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Original Article

Evaluation of the antiproliferative effect of histone deacetylase inhibitor (HDACi) CI-994 and Liposomal Cisplatin LipoPlatin on MCF-7 and MDA-MB-231 cells as monotherapy and combined therapy

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Abstract

In this study, the effects of histone deacetylase inhibitor CI-994 and nanotechnological drug liposomal cisplatin LipoPlatin on Luminal A breast cancer and triple-negative breast cancer were explored using agents alone and in combination. MCF-7 and MDA-MB-231 cell lines were used. Cell viability, and cell index values obtained from xCELLigence System, MI, BrdU LI and AI were evaluated in experiments. In monotherapy applications, 10 µM, 20 µM and 40 µM concentrations of CI-994 and 80 µM, 160 µM, and 250 µM concentrations of LipoPlatin were applied to both cell lines. IC_{50} concentration of CI-994 was 10 µM for both cell lines. While IC_{50} concentration of LipoPlatin was 80 µM for Luminal A breast cancer cell line, this concentration was 160 µM for triple-negative breast cancer cell line. The application of a combination of CI-994 and Lipoplatin for each cell line has shown that while there was a significant decrease in cell viability, mitotic index (MI), and BrdU labeling index (LI), there was a significant increase in AI. Consequently, it was thought that combined treatments were more efficient than single medication applications and decreased the cell prognosis with a synergistic and additive impact.

Keywords: Cancer, Breast cancer, Treatment, Histone deacetylase inhibitor, Liposomal cisplatin, *In vitro*

1. Introduction

Chemotherapy, along with surgery and radiotherapy, is the standard treatment method for cancer. However, it has a wide variety of side effects that cause a decrease in the patient's quality of life [1]. Targeting specific pathways involved in cancer formation and progression with two or more chemotherapeutic agents is the basis of cancer therapy with drugs [2, 3]. Although the treatment of various cancer types has been tried to be carried out using a single chemotherapeutic agent, it shows less effectiveness compared to combination therapy. In treatment methods using a single agent, proliferating cells are targeted without making any distinction between healthy cells and cancer cells. Destruction of healthy cells also negatively affects the patient's quality of life by causing various side effects [4, 5]. Although side effects are seen in combination therapy, both targeting different pathways and using lower doses than the dose required for the expected effect as result of applying single agent will reduce these side effects [6, 7]. Genes belonging to various pathways that contribute to cancer formation are silenced by epigenetic mechanisms [8, 9]. Changes in the activity of histone deacetylases, a significant class of enzymes involved in epigenetic processes, aid in the development of numerous cancer-related traits in the cells [10]. In a number of cancers, overexpression of histone deacetylase has been linked to a poor prognosis [11-14].

In medicine, platinum complexes are utilized as a cancer adjuvant treatment. One of the most popular drugs, cisplatin is used to treat a variety of human cancers [15, 16]. The primary target of cytotoxicity induced by cisplatin is nuclear DNA. This reaction can induce apoptosis, necrosis and autophagy in the cell [17-20]. Platinum compounds are poisonous despite their positive effects. Patients receiving these agents show a wide variety of side effects, including nausea and vomiting, suppression of bone marrow, decreased response to infection, and more specifically kidney and neuron damage and hearing loss. These side effects limit the dose that should be given to the patient [16, 21-24]. In order to reduce these side effects and increase the efficacy of the treatment, liposomal cisplatin, which is the encapsulated form of cisplatin in the liposome, has been produced [25].

This study, it was aimed to evaluate the effects of his-

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tone deacetylase inhibitor CI-994 used in epigenetic cancer treatment and LipoPlatin, the nanotechnological derivative of Cisplatin, which is widely used in the clinic for the treatment of a wide variety of malignancies, but whose use has been limited due to its side effects, as monotherapy and combined therapy on MCF-7 breast cancer cells that are estrogen receptor positive and triple-negative breast cancer cell line MDA-MB-231.

