Original
ISSN 2413-0516

Synergistic Antitumor and Apoptotic Activity of Sitagliptin or Linagliptin Plus Cisplatin Against A549 Lung Cancer Cells (*An Invitro Study*)

Ameer M. Al Khafaji*, Ahsan F. Bairam

Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq.
*Correspondence to: Ameer M. AL Khafaji (E-mail: ameer.alkhafaji@student.uokufa.edu.iq)
(*Submitted: 10 April 2024 – Revised version received: 26 April 2024 – Accepted: 23 May 2024 – Published online: 26 June 2024*)

Abstract

Objective: Lung cancer (LC) has the highest mortality rate globally. Most chemotherapeutic medicines now in use are cytotoxic, prompting the search for novel compounds with anticancer properties and improved safety profiles for normal cells. Dipeptidyl peptidase-4 inhibitors have demonstrated anticancer effects and apoptotic properties by specifically inhibiting dipeptidyl peptidase-4, a glycoprotein found in various organs that support the spread of cancer and tumor formation. Based on that, this study aimed to evaluate the cytotoxicity and apoptotic activity of sitagliptin (SITA) and linagliptin (LINA) against the lung cancer cell line (A549) alone and in combination with cisplatin (CP).

Methods: A549 cells were categorized into six groups: control (untreated cells), CP-treated cells, SITA-treated cells, LINA-treated cells, CP plus SITA treated cells (1:1 ratio), and CP plus LINA treated cells (1:1 ratio). After 72 hours of incubation, cell viability (or cytotoxicity) and concentration required to inhibit 50% of cell viability (IC50) for each group were determined using an MTT assay. This method is safe and easy to use, has more reproducibility, and is commonly used for cell viability and cytotoxicity tests. Later, A549 cells were cultured in six flasks and exposed to the IC_{50} for 36 hours. Afterward, the cells were harvested and centrifuged, and the supernatant was removed. The remaining cell pellets were collected and lysed using a lysis buffer to measure B-cell lymphoma type 2 (BCL-2) levels with ELISA test kits. The data was collected and subjected to statistical analysis techniques.

Results: MTT assay results determined that SITA and LINA significantly increased A549 cell cytotoxicity compared to the control group ($P<0.0001$). Moreover, combining SITA or LINA with CP showed markedly increased antitumor efficacy and more significant cytotoxicity directed toward A549 cells. Additionally, these combinations highly reduced IC_{50} in comparison to monotherapy. Considerably, both drugs showed remarkable apoptotic activity on A549 cells when used alone or combined with CP by decreasing BCL2 levels. Consequently, it potentiates apoptotic effects and cytotoxicity of CP against cancer cells. Interestingly, Lina was more potent than Sita regarding cytotoxicity and apoptotic activity on A549 cells.

Conclusion: SITA and Lina exhibited significant cytotoxic and apoptotic effects against A549 cells through the induction of apoptosis. Notably, the results suggest a potential synergistic anticancer impact on CP.

Keywords: Antitumor, A549 cell line, sitagliptin, linagliptin, MTT assay

Introduction

Lung or pulmonary cancer is a cancerous tumor that forms in the lungs. It is defined by the rapid and aberrant proliferation of cells, leading to the creation of tumors. The tumors progressively increase in quantity and size, impeding the lung's ability to exchange oxygen efficiently.[1]

Metastatic lung tumor is the spread of lung cancer to other bodily tissues such as adjacent lymph nodes and other locations within the lung, thoracic cavity, brain, skeletal system, neurological system, liver, bone, and adrenal glands. Individuals diagnosed with non-metastatic lung cancer had a median survival time of 13 months, while those with metastatic lung cancer had a median survival time of five months.[2]

Lung cancer is a significant factor in global morbidity and mortality, accounting for 12% of new cancer cases and 18% of annual cancer-related deaths. Lung cancer has the highest fatality rate globally because of its poor prognosis.[3]

In 2020, there were 2,206,771 new instances of lung cancer diagnosed globally, according to the latest Global Cancer Observatory (GLOBOCAN) estimates. Lung tumors are the primary cause of cancer occurrence and death in men, with 1,435,943 new cases and 1,188,679 fatalities. Among women, it is the third most prevalent type of cancer, with 770,828 cases reported, and the second related cause of cancer deaths, with 607,465 fatalities after breast cancer.[4]

Lung cancer is divided into two main histological classes: Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancer cases, while small cell lung cancer (SCLC) comprises 15%. NSCLC is divided into three principal pathological subtypes: Squamous cell cancer, adenocarcinoma, and large cell carcinoma.[5]

Therapeutic strategies for lung cancer must include the stage of the disease, histology, molecular pathology, age, comorbidities, and patient preferences. Therapeutic choices include one or more of the following: surgery, radiation, immunotherapy, and chemotherapy, including CP.[6]

Cisplatin (CP) is a platinum-derived chemotherapeutic medication commonly used intravenously as the primary chemotherapy for many cancer types.[7] It becomes functional when entering a cellular component. CP cytoplasmic chloride atoms are replaced by water molecules. After hydrolysis, the above compound exhibits high electrophilicity, enabling it to react with various nucleophiles in nucleic acids. CP hinders the growth of cancer cells and triggers apoptosis by attaching to the N7 position on purine molecules, destroying DNA.[8,9]

Anticancer medications cause oxidative stress in living organisms, forming lipid peroxidation and various aldehydes that possess electrophilic characteristics. The implications of Oxidative stress may limit the effectiveness of anticancer treatments by stimulating the growth of cancer cells.[10] While CP

monotherapy has shown positive therapeutic results, other studies have reported notable side effects. Moreover, some cancer patients have shown significant resistance to drugs and toxicity in normal tissues, such as neurotoxicity and nephrotoxicity, which diminish the quality of life in patients and necessitate reducing the drug dosage, perhaps leading to the cessation of therapy and diminishing its effectiveness.[11] Therefore, Novel formulations and combination therapy with other medications have been studied to improve the therapeutic efficacy of CP.

