RESEARCH ARTICLE

Comparison of antiproliferative effects of amlodipine and nebivolol against MCF-7 cells: An in vitro study

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ABSTRACT

Background: Breast cancer (BC) has become the most common cancer among women in India. Both amlodipine, belonging to calcium channel blockers, and nebivolol, belonging to the beta blockers class have proven anticancer mechanisms. However, the effect has not been compared between these drugs till now. Aim and Objectives: The present study aims to compare the antiproliferation effects of amlodipine and nebivolol against human breast adenocarcinoma MCF-7 cells. Materials and Methods: Cell viability assay was used to screen the antiproliferation effects of the study drug molecules in MCF-7 cell cultures. The IC50 values were derived graphically by plotting the cell viability against drug concentration. Results: Both amlodipine and nebivolol exhibited dose-dependent cytotoxicity against BC cells. The IC50 of amlodipine was 166.6 µg/mL and nebivolol was 114.6 µg/mL. Conclusion: In this study, nebivolol showed potent cytotoxicity compared to amlodipine. This inference may provide further insights into future drug research.

KEY WORDS: Breast Cancer Cells; Cell Viability Assay; Beta Receptors; Calcium Channels

INTRODUCTION

Breast cancer (BC) remains the most frequent cancer among women. At an anticipated 2.3 million newly diagnosed cases, or 11.7% of the total number of cases of cancer, it overtook lung carcinoma as the primary cause of cancer incidence worldwide in 2020.[1] The incidence rose by over 50% in India from 1965 to 1985. In India, there were an estimated 118000 incident instances in 2016; 98.1% of these incidents involved females, and 526000 cases were the most prevalent.[2,3] Every state in the nation has seen an increase in the age-standardized incidence rate of BC in females over the past 26 years, which rose by 39.1% between 1990 and 2016.[4] According to Globocan Factsheet 2020, BC was responsible for 10.6% of all fatalities and 13.5% of the total cases of cancer in India.[5]

Several forms of human cancer have been linked to calcium channel blockers (CCBs) as anti-cancer agents. Amlodipine, a CCB of class dihydropyridine, for instance, has been demonstrated in several studies to both decrease the growth of malignant cells and cause apoptosis, leading to cell cycle arrest.[6-8] Furthermore, Ji et al. demonstrated that the use of an amlodipine derivative might improve leukemic cells’ p-glycoprotein-mediated multidrug resistance, which in turn inhibited doxorubicin efflux and increased its effectiveness.[9] Furthermore, research on human epidermoid malignant cells conducted both in vitro and in vivo has demonstrated that a number of CCBs, such as amlodipine, nicardipine, and nimodipine, can stop the proliferation of cancer cells.[10]
In 2007, the FDA-approved nebivolol, a third-generation β-blocker, to treat hypertension.\cite{11,12} With a high selectivity for β1-receptors, it also exhibits an additional vasodilatory action through β3-receptor agonism that interferes in the L-arginine/nitric oxide pathway, perhaps reducing oxidative stress.\cite{13-17} Nebivolol was recently demonstrated to have a tumor-inhibiting effect on tumors such as oral squamous cell carcinoma, lung cancer, and BC.\cite{18-20} In human malignancies, including BC, the fibroblast growth factor receptor (FGFR) signaling pathway is aberrantly activated. The combination of nerivolol, and erdafitinib, a strong and selective FGFR inhibitor, enhanced the responsiveness of BT-474 breast carcinoma cells.\cite{21}

One popular in vitro method for drug development, discovery, and biomedical research is cell culture.\cite{22} Researchers have been using two-dimensional cell culture for decades, where the technique includes cell proliferation on plate surfaces as monolayers. MCF-7 constitutes an estrogen-dependent breast carcinoma cell line that has been widely used for more than 40 years by many research groups conducting BC research.\cite{23,24} Because MCF-7 expresses the estrogen receptor, it has exceptional hormone sensitivity and is hence a prominent model for studying hormone response.\cite{25} Thus, for the first time, the present study compares the antiproliferation activity of nebivolol and amlodipine using MCF-7 cells.