2. Materials and methods

2.1. Cell culture

MCF-7 and MDA-MB-231 cells were cultured DMEM (high glucose) (Sigma) containing 10% FBS (Gibco Lab.) at 37°C in humidified atmosphere of 5% CO_2. The pH of the medium was 7.2.

2.2. Agent concentrations

10 µM, 20µM and 40 µM concentrations of histone deacetylase inhibitor CI-994 were prepared by diluting a total of 1 mM stock solution. Likewise, 80 µM, 160 µM and 250 µM concentrations of liposomal cisplatin LipoPlatin were prepared by diluting a total of 1 mM stock solution.

2.3. Cell viability (MTT)

The MTT assay was used to investigate the cytotoxicity of CI-994 and LipoPlatin on the cells as a consequence of the application of planned concentrations. By using the 690 nm wavelength as a reference, the spectrophotometer was used to measure the absorbance values of the experimental groups at 570 nm.

2.4. Cell index (CI)

Using the RTCA xCELLigence DP System, dynamic and real-time cell proliferation was examined in this work. The E-plate was seeded with cancerous cells. 10,000 cells of MCF-7 and 5000 of MDA-MB-231 were seeded into the E-plates of the real-time cell analysis system under all circumstances. For 20 hours, the cells were permitted to stay attached to the plate. After this time, the cells were exposed to different doses of LipoPlatin (80 M, 160 M, and 250 M) and CI-994 (10 M, 20 M, and 40 M). 72 hours of cell proliferation measurement.

2.5. Mitotic index (MI)

For both cell lines, 3x104 cells were sown in 24-well plates to identify mitotic cells. For 24 hours, these cells were treated. The cells were fixed with Carnoy's fixative, the Feulgen technique was used, and then they were stained with Giemsa and counted at the conclusion of the periods of applying the optimal INH1 concentration to the cells. Each slide had about 3000 cells counted for the MI analysis.

2.6. BrdU labelling index (BrdU)

The BrdU labeling index, which is used to measure DNA synthesis during cell proliferation, is based on the fact that bromodeoxyuridine, a nitrogen base analog, labels cells in proliferation. BrdU was determined according to the manufacturer's protocol and determined by spectrophotometric method.

2.7. Apoptotic index (AI)

DAPI was used as a fluorescent dye for the analysis of apoptotic cells. After culturing and inhibitor treatments, cells were fixed with a methanol: FTS mixture. PBS was used to remove the dye. Apoptotic cells were counted by fluorescence microscopy.

2.8. Statistical evaluation

An unidirectional Anova test was used to compare the data from the experimental groups. Version 6 of GraphPad Prism was used to conduct the statistical analyses. San Diego, California, USA: GraphPad Software. In the tests, a significance level of $p<0.05$ was 112 acceptable.

3. Results

3.1. Cell viability

The absorbance values of MCF-7 cell line for CI-994 were $742.14x10^{-3}$, $368.64x10^{-3}$, $264.94x10^{-3}$ and $257.18x10^{-3}$ respectively for control, 10 µM, 20 µM and 40 µM for 24 h (Figure 1A). The absorbance values of MDA-MB-231 cell line for CI-994 were $665.8x10^{-3}$, $330.92x10^{-3}$, $312.68x10^{-3}$ and $298.76x10^{-3}$ respectively for control, 10 µM, 20 µM, 40 µM for 24 h (Figure 1B).

The absorbance values of MCF-7 cell line for LipoPlatin were $225.1x10^{-3}$, $109.1x10^{-3}$, $102.7x10^{-3}$ and $84.54x10^{-3}$ respectively for control, 80 µM, 160 µM, 250 µM for 24 h (Figure 2A). The absorbance values of MDA-MB-231 cell line for LipoPlatin were $534.28x10^{-3}$, $467.84x10^{-3}$, $262.42x10^{-3}$ and $149.67x10^{-3}$ respectively for control, 80 µM, 160 µM, 250 µM for 24 h (Figure 2B).

