MIM1 augments proapoptotic activity of moxifloxacin toward MDA-MB-231 triple-negative breast cancer cells

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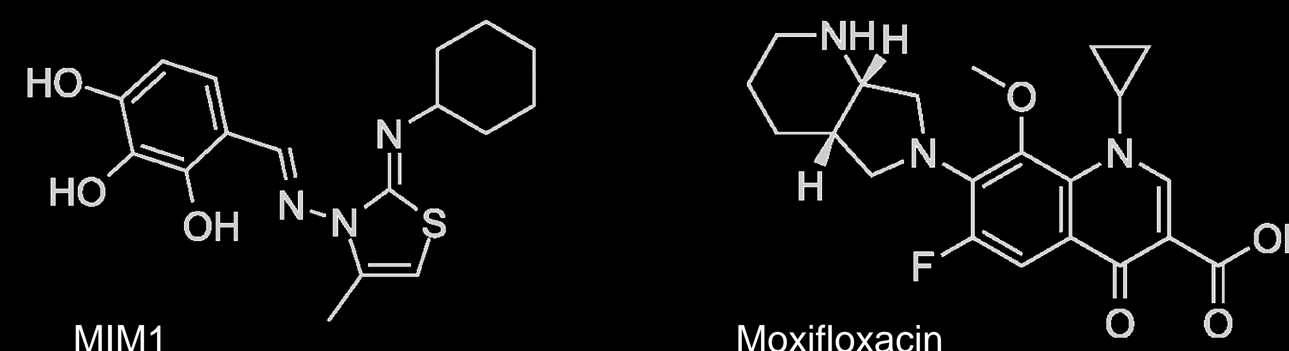
Background

The adverse effects related to currently applied polytherapy, the unsatisfactory effectiveness of the treatment as well as epidemiological data indicate there is a need to search and develop a new method of cancer treatment. Overexpression of Bcl-2 family proteins is a common event in cancer. Earlier conducted studies indicated that Mcl-1 protein was a crucial player in breast cancer. Thus, the significant role of Mcl-1 makes the possibility of using its inhibitors. Among the identified BH3 mimetics there is one low molecular Mcl-1 inhibitor – MIM1 (Mcl-1 Inhibitor Molecule 1). MIM-1 may selectively inhibit Mcl-1 protein and finally induce Mcl-1-dependent cancer cells death. Previously we have demonstrated that moxifloxacin – the fluoroquinolone antibiotic may induce high cytotoxic and proapoptotic effect on MDA-MB-231 breast cancer cells via Mcl-1 protein interaction as a molecular target.

Aim of the study

The aim of this study was to assess the possible synergistic activity and anticancer effect triggered by BH3 mimetic MIM-1 and moxifloxacin in a multi-component system. The culture of MDA-MB-231 breast cancer cells was used as an experimental model.



