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Antitumor Activity of Tubulin-Binding Agent MPC-6827 on Different Types of Cancer Cell Lines

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ABSTRACT

In this study, the antitumor effects of tubulin-binding agent MPC-6827 on HeLa, MCF-7 and A549 cell lines originated from cervix carcinoma, metastatic breast adenocarcinoma and adenocarcinomic human alveolar basal epithelial cells respectively were determined. Cell index, BrdU labelling index, mitotic index and apoptotic index were evaluated in experiments. In cell index experiment 2 nM, 4 nM, 6 nM, 8 nM, 10 nM MPC-6827 applied to three cell lines. These parameters showed that 4 nM was the optimum concentration for HeLa and A549 cells, while 2 nM was the optimum concentration for MCF-7 cells. The use of optimum concentrations for each cell line has shown that while there was a significant decrease in mitotic index, BrdU labelling index, there was a significant increase in apoptotic index.

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Introduction

Apoptotic mechanisms have an important role in the treatment of cancer. Agents that target tubulin such as taxanes (paclitaxel, docetaxel) and vinca alkaloids (vinblastine, vincristine, and vinorelbine) are commonly used in the treatment of a wide variety of cancer types as proapoptotic agents. The clinical success of these agents has led tubulin to be an important tumor target (1, 2). Disruption of microtubule dynamics leads to the arrest of cells in the G_2-M phase. This arrest ultimately results in apoptotic cell death (3).

Despite the success of the drugs used in treatment, the biggest obstacle to chemotherapy is the formation of chemo-resistance in cancer that has been recovered after exposure to chemotherapeutic agents. The effect of resistance to an antineoplastic agent in the destruction of cancer cells is a known difficulty. However, it plays an important role in the failure of chemotherapy in multiple drug resistance (MDR) which has a different structure and action mechanisms (4). Therefore, the discovery of new agents that can overcome drug resistance mechanisms has become an important issue.

MPC-6827 is a member of a new generation of tubulin-binding agents (5). Small-molecule MPC-6827 binds to the same or nearby site on beta-tubulin as colchicine. Thus, it exerts antitumor activity by inhibiting the microtubule assembly in a manner similar to that of colchicine and vinca alkaloid drugs (6). MPC can be effective at very low concentrations on many types of cancers including breast, ovarian, pancreas, non–small cell lung, small cell lung, colon, prostate, melanoma, brain cancers and leukemia *in vitro* (6, 7).

The aim of the present study was to investigate the effect of MPC-6827 on HeLa cell lines originated from cervix carcinoma, MCF-7 cell line originated from metastatic breast adenocarcinoma A549 cell line originated from adenocarcinoma human alveolar basal epithelial cells.

Materials and methods

Cell Culture

The experiment was performed on a different types of cell lines (HeLa, MCF-7 and A549). HeLa and MCF-7 cell lines were maintained in high glucose DMEM and A549 was maintained in RPMI-1640 supplemented with 10% fetal bovine serum, at 37°C, in a humidified atmosphere containing 5% CO_2.

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Agent Concentrations

2 nM, 4 nM, 6 nM, 8 nM, 10 nM MPC-6827 concentrations prepared by diluting a total of 1 mM stock solution.

Cell Index

The cells were seeded on the E-plate. Under all conditions, 5,000 cells for A549 and 10,000 cells for MCF-7 and HeLa were seeded into the each well of E-plate. The cells were allowed to attach to the plate for 20 hours. At the end of this period, cells were exposed to varying concentrations of MPC6827. Cell proliferation measured for 72 h.

Mitotic index (MI)

At the end of the experimental period, Carnoy fixative (ethanol: acetic acid, 3:1) was used to fix cells and Feulgen method has been applied. After hydrolysis, it was dyed with Giemsa. The number of mitotic phase cells was determined as 3000-3500. The mitotic index has been estimated by: MI = (n/C)×100 formula (number of n: dividing cells, number of C: total cells).

BrdU Labelling Index

BrdU (5-bromo-2'-deoxyuridine) was used to determine the DNA synthesis rate of HeLa, MCF-7 and A549 cells after administration of optimum concentration of MPC6827. This test is based on the determination of BrdU that binds to the genomic DNA of proliferating cells. BrdU was prepared according to the manufacturer's protocol (Sigma-Aldrich, Catalogue Number: 2752) and then detected via the spectrophotometric method.