When the absorbance values belonging to CI-994 were analyzed viability values were 49.67% for 10 µM; 35.69 % for 20 µM and 34.65 % for 40 µM compared to control group which was considered as 100 % for MCF-7 (Figure 1C). For MDA-MB-231, these values belong to CI-994 were 49.7% for 10 µM; 46.96 % for 20 µM and 44.87 % for 40 µM (Fig. 1D). The differences between control and all experimental groups were statistically significant ($p<0.05$).

As a result of lipoplatin application viability values of MCF-7 cells were 48.46% for 80 µM; 45.62% for 160 µM and 37.55% for 250 µM compared to control group which was considered as 100 % (Figure 2C). Cell viability values of MDA-MB-231 cells were 87.56% for 80 µM; 49.11%

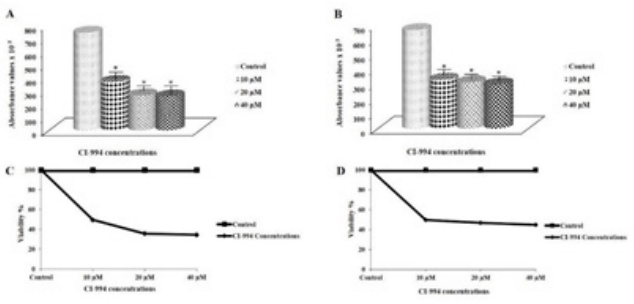

Fig. 1. A: Absorbance values of mitochondrial dehydrogenase activity (450-690 nm) of MCF-7 cells treated with 10 µM, 20 µM, and 40 µM concentrations of CI-994 for 24 h ($p<0.05$). B: Absorbance values of mitochondrial dehydrogenase activity (450-690 nm) of MDA-MB-231 cells treated with 10 µM, 20 µM, and 40 µM concentrations of CI-994 for 24 h ($p<0.05$). C: Percent viability values of MCF-7 cells treated with 10 µM, 20 µM, and 40 µM concentrations of CI-994 for 24 h ($p<0.05$). D: Percent viability values of MDA-MB-231 cells treated with 10 µM, 20 µM, and 40 µM concentrations of CI-994 for 24 h ($p<0.05$).

for 160 µM and 28.01% for 250 µM compared to control group which was considered as 100 % (Figure 2D).

The IC_{50} concentrations determined when the data obtained as a result of the application of the histone deacetylase inhibitor CI-994 and liposomal cisplatin LipoPlatin alone to MCF-7 and MDA-MB-231 are evaluated are shown in Table 1.

After determining the IC_{50} concentrations for both cell lines, 10 µM CI-994, 80 µM LipoPlatin and combination (5 µM CI-994 + 40 µM LipoPlatin) concentrations were applied to MCF-7 cells to determine the change in cell viability depending on time. These concentrations were 10 µM CI-994, 160 µM LipoPlatin and combination (5 µM CI-994 + 80 µM LipoPlatin) concentrations for MDA-MB-231. Absorbance values for MCF-7 decreased from 742.543 $x10^{-3}$ to 361.67 $x10^{-3}$ for CI-994, to 346.76 $x10^{-3}$ for LipoPlatin and to 218.87 $x10^{-3}$ for combination at 24 h; decreased from 751.91 $x10^{-3}$ to 343.865 $x10^{-3}$ for CI-994, to 336.187 $x10^{-3}$ for LipoPlatin and to194.83 $x10^{-3}$ for combination at 48 h; decreased from 767.65 $x10^{-3}$ to 297.54 $x10^{-3}$ for CI-994, to 251.564 $x10^{-3}$ for LipoPlatin and to 101.53 $x10^{-3}$ for combination at 72 h (Figure 3A). Absorbance values for MDA-MB-231cell line decreased from 688.321 $x10^{-3}$ to 341.793 $x10^{-3}$ for CI-994, to 343.682 $x10^{-3}$ for LipoPlatin and to 195.854 $x10^{-3}$ for combination at 24 h; decreased from 698.456 $x10^{-3}$ to 221.44 $x10^{-3}$ for CI-994, to 219.543 $x10^{-3}$ for LipoPlatin and to143.53 $x10^{-3}$ at 48 h; decreased from 721.086 $x10^{-3}$ to 197.651 $x10^{-3}$ for CI-994, to 193.623 $x10^{-3}$ for LipoPlatin and to 113.469 $x10^{-3}$ for combination at 72 h (Fig. 3B).