Results

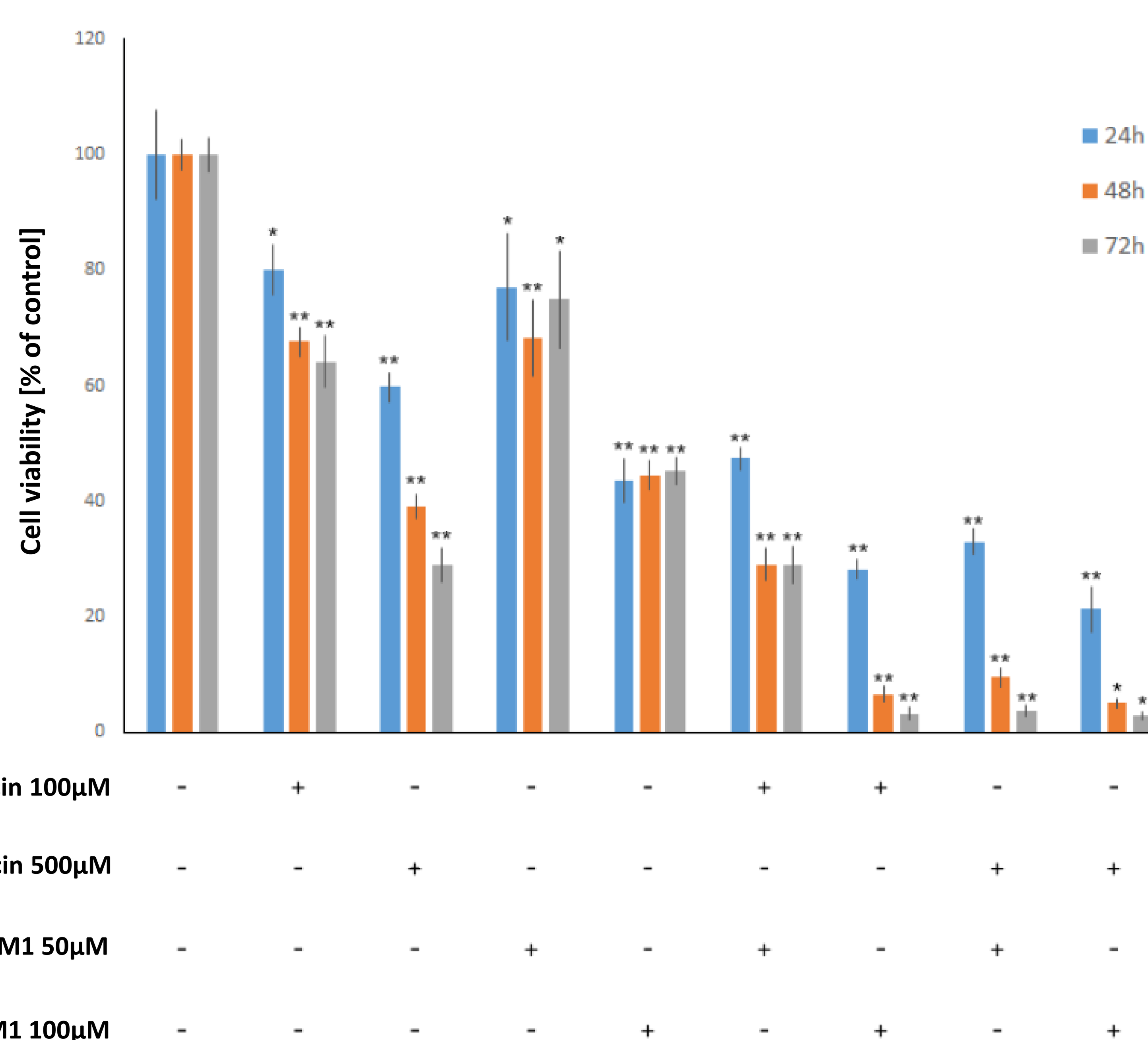


Figure 1. Screening analysis of MDA-MB-231 cells viability after incubation with MIM1 (MIM1) and/or moxifloxacin. The cells were treated for 24 h, 48 h and 72 h. Mean values ± SD from three independent experiments are presented; * $p < 0.05$, ** $p < 0.005$ vs untreated control.