Apoptotic index (AI)

A fixation process has been continued at room temperature for 3 minutes, first with the addition of methanol / PBS in a 1: 1 ratio, and then with the use of pure methanol. After fixation cells of both nuclei have been stained using 1 µg / ml solution of DAPI within 20 minutes at room temperature. The stained nucleus was washed with PBS, and the cells were analyzed under a fluorescent microscope (magnification, ×1000). Apoptotic index percent has been determined due to the formula given here: AI = (Apoptotic cell number / total number of cells) × 100).

Statistical Evaluation

In the study, the cells had values that were determined both in relation to each other and to the control groups created. These values were obtained by applying cellular kinetic parameters, and the ANOVA test was applied to all values to verify that there was no significant statistically significant difference. Instead of comparing all groups in pairs and multifaceted in the experiment, the DUNNETT`S test used the control group to measure the importance of the experimental group, and the t-test assessed the significance of the experimental group. Statistical values were obtained based on the level of $p < 0.05$.

Results and discussion

Cell Index

Cell index values obtained from xCelligence real-time cell analysis system (Roche ®) showed that the application of MPC6827 on HeLa, MCF-7 and A549 cells had significant antiproliferative effects. For all three cell lines, 2 nM, 4 nM, 6 nM, 8 nM and 10 nM concentrations of MPC6827 were used throughout the experimental period. When the graphical curves of Hela cells were examined, it was thought that from 4 nM concentration of MPC6827 showed antimitotic effects (Figure 1). Graph curves of MCF-7 cells showed cytoskeletal effects 4 nM concentration of MPC6827 (Figure 2). Graph curves belonging to A549 cells showed that while 2 nm concentration of MPC6827 had no effect on cells, other concentrations had antimitotic effects (Figure 3).

Mitotic Index

The values obtained by applying the 4 nM concentration of MPC6827 to all three cell lines were shown in Figures 4A, 4B, 4C. The results showed that this concentration decreased the mitotic index values of HeLa cell line from 8.24 % to 4.53% at 24 h; from 8.27% to 3.01% at 48 h and 8.96 % to 2.09 % at 72 h. Mitotic index values belong to MCF-7 cells decreased from 6.53% to 2.96% at 24 h; from 7.21% to 2.18% at 48 h and 7.89% to 1.97% at 72 h. Mitotic index values of A549 cell line from 6.78% to 4.12% at 24 h; from 6.63% to 3.56% at 48 h and 7.12% to 2.94% at 72 h. The differences between the control and all experimental groups were statistically significant ($p<0.05$).

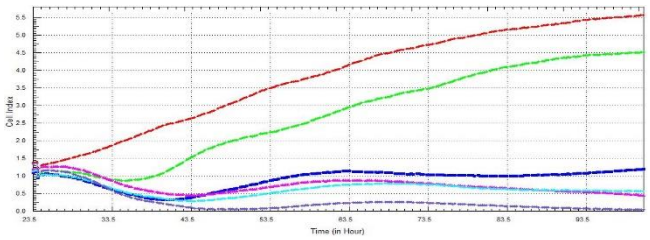

Figure 1. Cell index values of HeLa cells treated with 2 nM, 4 nM, 6 nM, 8 nM and 10 nM concentrations of MPC6827 obtained from xCelligence Real-Time Cell Analysis (RTCA) system (Red Line: Control, Green Line: 2 nM, Dark Blue Line: 4 nM, Light Blue Line: 6 nM, Pink Line: 8 nM and Purple Line: 10 nM).

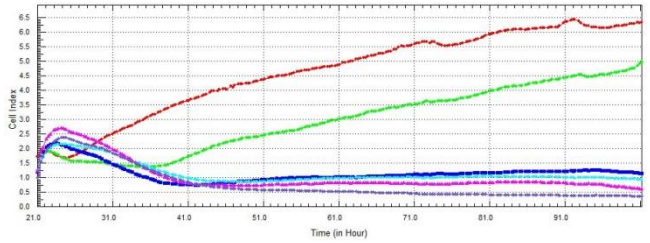

Figure 2. Cell index values of MCF-7 cells treated with 2 nM, 4 nM, 6 nM, 8 nM and 10 nM concentrations of MPC6827 obtained from xCelligence Real-Time Cell Analysis (RTCA) system (Red Line: Control, Green Line: 2 nM, Dark Blue Line: 4 nM, Light Blue Line: 6 nM, Pink Line: 8 nM and Purple Line: 10 nM).

