RESEARCH ARTICLE

Anastrozole and Paclitaxel Loaded Nanocrystals: Evaluation of Anticancer Activity

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ABSTRACT

To study the cytotoxic study of anastrozole and paclitaxel-loaded nanocrystals on MDA-MB-231 cell lines for enhanced anticancer activity. Paclitaxel is a tubulin-targeting cytoskeletal drug that interferes with microtubule structures, and anastrozole's mechanism of action is apoptosis in living cells. Methylthiazol tetrazolium (MTT) assay was used to determine anticancer activity. After 24 hours of treatment, the highest ALN and PLN concentrations (100 µg/mL) showed a cytotoxic effect. The inhibition rate of doxorubicin (standard) was found at 24 hours with 89.6. The viability values of Anastrozole loaded nanocrystals (ALN) were 14.03 (100), 22.76 (80), 28.23 (40), 34.9 (20), 40.26 (10), 50.13 (5), 60.53 (2), 80.66 (1), and 99.11 (0) µg/mL. When compared to the standard, viability was 38.23% and drug unloaded nanocrystal was 86.06% (100 µg/mL) 24 hours after anastrozole (100 µg/mL) administration to MDA-MB-231 cells. All experimental groups showed significantly from the control group. The cytotoxicity effect was determined with low dose of anastrozole and paclitaxel-loaded nanocrystals. The cell viability and toxic effects of the ALN and PLN also tested. As ALN and PLN concentrations grew, so did the harmful effect on MDA-MB-231 cell viability. Cell count was significantly reduced ($p < 0.05$) at high ALN and PLN concentrations (100 µg/mL). Finally, the MTT assay revealed that ALN and PLN cytotoxic to MDA- MB-231. Exposure to dose-dependently hazardous doses of ALN and PLN nanocrystal formulations resulted in increased nuclear intensity, cytochrome c, and permeability of the cell membrane in the MDA-MB-231 cell line.

Keywords: Anastrozole, Paclitaxel, Nanocrystals, Methylthiazol tetrazolium.

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INTRODUCTION

Globally, breast cancer is the leading cause of death for women.[1] A range of drugs are possible adjuvants for postmenopausal women with hormone-sensitive advanced metastatic breast cancer.[2,3] Certain medications, such as anastrozole (ANZ), a third-generation non-steroidal aromatase inhibitor used to treat hormones, work as aromatase inhibitors.[4] It mainly inhibits the enzyme aromatase by reversibly binding to it and suppressing the generation of estrogen. It is most commonly prescribed to menopausal women with hormone-dependent breast cancer.

Paclitaxel (PTX) used in of cancers like the cervix, breast, lung, ovaries, esophagus, pancreas, and sarcomas.[5] Paclitaxel is one of several cytoskeletal drugs that target tubulin. Paclitaxel function is by interfering with the microtubule structures that support the growth and division of cancer cells. This finally stops cancer cells from growing and destroys them. Presently ANZ has been widely prescribed as a tablet formulation for cancer treatment, while PTX has been widely prescribed as an injection form.[6,7] Lipidic systems have proven to be effective at delivering various anticancer drugs over the last two decades. Lipidic drugs are excellent lipidic Nano systems candidates.[8] The physiological and biodegradable properties of certain lipidic molecules may be able to mitigate a variety of negative effects.

Furthermore, the long-term toxicity of the anticancer delivery systems that are now in use can be reduced in comparison to previously described drug delivery techniques.[9] As a result, the work in this investigation was planned to formulate nanocrystals containing ANZ and PTX in order to improve ANZ and PTX formulation oral and intravenous delivery. Finally, an *in-vitro* cytotoxicity study revealed that the developed ANZ and PTX had anticancer activity.

