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Research Article

Phenformin Inhibits the Proliferation of MCF-7 and MDA-MB-231 Human Breast Cancer Cell Lines

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Abstract

Aim: Breast cancer is the most common malignancy in women. This disease is a critical public health problem and further research at the molecular level would define its prognosis and specific treatment. Phenformin is an antidiabetic agent. Recent studies showed that it should be considered as a potential agent for the prevention and treatment of cancer cell lines. This study aimed to investigate the antiproliferative effect of phenformin on MDA-MB-231 and MCF-7 breast cancer cell lines.

Materials and Methods: MDA-MB-231 and MCF-7 breast cancer cell lines were used in the study. Experimental groups; control group, 1 µM, 2.5 µM, and 5 µM phenformin administered groups were planned. WST-1 analysis was performed to evaluate the viability of cells 24 h after the treatments. Statistical analysis was carried out using the SPSS 17.0 statistical program. $p<0.05$ was considered to indicate a statistically significant difference.

Results: In the MDA-MB-231 breast cancer cell line, there were statistically significant differences among all groups ($p<0.05$, for all), except between the 1 µM phenformin-treated and control groups ($p>0.05$). In the MCF-7 breast cancer cell line; there were statistically significant differences between the control group and 5 µM phenformin-treated, and between 1 µM phenformin-treated and 5 µM phenformin-treated ($p<0.05$, for all) groups.

Conclusion: Phenformin seems to exert antiproliferative effects on MDA-MB-231 and MCF-7 breast cancer cell lines. It was observed that the antiproliferative effect was stronger in the MDA-MB-231 cell line. It showed a stronger antiproliferative effect in the MDA-MB-231 cell line of antiproliferative effect.

Keywords: Breast cancer, MDA-MB 231, MCF-7, phenformin

INTRODUCTION

Cancer is a chronic life-threatening disease that greatly impacts all spheres of life, so we can mention that breast cancer is one of the vicious diseases which changes millions of women's life (1). Breast cancer is classified according to the type of tissue from which it is originated and according to the extent. Breast cancer can start in the mammary glands, mammary ducts, adipose, or connective tissue (2). It has been explained that it occurs in epithelial cells of ducts (85%), or lobules (15%) in the breast tissue (3). Some grow very slowly and spread to other body parts only when they become significant. Others are more aggressive, grow and spread quickly (2). The reason why women die from breast cancer is mainly due to widespread metastasis. According to the World Health Organization (WHO), in 2020 alone, 2.3 million women were diagnosed with breast cancer, with approximately 685,000 deaths (3).

However, recent advances in medicine, and technology, have led to significant advances, improvements in the early detection and treatment of cancer through radiation and targeted chemotherapy, surgery, and drugs such as hormone therapy or targeted biologic therapy. This type of treatment can prevent the growth and spread of cancer and thus save lives (3, 4). Phenformin is an antidiabetic drug of the biguanide group. Phenformin (phenylethyl biguanide; an antidiabetic agent) shows that it is successful in combined cancer treatments (5). Phenformin has been recognized as a drug with antiproliferative potential. It has been observed the introduction of -Cl and -OCF3 substituents significantly increase the antiproliferative activity of phenformin (6). Phenformin has been shown to have an antitumor effect, not only through AMPK (AMP-

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activated protein kinase) but also as a blocker of the mTOR regulatory complex. Moreover, phenformin abolishes resistance to antiangiogenic tyrosine kinase inhibitors (TKI), which prevents uncontrolled glucose metabolism in tumor cells (7). Studies on various tumor types (eg, breast, lung, prostate, and colon cancer) indicate that phenformin is more potent than metformin (biguanide; an antidiabetic agent) in inhibiting cell proliferation and tumor growth both in vitro and in vivo (8). Therefore, phenformin should be considered as a potential agent for the prevention and treatment of breast cancer in cell line research (9).

The aim of the present study was to investigate the antiproliferative effect of phenformin on MDA-MB-231 and MCF-7 breast cancer cell lines and also to compare effectiveness of phenformin in different concentrations.