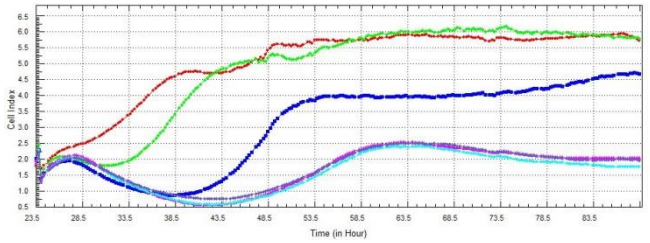

Figure 3. Cell index values of A549 cells treated with 2 nM, 4 nM, 6 nM, 8 nM and 10 nM concentrations of MPC6827 obtained from xCelligence Real-Time Cell Analysis (RTCA) system (Red Line: Control, Green Line: 2 nM, Dark Blue Line: 4 nM, Light Blue Line: 6 nM, Pink Line: 8 nM and Purple Line: 10 nM).

BrdU Proliferation Assay

The bromodeoxyuridine (BrdU) values that enable the labeling of cells in the synthesis stage are shown in Figures 5A, 5B, 5C. For HeLa cell line BrdU % values were 48%, 37% and 27%; for MCF-7 cell line BrdU % values were 45%, 32% and 23% and for A549 cell line BrdU % values were 47%, 36% and 29% compared to the control groups which were considered 100%. There were significant differences between the control and the experimental groups ($p<0.05$).

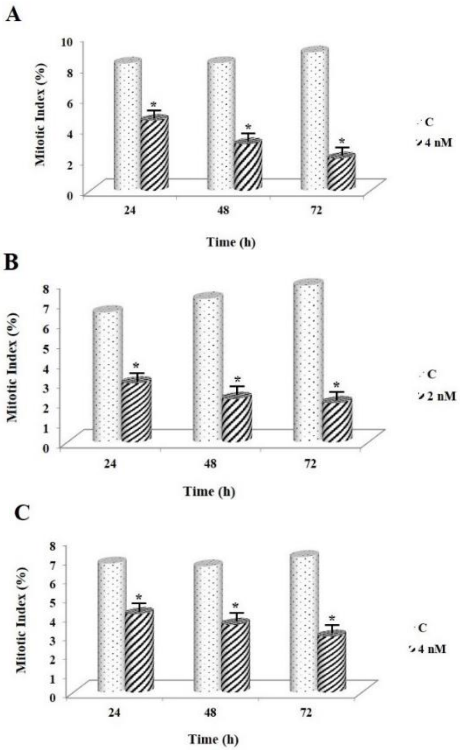

Figure 4. MI values of HeLa cells treated with 4 nM MPC6827 for 0-72 h ($p<0.05$) (A), MI values of MCF-7 cells 4 nM MPC6827 for 0-72 h ($p<0.05$) (B), MI values of A549 cells treated with 4 nM MPC6827 for 0-72 h ($p<0.05$) (C).

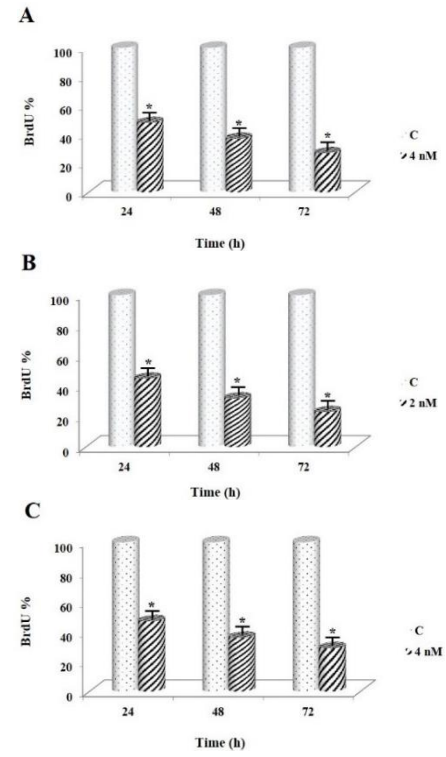

Figure 5. BrdU % values of HeLa cells treated with 4 nM MPC6827 for 0-72 h ($p<0.05$) (A), BrdU % values of MCF-7 cells 4 nM MPC6827 for 0-72 h ($p<0.05$) (B), BrdU % values of A549 cells treated with 4 nM MPC6827 for 0-72 h ($p<0.05$) (C).