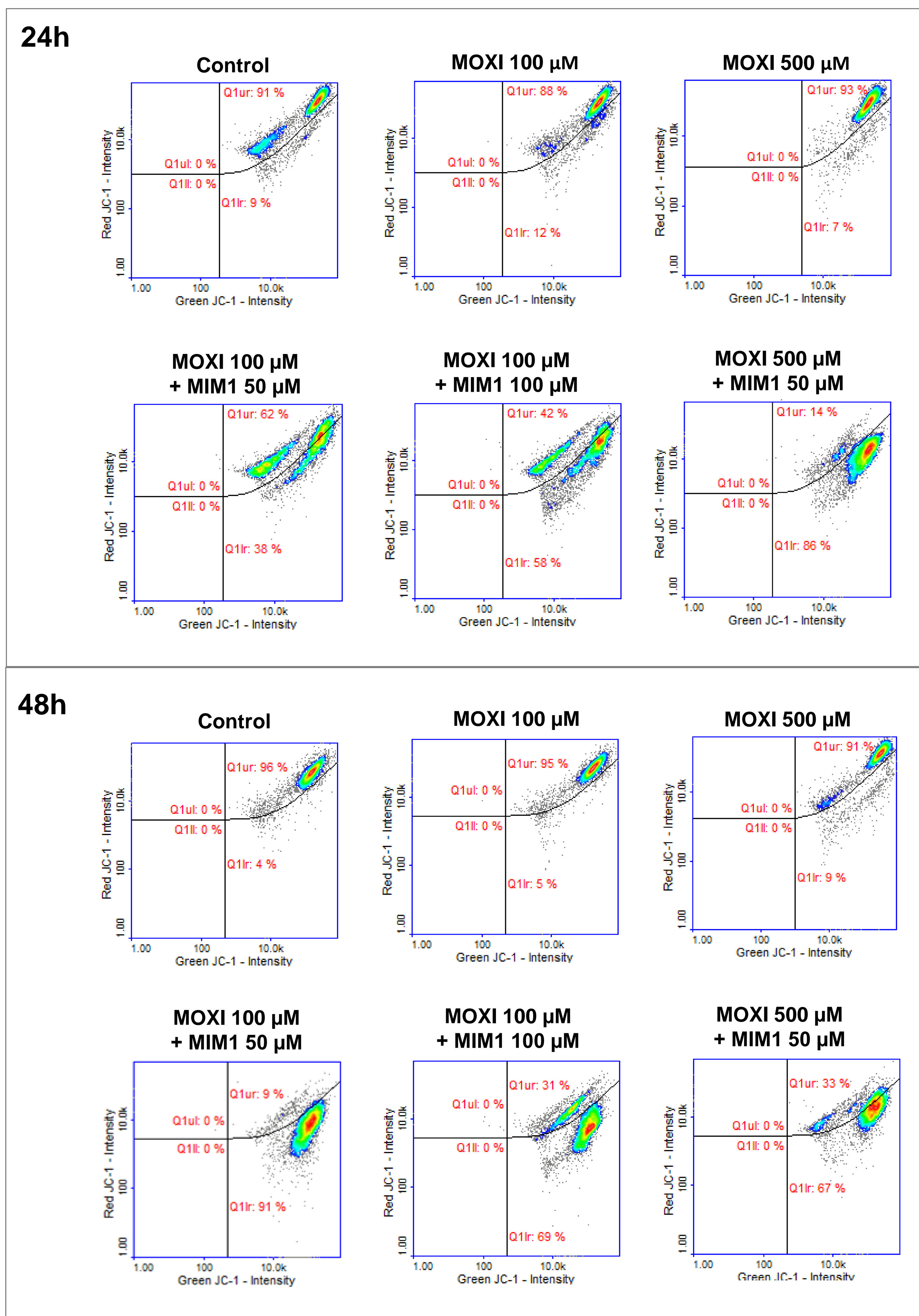


Figure 2. The impact of MIM1 (MIM) and or moxifloxacin (MOXI) on mitochondrial potential of MDA-MB-231 breast cancer cells. The analysis was performed after 24 h or 48 h incubation. Representative scatter plots showing tested populations of stained cells divided by a gate into the subpopulations of cells with polarized (Q1ur) and depolarized (Q1lr) mitochondria.

Methods

CELL CULTURE

MDA-MB-231 cell line (ATCC) was cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS), and antibiotics: penicillin (100 µg/mL), amphotericin B (0.25 µg/mL), and neomycin sulfate (10 µg/mL). Cells were cultured at 37˚C in a 5% CO_2 humidified atmosphere.

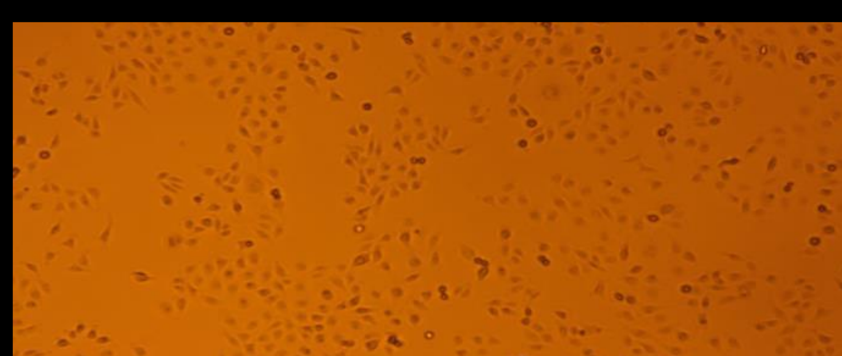


Figure 3. Microphotographs of MDA-MB-321 cells in culture. The images obtained with light inverted microscope NIKON TS100F.

SCREENING ANALYSIS OF CELLS PROLIFERATION

Proliferation of breast cancer cells was estimated by the Cell Proliferation Reagent WST-1. The reagent was added to cells cultured in 96-well microplates in an amount of 10 µL/well 3 h before the measurement.

MITOCHONDRIAL POTENTIAL ANALYSIS

The mitochondrial transmembrane potential was measured using the NucleoCounter NC-3000 fluorescence image cytometer. Cell pellets were stained with JC-1 solution at 37 °C for 15 min. At the end of analysis, cell pellets were resuspended in DAPI solution and analysed immediately using NucleoView NC-3000 software (ChemoMetec). The obtained scatter plots were used to demarcate the percentage of polarized/healthy cells and depolarized/apoptotic cells.

STATISTICAL ANALYSIS

In all experiments, mean values of at least three separate experiments performed in triplicate (n = 9) ± standard deviation (SD) were calculated. Statistical analysis was performed using GraphPad Prism 7. Differences among groups were assessed using two-way ANOVA analysis of variance followed by Dunnett's test; $p < 0.05$ was determined to indicate a significant difference.

Conclusions

- **The obtained data from both WST-1 and image cytometry analysis show that MIM1 potentiates moxifloxacin impact on MDA-MB-231 cells viability and mitochondrial depolarization suggesting the possible synergistic effect.**
- **Summarizing, the obtained results i/ indicate that MIM1 augments proapoptotic activity of moxifloxacin as a result of Mcl-1 protein interaction and ii/ consist the basis for further in vitro as well as in vivo panel of experiments to confirm the anti-breast cancer activity of MIM1 and moxifloxacin especially when used in multi-component system.**

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