MATERIAL AND METHOD

MDA-MB-231 and MCF-7 breast cancer cells (ATCC, Manassas, VA, USA) were cultured in RPMI 1640 culture medium containing 10% heat-inactivated fetal bovine serum, streptomycin, and 1% penicillin. The cells were cultured in 75 cm^2 polystyrene flasks and maintained in an incubator at 37°C in 5% CO^2. Their morphology and proliferation were checked microscopically. The cells were split-passaged when they had reached a confluency of approximately 90%. Cells in semi-confluent flasks were harvested using 0.05% trypsin and centrifuged after the addition of RPMI 1640 for trypsin inactivation. After centrifugation, they were resuspended in a culture medium. Phenformin (CAS Number: 834-28-6, Sigma-Aldrich, St Louis, MO, USA) was prepared as a 5 µM stock solution.

WST-1 cell viability assay

The viability of the cells was evaluated with the WST-1 solution (Water-soluble tetrazolium salt) assay. Briefly, the cells were seeded in triplicate in 96-well plates at a density of 1×10^5 cells/well. The experimental groups are planned as follows for both cell lines: control group, 1 µM, 2.5 µM, and 5 µM phenformin administered groups. After 24 h of incubation, each cell line was exposed to increasing concentrations of phenformin (0 µM, 1 µM, 2.5 µM, 5 µM). Then, the plates were incubated at 37°C in a 5% CO^2 incubator for 24 h. A solution of WST-1 (Water-soluble tetrazolium salt) equal to 1:10 of the medium it contained was added to each well of the culture dish, and the plates were incubated at 37°C for 3 h. At the end of the incubation, absorbance was measured on an ELISA plate reader with the wavelength of 450 nm and a reference wavelength of 650 nm (10).

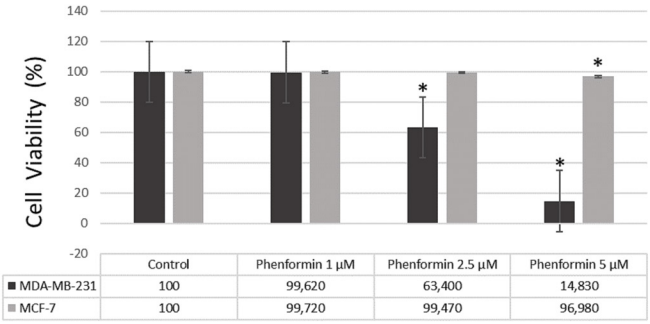
Statistical analysis

Statistical analysis was carried out using the SPSS 17.0 statistical program (SPSS Inc., Chicago, Ill., USA). All experiments were carried out in triplicate and presented as mean±SEM. Statistical analysis was performed by using a one-way analysis of variance, followed by Tukey's or Dunnett's post hoc test. $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

The percentage of MDA-MB-231 and MCF-7 breast cancer cells in the control group was accepted as 100%, and the percentages of cells in the other groups were proportional accordingly. According to the results of the WST-1 analysis, it was found that the percentage of cell viability in MDA-MB-231 cells was 100% (control), 99.62% (1 µM phenformin), 63.40% (2.5 µM phenformin), 14.83% (5 µM phenformin), while according to the results of WST-1 analysis, it was found that the percentage of cell viability in MCF-7 cells was 100% (control), 99.72% (1 µM phenformin), 99.47% (2.5 µM phenformin), 96.98% (5 µM phenformin). Cell viability was reduced in a dose-dependent manner in the MDA-MB-231 and MCF-7 breast cancer cell lines.

In the MDA-MB-231 breast cancer cell line, there were statistically significant differences among all groups ($p<0.05$, for all), except between 1 µM phenformin-treated and control groups ($p>0.05$). In the MCF-7 breast cancer cell line; there were statistically significant differences between the control group and 5 µM phenformin-treated, and between 1 µM phenformin-treated and 5 µM phenformin-treated groups ($p<0.05$, for all) (Figure 1).



	Control	Phenformin 1 µM	Phenformin 2.5 µM	Phenformin 5 µM
MDA-MB-231	100	99,620	63,400	14,830
MCF-7	100	99,720	99,470	96,980

Figure 1. Effects of phenformin on cell viability in the MDMB-231 and MCF-7 breast cancer cell lines after 24h. * Significant difference when compared with control $p<0.05$.