3.2. Cell index

The cell index values obtained as a result of the application of CI-994 and Lipoplatin alone to MCF-7 and MDA-MB-231 cells were shown in Figures 4A, 4B, 5A, and 5B. Cell index values showed that CI-994 had antiproliferative effect on MCF-7 and MDA-MB-231 cells. Cell index curves compared with control curves show that 10 µM concentrations of CI-994 had cytostatic effects and 20 µM and 40 µM concentrations of CI-994 had cytoskeletal effects on MCF-7 cells. For MDA-MB-231 cells while 10 µM concentrations of CI-994 had antimitotic effects, 20 µM and 40 µM concentrations of CI-994 had cytostatic effects. When the cell index values obtained from Xcelligence DP are examined; antiproliferative effects of LipoPlatin on MCF-7 cells are seen. When the obtained cell index values are compared with the standard curves, it is seen that all applied LipoPlatin concentrations cause DNA damage to the cells. Cell index values of LipoPlatin for MDA-MB-231 cells are compared with the standard curves, it is thought that 160 µM and 250 µM LipoPlatin concentrations cause DNA damage. However, 80 µM LipoPlatin concentration had no effect on MDA-MB-231 cells.

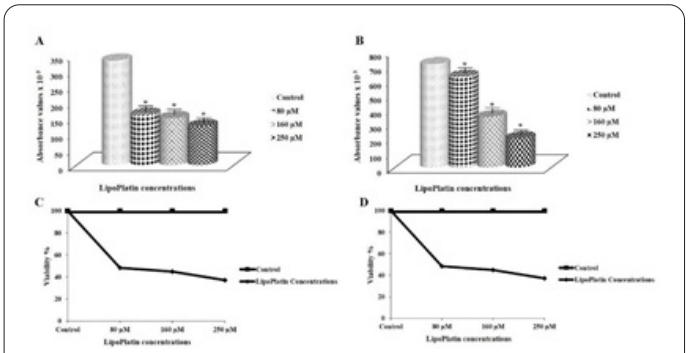

Fig. 2. A: Absorbance values of mitochondrial dehydrogenase activity (450-690 nm) of MCF-7 cells treated with 80 µM, 160 µM, and 250 µM concentrations of LipoPlatin for 24 h ($p<0.05$). B: Absorbance values of mitochondrial dehydrogenase activity (450 690 nm) of MDA-MB-231 cells treated with 80 µM, 160 µM, and 250 µM concentrations of LipoPlatin for 24 h ($p<0.05$). C: Percent viability values of MCF-7 cells treated with 80 µM, 160 µM, and 250 µM concentrations of LipoPlatin for 24 h ($p<0.05$). D: Percent viability values of MDA-MB-231 cells treated with 80 µM, 160 µM, and 250 µM concentrations of LipoPlatin for 24 h ($p<0.05$).

Table 1. IC_{50} concentrations of CI-994 and LipoPlatin.

	MCF-7	MDA-MB-231
CI-994	10 µM	10 µM
LipoPlatin	80 µM	160µM

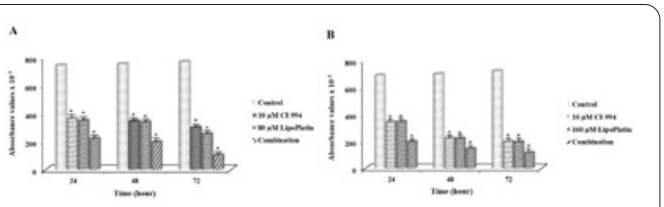

Fig. 3. A: Absorbance values of mitochondrial dehydrogenase activity (450-690 nm) of MCF-7 cells treated with 10 µM CI-994, 80 µM LipoPlatin and combine (5 µM CI 994+ 40 µM LipoPlatin) concentrations for 0-72 h ($p<0.05$). B: Absorbance values of mitochondrial dehydrogenase activity (450-690 nm) of MDA-MB-231 cells treated with 10 µM CI-994, 160 µM LipoPlatin and combine (5 µM CI-994+ 80 µM LipoPlatin) concentrations for 0-72 h ($p<0.05$).