Apoptotic Index

The values obtained by applying the 4 nM concentration of MPC6827 to all three cell lines were shown in Figures 6A, 6B, 6C. The results showed that this concentration increased the apoptotic index values of HeLa cell line from 3.21 % to 10.11 % at 24 h; from 3.89 % to 18.13 % at 48 h and 4.02 % to 21.79 % at 72 h. Mitotic index values belong to MCF-7 cells increased from 3.51 % to 18.48 % at 24 h; from 4.64 % to 23.35 % at 48 h and 4.89 % to 34.35 % at 72 h. Mitotic index values of A549 cell line from 4.14 % to 17.12 % at 24 h; from 4.67 % to 20.18 % at 48 h and 5.12 % to 27.31 % at 72 h. The differences between the control and all experimental groups were statistically significant ($p<0.05$).

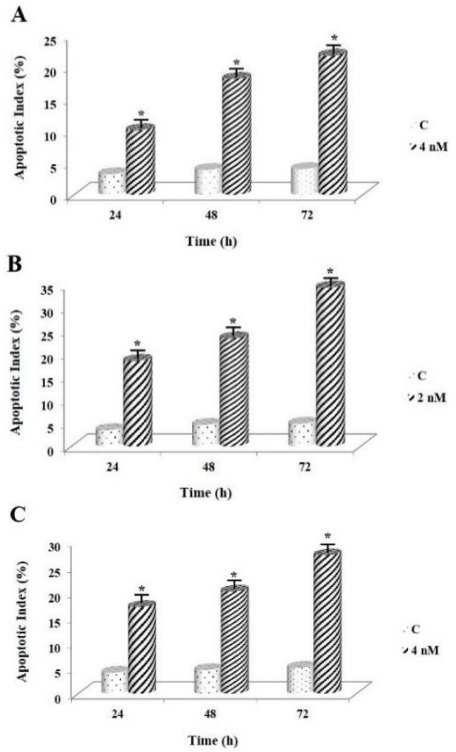

Figure 6. AI values of HeLa cells treated with 4 nM MPC6827 for 0-72 h ($p<0.05$) (A). AI values of MCF-7 cells 4 nM MPC6827 for 0-72 h ($p<0.05$) (B), AI values of A549 cells treated with 4 nM MPC6827 for 0-72 h ($p<0.05$) (C).

In this current study, the antiproliferative effects of tubulin-binding agent MPC6827 on cancer cell lines (HeLa, MCF-7, A549) originating from different tissues were evaluated using various cell kinetic parameters.

Microtubules are major components of the cytoskeleton and are involved in important cell functions such as maintenance of cell shape, signal transduction, and mitosis (8, 9). Microtubule targeting agents are one of the most successful groups of agents used in cancer treatment (10, 11).

MPC-6827 has been shown to inhibit the proliferation of many different cell lines, including human breast, colon, pancreatic, ovarian, and mouse melanoma (6). A study on OVCAR-3 xenograft models showed that MPC-6827 administration greatly promoted tumor necrosis (12). MPC-6827 treatment causes rapid G_2-M mitotic arrest and apoptosis in different cell lines (6). In vivo studies have also shown that MPC6827 causes tumor regression (6, 7).

As a result; when the antiproliferative effects of MPC6827 on all three cell lines were evaluated, it was observed that different concentrations caused different types of cell death. The 2nM concentration did not cause any effect in A549 cells, the antiproliferative effect only started from the 4nM concentration. However, antiproliferative effects started to be seen in HeLa and MCF-7 cells from 2nM concentration. In addition, it was concluded that in all cell types optimum concentrations cause a significant regression in the percentages of cells in the mitosis and synthesis phase while increasing the percentage of apoptotic cells, thereby reducing tumor prognosis.

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Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

Author's contribution

All authors responsible for the manuscript equally.

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