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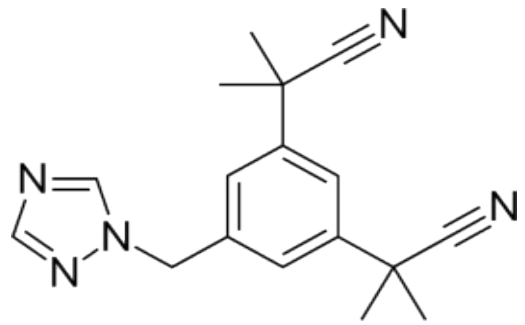

Figure 1: Anastrozole structure[10]

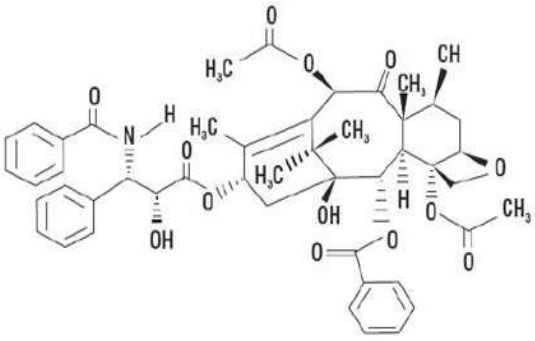

Figure 2: Paclitaxel structure[11]

MATERIALS AND METHODS

The anastrozole (Figure 1) and paclitaxel (Figure 2) was supplied by Neutral Pharma Pvt. Ltd. of Surat, Gujarat, India. All the chemicals were used AR grade only. MDA-MB-231 cells were procured from Amala Cancer Research Institute, Thrissur, Kerala.

For Accessing the Anticancer Activity

Weekly intraperitoneal (I.P) inoculations of 106 cells/mouse were used to maintain EAC cells. MDA-MB-231 cells were used as a test system. Details of MDA-MB-231 cells as shown in Table 1. The Amala Cancer Research Institute provided cell lines. The frozen lot of cells was used for the experiment after the absence of mycoplasma contamination was confirmed. Healthy cells divide and multiply indefinitely when cultured.[12] A toxic chemical, regardless of its site of action or mechanism of action, interfered with this process, resulting in a decrease in cell number growth rate.

Cytotoxicity Assay (MTT assay)

Anastrozole and paclitaxel were tested for their anticancer efficacy using the MTT assay on MDA-MB-231 cells. DMSO was used to dissolve anastrozole and paclitaxel to create the stock solution.[14] Every plate had doxorubicin added as a control, and it was then incubated. Following the removal of the medium, DMSO was used to stabilize the formazan crystals that had formed.[15] The absorbance was measured and the growth inhibition was calculated using a plate reader. Pipette tips (10–100 µL), sterile HCl, phosphate-buffered saline, (2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT; 10 × 5 mg, 0.01 M dimethyl sulfoxide and 5% CO_2.[16]

Table 1: Details of MDA-MB-231 cells[13]

Name of the cell line	*MDA-MB-231 cells Amala Cancer research Institute [ACRI]*
Species	Mouse
Tissue	intraperitoneal (i.p) inoculation of 10^6 cells/ mouse
Cell type, Morphology	Adherent , Fibroblast
Growth Medium	Minimum Essential Medium (MEM) with 10% FBS
Growth Conditions	Temperature : 37 ± 1°C Carbon dioxide: 5%
Storage condition of testsystem	The cell line is cryopreserved in cryovials and stored as stockcultures in liquid nitrogen (Below -130°C).

Cell Culture Formation

The cell proliferation assay with MTT Reagent was used to assess the cytotoxicity of anastrozole and paclitaxel-loaded nanocrystals on breast cancer cells.[17] The 3-(4, 5-dimethylthialzol-2-yl)-2, 5-diphenyl tetrazolium bromide, the cytotoxic effects of free nanocrystals, anastrozole, paclitaxel, anastrozole loaded nanocrystals (ALN), and paclitaxel loaded nanocrystals (PLN) on breast cancer cell were assessed. Breast cancer cells were treated for 24 hours with drug-free nanocrystals, anastrozole, paclitaxel, ALN, PLN at concentrations ranging from 0 to 40 µg/mL. MDA-MB-231 cells were placed in 5000 cells/well and growned in serum free medium overnight before being treated for 24 hours at 37°C with 0, 2, 5, 10, 20, 40, 80, 100 g/mL of drug unloaded nanocrystals, anastrozole, paclitaxel, ALN, PLN, and doxorubicin standard.[18] All of the cells were cultured for 4 hours at 370°C with 0.1% MTT after the media was discarded. The formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and a microplate reader was used to quickly detect the absorbance at 496 nm.[19] In this experiment, equal volumes of DMSO were utilized as controls. Before reading, the plates were shaking for 2 minutes.