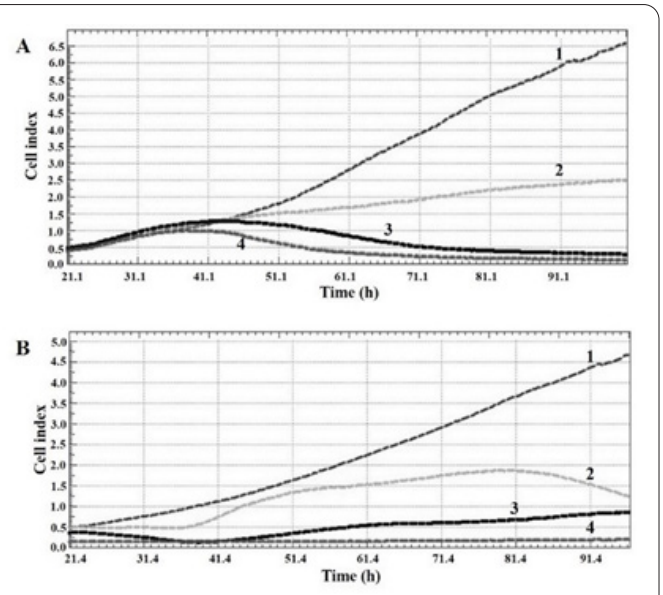

Fig. 4. A: Cell index values of MCF-7 cells treated with 10 µM, 20 µM, and 40 µM concentrations of CI-994 obtained from xCelligence Real-Time Cell Analysis (RTCA) system (Line1: Control, Line 2: 10 µM, Line 3: 20 µM and Line 4: 40 µM). B: Cell index values of MDA-MB-231 cells treated with 10 µM, 20 µM, and 40 µM concentrations of CI-994 obtained from xCelligence Real-Time Cell Analysis (RTCA) system (Line1: Control, Line 2: 10 µM, Line 3: 20 µM and Line 4: 40 µM).

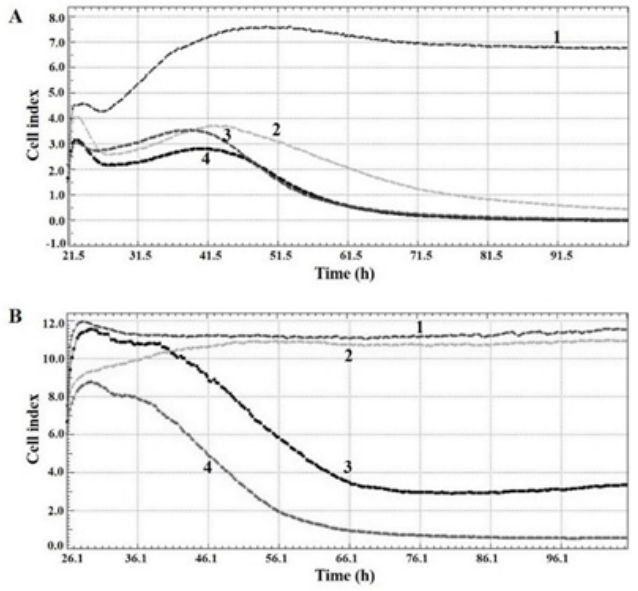

Fig. 5. A: Cell index values of MCF-7 cells treated with 80 µM, 160 µM, and 250 µM concentrations of LipoPlatin obtained from xCelligence Real-Time Cell Analysis (RTCA) system (Line1: Control, Line 2: 80 µM, Line 3: 160 µM and Line 4: 250 µM). B: Cell index values of MDA-MB-231 cells treated with 80 µM, 160 µM, 250 µM concentrations of LipoPlatin obtained from xCelligence Real-Time Cell Analysis (RTCA) system (Line1: Control, Line 2: 80 µM, Line 3: 160 µM and Line 4: 250 µM).