For MDA-MB-231; Control vs 2.5 µM Phenformin and 5 µM Phenformin $p<0.05$, 1 µM Phenformin vs 2.5 µM Phenformin and 5 µM Phenformin $p<0.05$, 2.5 µM Phenformin vs 5 µM Phenformin $p<0.05$. For MCF-7; Control vs 5 µM Phenformin $p<0.05$, 1 µM Phenformin vs 5 µM Phenformin $p<0.05$.

DISCUSSION

The antidiabetic drug phenformin has been considered as a potential agent for treatment in various in vitro cancer studies (7). Coperchini et al. (2019) 's TPC-1 (Human Papillary Thyroid Carcinoma) and 8505C (Anaplastic thyroid carcinoma) cell lines after phenformin applications, cell viability was evaluated with the WST-1 assay performed on the 7th, 14th, and 24th hours; For both cell lines, administration of 10 mM phenformin significantly decreased cell viability at all hours (11). In addition, in

one study, phenformin showed higher cytotoxicity than metformin. The EC50 of metformin was 25 to 15,200,000 times higher than phenformin in B16F10 cells (melanoma), MCF7 cells (breast cancer), CT26 cells (colon cancer), A549 cells (lung cancer), and DU145 cells (prostate cancer) (12).

In a study examining miR-27a-mediated antiproliferative effects of metformin on the breast cancer cell line MCF-7, they reported that metformin inhibits the proliferation of MCF-7 cells at concentrations of 1, 2, 5, 10, and 20 mmol/l (13). In the present study we also investigated the effects of phenformin in different concentrations. We applied 1μM, 2.5μM, and 5μM phenformin and found that the antiproliferative effect of phenformin is increased in a dose dependent manner. Phenformin is a drug in the same antidiabetic group as metformin, and this study supports our data. In a study comparing the efficacy of phenformin and metformin in an in vivo experimental breast cancer model, it was shown that phenformin significantly inhibited both the growth and development of MCF-7 and MDA-MB-231 tumors with greater efficacy than metformin in mice, thus phenformin has clinical potential as an antineoplastic agent opinion has been reached (14).

It has been reported that phenformin reduces cell proliferation and impairs cell cycle progression in SKBR3 and 78617 breast cancer cell lines. This has been shown to occur by inhibiting the growth and epithelial-mesenchymal transition of ErbB2-overexpressing breast cancer cells by targeting phenformin to the IGF1R pathway (15).

Liu et al. (2015) used an intracardiac MDA-MB-231 cancer cells injection model to evaluate the role of phenformin in regulating breast cancer metastasis. They showed that phenformin inhibits the metastasis of MDA-MB-231 cancer cells in nude mice. The same study reported that the respective IC50 values of phenformin in MCF7 and MDA-MB-231 cancer cells were 1.184±0.045 mM and 2.347±0.010 mM. MDA-MB-231 cell line and MCF-7 cell line were used to test the antiproliferative effect of phenformin, so this study shows the efficacy of phenformin, due to its in vitro antitumor activity in preventing the proliferation of cancer cells (9). MDA-MB-231 cells are the most advanced type of breast cancer. In our study we investigated the antiproliferative effect of phenformin on MDA-MB-231 and MCF-7 breast cancer cell lines and observed that phenformin has a more potent antiproliferative effect in the MDA-MB-231 cell line than in the MCF-7 cell line.

CONCLUSION

In this study, it was revealed that phenformin showed strong antiproliferative effect on MDA-MB-231 and MCF-7 breast cancer cell lines and its effect was increased in higher doses. In addition, MDA-MB-231 cancer cells were found to be more sensitive to phenformin compared to MCF-7 cancer cells. In line with data from the literature indicating a controlling inhibiting activity of phenformin on tumor proliferation in vivo, we provide evidence that using a smaller concentration of phenformin exerts a direct lowering effect on tumor proliferation at the cellular level. More studies are certainly required to reveal the reasons behind the difference occurring among breast cancer subtypes. From a future perspective analysis, we can understand that even valuable information about the lower concentrations of phenformin helps us to look in front for solving future problems of our diseases.

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