$$\%\ \text{Cell Viability} = \frac{\text{Purple coloured cells}}{\text{No, of. Total cell}} \times 100$$

The equation was used to calculate the percentage of inhibition of cell proliferation.[15] The concentration needed to lower 50% of the untreated control's absorbance value was referred to as the IC_{50} concentration. IC_{50} values were calculated and curves were plotted.

$$\%\text{Cell growth inhibition} = \frac{(\text{Abs Zero} - \text{Abs sample})}{\text{Abs Zero}} \times 100$$

RESULTS

The MDA-MB-231 cell line was used to test the cytotoxicity of anastrozole and paclitaxel (shown in Tables 2-5, Figures 3-5).

Table 2: %Cell viability of anastrozole loaded nanocrystals

Con^ (µg/mL)	*ControlDMSO*	*Drug unloaded nanocrystals*	*Anastrozole*	*ALN*	*Doxorubicin (Std. Drug)*
0	100 ± 0	99.33 ± 0.28	99.2 ± 0.34	99.11 ± 0.15	99.5 ± 0.45
1	100 ± 0	98 ± 0.2	93.83 ± 0.47	80.66 ± 0.76	61.4 ± 1.04
2	100 ± 0	97.8 ± 0.25	80.5 ± 0.95	60.53 ± 0.71	50.33 ± 0.25
5	100 ± 0	96.36 ± 0.81	72.26 ± 0.30	50.13 ± 1.20	40.73 ± 0.37
10	100 ± 0	94.26 ± 0.55	60.96 ± 0.40	40.26 ± 0.60	35.46 ± 0.45
20	100 ± 0	92.33 ± 0.66	50.1 ± 0.45	34.9 ± 0.6	32.9 ± 0.17
40	100 ± 0	90.2 ± 0.62	46.33 ± 0.75	28.23 ± 0.58	26.83 ± 0.25
80	100 ± 0	88.6 ± 0.88	40.13 ± 0.35	22.76 ± 0.32	20.76 ± 0.25
100	100 ± 0	86.06 ± 0.21	38.23 ± 0.35	14.03 ± 0.40	10.4 ± 0.4

Table 3: Percent cell Inhibition of Anastrozole loaded nanocrystals (ALN)

Con^ (µg/ml)	*Control DMSO*	*Drug unloaded nanocrystals*	*Anastrozole*	*ALN*	*Doxorubicin (Std. Drug)*
0	0	0.66 ± 0.28	0.36 ± 0.05	0.16 ± 0.15	0.5 ± 0.45
1	0	4 ± 0.2	4.29 ± 2.79	19.06 ± 0.51	38.6 ± 1.04
2	0	11.6 ± 0.25	19.5 ± 0.95	39.53 ± 0.60	49.66 ± 0.25
5	0	19.63 ± 0.81	27.73 ± 0.30	49.83 ± 0.47	59.26 ± 0.37
10	0	25.73 ± 0.54	39.03 ± 0.40	59.73 ± 0.60	64.53 ± 0.45
20	0	29.66 ± 0.66	49.9 ± 0.45	65.1 ± 0.6	67.1 ± 0.17
40	0	33.8 ± 0.62	53.66 ± 0.75	66.67 ± 0.58	71.16 ± 0.25
80	0	40.4 ± 0.88	59.86 ± 0.35	69.23 ± 0.32	79.23 ± 0.25
100	0	49.93 ± 0.20	61.76 ± 0.35	75.96 ± 0.40	89.6 ± 0.4

Table 4: Percent cell viability of Paclitaxel loaded nanocrystals (PLN)