3.3. Mitotic index (MI)

To observe the proportion of cells in the mitotic phase after agent application, 10 µM CI-994, 80 µM LipoPlatin and combination (5 µM CI-994 + 40 µM LipoPlatin) concentrations and 10 µM CI-994, 160 µM LipoPlatin and combination (5 µM CI-994 + 80 µM LipoPlatin) concentrations were applied to MCF-7 and MDA-MB-231 cells respectively. MI values of MCF-7 cells decreased from 5.12 % to 3.85 % for CI-994, to 3.25 % LipoPlatin and 2.13 % for combination at 24 h; decreased from 5.38 % to 2.25 % for CI-994, to 2.18 % LipoPlatin and 1.04 % for combination at 48 h and decreased from 6.01 % to 1.63 % for CI-994, to 1.5 % LipoPlatin and 0.41 % for combination at 72 h (Figure 6A). MI values of MDA-MB-231 cells decreased from 3.4% to 2.76% for CI-994, to 2.87% LipoPlatin and 1.27% for combination at 24 h; decreased from 3.8% to 1.96% for CI-994, to 2.01% LipoPlatin and 0.96% for combination at 48 h and decreased from 4.65% to 1.87% for CI-994, to 1.79% LipoPlatin and 0.58% for combination at 72 h (Figure 6B). When these values were analyzed, it was seen that IC_{50} concentrations caused a significant decrease in both cell lines for both CI-994 and LipoPlatin, while the combined administration caused a higher decrease than the single administration.

3.4. BrdU labelling index (BrdU LI)

After application of 10 µM CI-994, 80 µM LipoPlatin and combination (5µM CI-994 + 40 µM LipoPlatin) concentrations and 10 µM CI-994, 160 µM LipoPlatin and combination (5 µM CI-994 + 80 µM LipoPlatin) concentrations to MCF-7 and MDA-MB-231 cells respectively, in order to determine the proportion of cells in the synthesis phase during cell proliferation, labeling with BrdU was performed. The results showed that while the BrdU labelling index of MCF-7 cells decreased from 100 % to 51% for CI-994, to 49 % LipoPlatin and 27 % for combination at 24 h; decreased from 100 % to 41% for CI-994, to 39% LipoPlatin and 19 % for combination at 48 h and decreased from 100 % to 35 % for CI- 994, to 28 % LipoPlatin and 11% for combination at 72 h (Figure 7A), BrdU labeling index of MDA-MB-231cells decreased from 100 % to 49% for CI-994, to 47 % LipoPlatin and 25 % for combination at 24 h; decreased from 100 % to 38% for CI-994, to 36% LipoPlatin and 20 % for combination at 48 h and decreased from 100 % to 33 % for CI- 994, to 28 % LipoPlatin and 14 % for combination at 72 h (Figure 7B).

3.5. Apoptotic index (AI)

To evaluate the apoptotic index 10 µM CI-994, 80 µM LipoPlatin and combination (5 µM CI- 994 + 40 µM LipoPlatin) concentrations and 10 µM CI-994, 160 µM LipoPlatin and combination (5 µM CI-994 + 80 µM LipoPlatin) concentrations were applied to MCF-7 and MDA-MB-231 cells respectively. AI of MCF-7 cells increased from 2.24 % to 6.27 % for CI-994, to 5.96 % LipoPlatin and 14.15 % for combination at 24 h; increased from 3.12% to 12.34 % for CI-994, to 13.14 % LipoPlatin and 22.76 % for combination at 48 h and increased from 3.65 % to 15.36 % for CI-994, to 17.98 % LipoPlatin and 31.78 % for combination at 72 h (Figure 8A), AI of MDA-MB-231cells increased from 3.43 % to 8.65% for CI-994, to 7.94 % LipoPlatin and 17.63 % for combination at 24 h; increased from 3.79 % to 14.24% for CI-994, to 15.63% LipoPlatin and 23.02 % for combination at 48 h and increased from 4.18 % to 17.36 % for CI-994, to19.71% LipoPlatin and to 33.43 % for combination at 72 h (Figure 8B).

