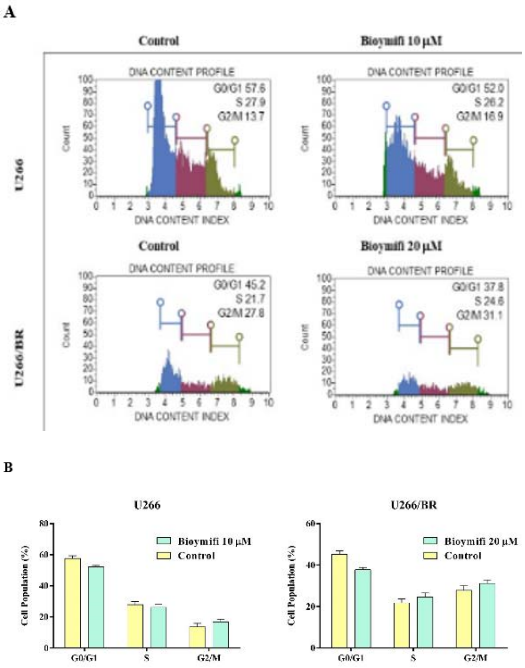

Figure 3: Effects of bioymifi on cell cycle distribution (A): Bioymifi at different concentrations was added to U266 and U266/BR cells. Then, the flow cytometric analysis was run for 24 h. (B): The histogram shows the percentages of U266 and U266/BR cells by the cell cycle phases

DISCUSSION

The development of drug resistance in MM increases the need for alternative therapeutic strategies [6] because the undesirable consequences of resistance to bortezomib and lenalidomide in relapsing or refractory MM patients are associated with poor prognosis [7].
The first-line proteasome inhibitor bortezomib has a remarkable anti-myeloma effect of 43% for relapsed/refractory MM. Furthermore, bortezomib is a preferred drug in geriatric patients, who are not eligible for a bone marrow transplant. However, bortezomib is associated with untoward effects such as thrombocytopenia, toxicity to active T cells, and significant impairments in the immune-stimulating capacity of human dendritic cells. Despite the current treatment strategies with new generation PIs, IMIDs, monoclonal antibodies, or histone

deacetylase inhibitors that can overcome bortezomib resistance, new approaches are still needed in refractory myeloma treatment. Also, novel therapeutic agents are necessary for the treatment of MM to achieve the highest possible response rates in patients with primary or acquired drug resistance [8]. In the present study, DR5 receptor-mediated apoptosis induction and anti-myeloma efficacy of bioymifi was investigated in both bortezomib-sensitive and bortezomib-resistant MM cell lines for the first time.

Apo2L/TRAIL, which acts on DR4 and DR5 receptors, selectively induces apoptosis in tumour cells but does not show any toxicity to normal cells [9,10]. It has been previously demonstrated that DR4 and DR5 expression is considerably increased in many haematological and non-haematological malignancies [11-14]. However, increasingly more studies have shown increased expression of DR4 and DR5 receptors in MM cells. Furthermore, many previous studies have shown that TRAIL/Apo2L promotes apoptosis in MM cell lines [15]. Also, it has been reported in the literature that DR4 and DR5 are involved in the treatment of myeloma. In the present study; bioymifi, a DR5 agonist, was applied at concentrations ranging from 0.01 µM to 50 µM in order to evaluate the cytotoxic effects on the bortezomib-sensitive and bortezomib-resistant U266 myeloma cell lines.

The study also determined the IC_{50} (10 µM for U266; 20 µM for U266/BR) values of bioymifi for the evaluation of apoptosis in the cell cycle. The half-maximal inhibitory concentration (IC_{50}) value was assigned as 10 µM in the bortezomib-sensitive U266 cell line and 20 µM in the bortezomib-resistant U266 cell line. In the literature, it has been previously demonstrated by Mitsiades *et al* [15] that TRAIL receptor agonists induce apoptosis in U266 cells and in MM cell lines resistant and non-resistant to RPMI-8226. In light of previous findings reported in the literature, our results are promising for the treatment of refractory myeloma cases [15].

Stimulation of membrane receptors such as DR4 and DR5 by the Apo2L ligand stimulates a reaction cascade causing caspase activation [16]. Mitsiades *et al* reported in their study that apoptosis of Apo2L is strongly induced in human multiple myeloma cells sensitive to dexamethasone, doxorubicin, melphalan, and mitoxantrone [15]. Ursini-Siegel *et al* showed in their study that Apo2L was expressed in plasma cells and that Apo2L might be responsible for the plasma cell apoptosis independent of the caspase pathway [17].

Activation of death receptors with Apo2L ligand through the cell surface receptors DR4 and DR5 activates subcellular caspase-mediated apoptotic pathways and induces cell apoptosis. Chen *et al* showed the Interferon-I-induced apoptosis occurred through the activation of the Apo2L pathway in MM cells [18]. Consistent with the reports in the literature, the stimulation of apoptosis in MM cells via DR4 and DR5 receptor targeting is supported by the present findings of the apoptotic effect of Bioymifi in this study.

CONCLUSION

The results of this study show that bioymifi inhibits multiple myeloma cell proliferation via the death receptor. Moreover, bioymifi treatment activates apoptosis and induces cell cycle arrest in U266 and U266/BR cells. Therefore, bioymifi has the potential for development as a treatment strategy for multiple myeloma.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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