MATERIALS AND METHODS

Cell Procurement and Cell Culture Techniques

The MCF-7 has been procured from NCCS, Pune, India. The cell culture technique was followed as per previous literature.\cite{26}

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay

After developing for 24 h in 96-well plates, the MCF-7 cells were exposed to different doses of amlodipine and nebivolol. Every 2 days, the media was changed, and on the 3rd day (72 h later), the cells were cleaned and subjected to MTT salts in accordance with established protocol. The cells’ vitality was assessed using the absorbance at 570 nm. The 50% cell viability (IC\textsubscript{50}) value was obtained from the cell viability-concentration plot. Cell viability percentage is obtained with.\cite{26}

\[
% \text{Cell viability} = \left( \frac{A570 \text{ of treated cells}}{A570 \text{ of control cells}} \right) \times 100
\]

RESULTS

Effects of Amlodipine against MCF-7 Cells Treated with MTT Salts

Amlodipine exhibited a dose-dependent inhibition of cell viability in MCF-7 cells treated with MTT salts [Table 1]. MTT treatment showed increased cytotoxicity with increased concentration. The IC\textsubscript{50} exhibited by amlodipine is deduced from a graphical representation which was estimated to be 166.6 µg/mL [Figure 1]. The cytology of the MCF-7 cells treated with amlodipine and MTT salts is represented in Figure 2.

Effects of Nebivolol against MCF-7 Cells Treated with MTT Salts

Like amlodipine, nebivolol also exhibited a dose-dependent inhibition of cell viability in MCF-7 cells treated with MTT salts [Table 2]. However, nebivolol was potent in vitro compared to amlodipine. The graphically obtained IC\textsubscript{50} value of nebivolol was 114.6 µg/mL. [Figure 3]. The cytology of MCF-7 cells treated with nebivolol and MTT salts is represented in Figure 4.

DISCUSSION

To the best of our current understanding, this is the first study to compare the antiproliferative properties of

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>OD value</th>
<th>Cell viability (%)</th>
</tr>
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<tbody>
<tr>
<td>1000</td>
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<tr>
<td>Cell control</td>
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OD: Optical density

Figure 1: Graphical representation of IC\textsubscript{50} of amlodipine against MCF-7 cells treated with 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide salts

Table 1: Anti-cancer activity of amlodipine in MCF-7 cells
Nebivolol and amlodipine against MCF-7 cells treated with MTT salts in vitro. Both the drugs have shown good inhibition of cell viability of BC cells, MCF-7. Nebivolol was potent and exhibited IC$_{50}$ of 114.6 µg/mL in comparison to amlodipine which exhibited IC$_{50}$ at 166.6 µg/mL.

Nebivolol in previous studies revealing antiproliferation properties in BC cells has demonstrated its effects on ATP homeostasis and mitochondrial respiration.\textsuperscript{[27]} Nebivolol also exhibited activation of caspase 3 which increased apoptotic activity.\textsuperscript{[17,28]} These properties might have contributed to the reduction in cell viability of MCF-7 cells in this present study. Amlodipine exhibited inhibitory effects in various cancer cells such as ovarian cancers, melanoma, hepatic cancers, and gastric cancers demonstrated in past studies.\textsuperscript{[29-32]} In BC cells, it was demonstrated that amlodipine exhibited silencing of calcium channel expression which reduced the motility and adhesion of cancer cells.\textsuperscript{[33]} Amlodipine also exhibited inhibition of filopodia which is essential for invasion of cancer cells, thus preventing metastasis.\textsuperscript{[34]} The present study results are also consistent with past research which could substantiate the antiproliferative effects of amlodipine and nebivolol in BC.

The present study was conducted in vitro cell cultures, and the results are independent from influences of the integration system of the body which can be considered a study limitation and further in vivo studies may be conducted to evaluate the effects of drugs.

### Table 2: Anti-cancer activity of nebivolol in MCF 7 cells

<table>
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<th>Cell viability (%)</th>
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<tr>
<td>Cell control</td>
<td>0.563</td>
<td>100</td>
</tr>
</tbody>
</table>

OD: Optical density

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**Figure 2:** (a-d) Morphology of MCF-7 cell morphology in 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay with inverted microscope at 40× magnification

**Figure 3:** Graphical representation of IC$_{50}$ of nebivolol against MCF-7 cells treated with 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide salts

**Figure 4:** (a-d) Morphology of MCF-7 cell morphology in 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay with inverted microscope at ×40 magnification
CONCLUSION

The current study’s findings demonstrated that nebivolol and amlodipine had a curative impact on cell growth in BC. This investigation presents nebivolol and amlodipine as promising therapeutic agents for the treatment of BC. It may also offer new evidence for investigations into the effects of amlodipine and nebivolol on the susceptibility of breast tumor cells to treatment. This study further provides the IC₅₀ differences between the study molecules as well as provides insights into their benefits as adjuvants or antihypertensives in BC treatments.

REFERENCES


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