4. Discussion

Preclinical studies investigating the efficacy of histone deacetylase inhibitors when administered as monotherapy have shown that this inhibitor is effective in many different

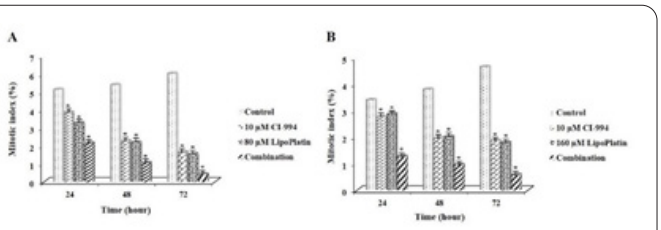

Fig. 6. A: MI values of MCF-7 cells treated with 10 µM CI-994, 80 µM LipoPlatin and combine (5 µM CI-994+ 40 µM LipoPlatin) concentrations for 0-72 h ($p<0.05$). B: MI values of MDA-MB-231 cells treated with 10 µM CI-994, 160 µM LipoPlatin and combined (5 µM CI-994+ 80 µM LipoPlatin) concentrations for 0-72 h ($p<0.05$).

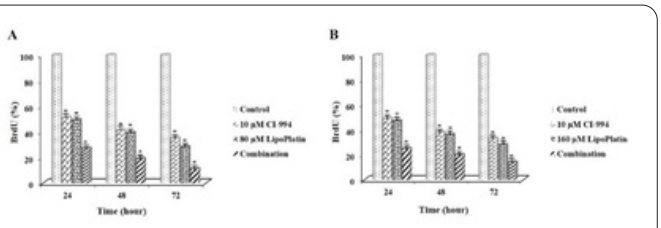

Fig. 7. A: BrdU LI values of MCF-7 cells treated with 10 µM CI-994, 80 µM LipoPlatin and combine (5 µM CI- 994+ 40 µM LipoPlatin) concentrations for 0-72 h ($p<0.05$). B: BrdU LI values of MDA-MB-231 cells treated with 10 µM CI-994, 160 µM LipoPlatin and combined (5 µM CI-994+ 80 µM LipoPlatin) concentrations for 0 72 h ($p<0.05$).

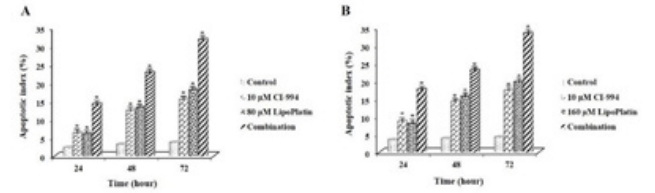

Fig. 8. A: AI values of MCF-7 cells treated with 10 µM CI-994, 80 µM LipoPlatin and combine (5 µM CI-994+ 40 µM LipoPlatin) concentrations for 0-72 h ($p<0.05$). B: AI values of MDA-MB-231 cells treated with 10 µM CI-994, 160 µM LipoPlatin and combined (5 µM CI-994+ 80µM LipoPlatin) concentrations for 0-72 h ($p<0.05$).

types of cancer. In preclinical models, targeted agent histone deacetylase inhibitor, has been shown to be non-toxic to healthy tissues as a single agent, but has a limited antiproliferative effect in clinical trials [26]. It is thought that the use of histone deacetylase inhibitors in combination with different anticancer agents can overcome this problem of limited efficacy [1]. One of these combinations is the use of DNA-damaging agents in combination with a histone deacetylase inhibitor [27]. HDACIs make cancer cells more sensitive to chemotherapeutic agents [28].