Con^ (µg/ml)	*Control DMSO*	*Drug unloaded nanocrystals*	*Paclitaxel*	*PLN*	*Doxorubicin (Std. Drug)*
0	100 ± 0	99.12 ± 0.32	99.76 ± 0.21	99.08 ± 0.28	99.5 ± 0.45
1	100 ± 0	98.04 ± 0.27	92.45 ± 0.17	79.06 ± 0.58	61.4 ± 1.04
2	100 ± 0	97.65 ± 0.19	79.38 ± 0.32	59.92 ± 0.67	50.33 ± 0.25
5	100 ± 0	96.28 ± 0.67	70.43 ± 0.42	49.32 ± 0.87	40.73 ± 0.37
10	100 ± 0	95.67 ± 0.53	59.75 ± 0.32	39.07 ± 0.45	35.46 ± 0.45
20	100 ± 0	94.18 ± 0.28	50.34 ± 0.29	33.25 ± 0.34	32.9 ± 0.17
40	100 ± 0	92.43 ± 0.31	44.22 ± 0.93	26.21 ± 0.30	26.83 ± 0.25
80	100 ± 0	90.49 ± 0.32	39.19 ± 0.24	19.78 ± 0.42	20.76 ± 0.25
100	100 ± 0	88.39 ± 0.64	34.16 ± 0.41	12.03 ± 0.40	10.4 ± 0.4

For the analyses, the MTT method was used. Using doxorubicin (20 µM) as a reference, anastrozole, paclitaxel, ALN, and PLN were examined at different concentrations ranging from 0 to 100 µg/mL. The obtained data has least significant differences after 1 and 2. After 24 hours of treatment, the highest ALN and PLNconcentrations (100 µg/mL) were the most cytotoxic to the cell line. After 24 hours, the conventional doxorubicin inhibition rate was 89.6. The viability values of anastrozole-loaded nanocrystals (ALN) were 14.03 (100), 22.76 (80), 28.23 (40), 34.9 (20), 40.26 (10), 50 .13 (5), 60.53 (2),80.66 (1), and 99.11 (0) µg/mL. When compared to the standard, viability was 38.23% and drug unloaded nanocrystal was 86.06% (100 µg/mL) 24 hours after anastrozole (100 µg/mL) administration to MDA-MB-231 cells. Each study group showed statistically significant changes from the control group.

Percent cell viability of paclitaxel-loaded nanocrystals (PLN): Paclitaxel and PLN were discovered to be cytotoxic in breast cancer cells. To analyses, the MTT method was used.

Table 5: % Cell inhibition of paclitaxel loaded nanocrystals (PLN)

Con^ (µg/ml)	*Control DMSO*	*Drug unloaded nanocrystals*	*Paclitaxel*	*PLN*	*Doxorubicin (Std. Drug)*
1	0	4.0 ± 0.28	5.23 ± 0.45	20.46 ± 0.25	38.6 ± 1.04
2	0	11.16 ± 0.25	20.46 ± 0.36	40.15 ± 0.32	49.66 ± 0.25
5	0	19.63 ± 0.81	28.46 ± 0.28	50.64 ± 0.52	59.26 ± 0.37
10	0	25.73 ± 0.54	40.6 ± 0.51	60.43 ± 0.43	64.53 ± 0.45
20	0	29.66 ± 0.66	50.21 ± 0.29	66.78 ± 0.74	67.1 ± 0.17
40	0	33.8 ± 0.62	55.46 ± 0.34	70.876 ± 0.43	71.16 ± 0.25
80	0	40.4 ± 0.88	60.52 ± 0.26	74.76 ± 0.22	79.23 ± 0.25
100	0	49.93 ± 0.20	64.48 ± 0.27	79.96 ± 0.40	89.6 ± 0.4

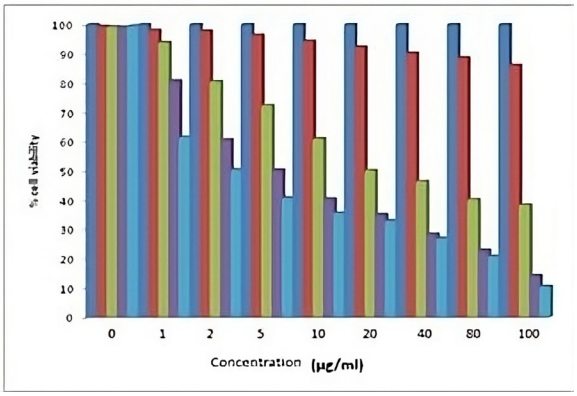

Figure 3: %cell inhibition activity of Anastrozole, ALN and doxorubicin

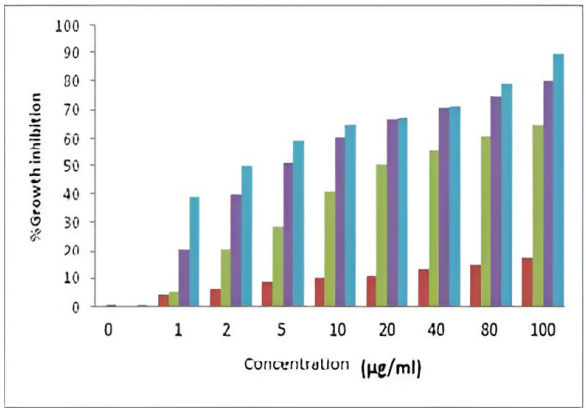

Figure 4: %Cell viability of Anastrozole, ALN, and doxorubicin

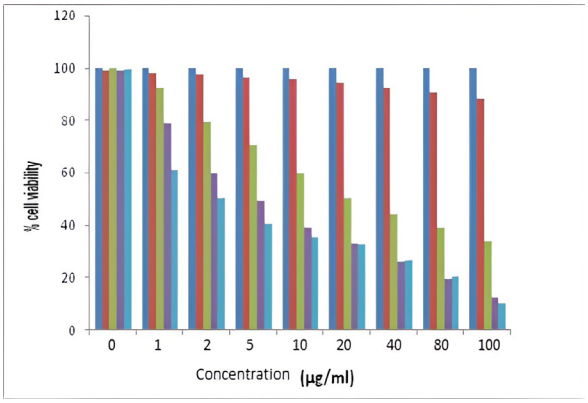

Figure 5: %cell viability of PLN and doxorubicin

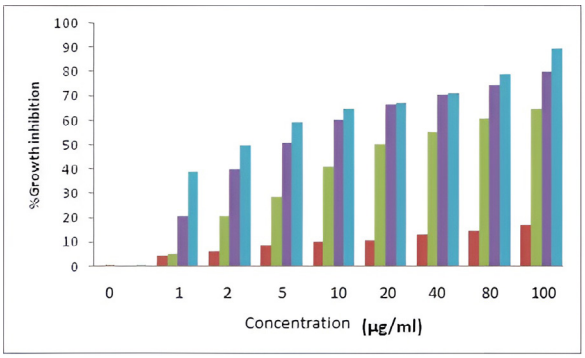

Figure 6: %cell inhibition activity of PLN and doxorubicin

Drug-unloaded nanocrystals, paclitaxel, and PLN were tested at various concentrations (0–100 µg/mL) and standard as doxorubicin (20 µM). The obtained data have less significant differences. After 24 hours of treatment, the highest PLN concentration (100 µg/mL) have cytotoxic to the cell line. The viability values of paclitaxel-loaded nanocrystals (PLN) were 12.03 (100), 19.78 (80), 26.21 (40), 33.25 (20), 39.07 (10), 49.32 (5), 59.92 (2), 79.06 (1), and 99.08 (0) µg/mL. When paclitaxel (100 g/mL) was used, viability was 34.16% when paclitaxel (100 µg/mL) was used and 88.39% (100 µg/mL) when drug unloaded nanocrystal was used when compared to the standard. Paclitaxel-loaded nanocrystals (PLN) were found to have a percent cell inhibition of 79.96% (100 µg/mL), whereas standard doxorubicin had a percent inhibition of 89.6% (100 µg/mL), (Figure 6) indicating that the PLN formulation has a higher percent inhibition when compared to the drug unloaded nanocrystals and paclitaxel.

CONCLUSION

The goal of the *in-vitro* cytotoxicity test is to ascertain the minimum concentration of nanocrystals loaded with anastrozole and paclitaxel that might be harmful to cells. Cell viability changes are linked to the negative effects of the tested PLN and ALN. There was a greater negative impact on MDA-MB-231 cell viability when PLN and ALN concentrations increased. Cell count was significantly reduced (p <0.05) at high ALN and PLN concentrations (100 µg/mL). During a 24-hour treatment period with different concentrations of ALN and PLN, MDA-MB-231 cell viability was clearly dose-dependent, affecting both cellular survival and cell count.

Finally, the MTT assay revealed that ALN and PLN cytotoxic to MDA-MB-231.

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