In a study evaluating the synergistic effect of the HDACi trichostatin A (TSA) with cisplatin, synergistic effects of these two agents were demonstrated in the inhibition of cell proliferation and induction of apoptosis of the human lung adenocarcinoma cell line A549 compared to single applications [29]. In another study with lung cancer cells, it was shown that TSA increased the sensitivity of cells to cisplatin [30]. The efficacy of HDACi CI-994 was investigated in a study with human lung adenocarcinoma cell line A549 and human hepatic stellate cell line LX-1. As a result, it was observed that cell viability decreased depending on concentration in single applications, and they showed synergistic effects when applied together with different antineoplastic agents [31]. A study with prostate cancer cell lines showed that HDAC inhibitors increase the effectiveness of various DNA-damaging antineoplastic drugs [32]. In a study with different breast cancer cell lines MCF-7, T47D and MDA-MB-231, it was determined that there was an increase in cell cycle arrest and induction of apoptosis as a result of the administration of different HDAC inhibitors alone or in combination with cisplatin [33]. Application HDAC inhibitor combination with PARP inhibitor increased rate of apoptosis in Hodgkin and non-Hodgkin lymphoma cell lines [34]. A study by Liu et al. has shown that HDAC inhibitors can increase apoptosis in triple-negative breast cancer when used in combination with other agents [35]. The study by Xu et al. highlights the efficacy of HDAC inhibitors combined with endocrine therapies for hormone receptor-positive breast cancer [36]. Histone deacetylases support DNA repair through chromatin regulation. Therefore, suppression of histone deacetylases makes cells sensitive to DNA-damaging agents [37, 38].

Liposomes, which have started to be used as drug carriers, increase antitumoral efficacy and reduce toxicity. Therefore, liposomal antineoplastic drugs are used clinically for various types of cancer [39]. In a study with cervical cancer cell lines HeLa, ME-180 and cisplatin-resistant R-ME-180, liposomal cisplatin lipoplatin was shown to induce apoptosis and suppress cell proliferation in a dose-dependent manner [40]. In another study, it was shown that lipoplatin promoted apoptosis in ovarian cancer cell lines, inhibited invasion and had a synergistic effect when used with different anticancer drugs [41].

In this current study *in vitro,* cytotoxic effect of histone deacetylase inhibitor CI-994 and nanotechnological drug liposomal cisplatin LipoPlatin on MCF-7 and MDA-MB-231 cell lines as monotherapy and combined therapy were evaluated. In monotherapy applications, 10 µM, 20 µM and 40 µM concentrations of CI-994 and 80 µM, 160 µM, 250 µM concentrations of LipoPlatin were applied to both MCF-7 and MDA-MB-231 cell lines. When cell viability values were examined it was shown that IC_{50} concentration of CI-994 was 10 µM for both cell lines. IC_{50} concentrations of LipoPlatin were 80 µM and 160 µM for MCF-7 and MDA- MB-231 cell lines respectively. Cell index values from xCELLigence RTCA System also showed similar results for IC_{50} values. Furthermore, application of CI-994 and Lipoplatin alone at scheduled concentrations produced different effects on cells. 10 µM concentrations of CI-994 had cytostatic effect and 20 µM and 40 µM concentrations of CI-994 had cytoskeletal effects on MCF-7 cells. While 10 µM concentrations of CI-994 had antimitotic effects, 20 µM and 40 µM concentrations of CI-994 had cytostatic effects on MDA-MB-231 cells. All LipoPlatin concentrations caused DNA damage on the cells MCF-7 cells. 160 µM and 250 µM LipoPlatin concentrations caused DNA damage on MDA-MB-231 cells. However, 80 µM LipoPlatin concentration had no effect on these cells. While there was a substantial decrease in cell viability, MI, and BrdU LI when monotherapy treatments were compared to combination applications, there was a large rise in AI. All of the data pointed to the fact that medication combinations work better than single drug applications at lowering cell prognosis through synergistic and additive effects.

5. Conclusion

The development of new strategies in the treatment of breast cancer is an important issue. In line with the data obtained, it is thought that the combined use of CI-994 and Lipoplatin will contribute to the development of new treatment strategies.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Mehmet R. Topçul: Research design and supervision; İdil

Çetin: Perform all laboratory procedures.

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