Recent research indicates that dipeptidyl peptidase 4 (DPP-4) inhibitors exhibit significant anticancer properties against cancer cells, such as ovarian and colon cancer cells, especially the medications used for diabetes treatment, sitagliptin (SITA) and linagliptin (LINA), which were approved by the FDA in 2006 and 2011, respectively.[12]

Glucagon-like peptide-1 (GLP-1) and Glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones that stimulate insulin release and are deactivated by the DPP-4 enzyme. The incretin hormone stimulates insulin release and inhibits glucagon release from beta cells.[13] DPP4 inhibitors reduce the activity of the DPP-4 enzyme, hence prolonging the effects of incretin hormones.[14] The DPP4 enzyme, called Cluster of Differentiation 26 (CD26), is a transmembrane protein found in several normal cell types. Its expression in different tumors varies depending on the specific kind of cancer.[15] Certain types of cancer, such as breast, melanoma, and endometrial cancers, show reduced DPP4 expression, indicating that DPP4 may act as a tumor suppressor. On the other hand, malignancies such as Mesothelioma and renal, colon, and lung tumors show higher DPP4 expression, indicating its role as a tumor activator.[16] It plays a vital role in the biology of cancer and the development of metastases in malignant cells[17,18] and the prognosis of cancer patients[19] represents essential tumor markers and serves as a novel treatment target for selected cancers.[20] Apoptosis is an essential biological process that inhibits unregulated cell growth and eliminates dangerous cells. The initiation of the apoptotic pathway within mitochondria is tightly regulated by proteins belonging to the B-cell lymphoma 2 (BCl-2) family.[21] BCL2 is a protein primarily found in the endoplasmic reticulum (ER) and mitochondria and has been recognized as a crucial regulator of both cell survival and apoptosis. It exhibits a strong correlation with the biological characteristics of many malignant neoplasms, such as breast cancer, hepatocellular carcinoma, and cervical cancer.[22,23] Hence, DPP4 inhibitors have been shown to improve the outlook for some forms of cancer, such as colon,[24] breast,[25] prostate,[26] renal,[27] and colorectal[28] cancers, in addition to adjusting the oxidative stress balance during chemotherapy. Direct comparison research on the impact of SITA and LINA alone or combined with standard chemotherapy against lung cancer cell lines A549 is limited. The precise molecular mechanisms through which these medicines exert their antitumor effects, particularly in lung cancers, still need to be fully understood. Investigating this variation could lead to understanding the potential impact of DPP-4 inhibitors in treating lung cancer and might aid in developing novel therapeutic approaches for lung cancer.

Therefore, this study aimed to evaluate SITA and LINA anticancer and apoptotic effects on the LC cell line (A549) and compare the resultant activity with CP, a standard chemotherapeutic agent used in anticancer protocols.

Methods

The study was conducted in the Cell Culture laboratory at the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, and lasted around two months. This investigation was conducted on the A549 cell line using the MTT technique previously described by Carmichael et al. and Van Meerloo, Kaspers, and Cloos.[29,30] The cells were cultivated in a 96-well plate and performed a 24-hour incubation period to encourage the development of a (cellular monolayer) at the 80% growth phase. The previous media was removed and replaced with a 200 µl medium containing the test drugs. Six main groups were used: control (untreated cells), cells treated with CP, cells treated with SITA, cells treated with LINA, cells treated with a combination of CP plus SITA in a (1:1 ratio), Cells treated with a combination of CP plus LINA in a (1:1 ratio). Each treatment group was exposed to six concentrations (500, 250, 125, 62.5, 31.25, 15.625 µg/ml) with four repetitions each. Plates were incubated for 72 hours. The medium was removed after drug exposure, and the wells were rinsed with phosphate-buffered saline (PBS). Formazan conversion was identified by using a blank control. To get the desired MTT concentration of 0.5 mg/ml, 10.8 ml of medium was mixed with 1.2 ml of stock MTT solution (5 mg/ml). Subsequently, 200 µl of the MTT solution was added to each well. Following a 3-hour incubation, the plate had intracellular-purple formazan crystals readily visible under an inverted microscope. The supernatant was removed, and 100 µl of DMSO was applied to each well to dissolve the formed formazan crystals. After incubating for 30 minutes at room temperature, the cells ruptured, and the crystals broke down.

They utilized a microplate reader at a wavelength of 570 nm to measure absorbance (optical density), and Cell viability % was determined using the following formula:

$$(A\ Treated - A\ Blank) / (A\ Control - A\ Blank) \times 100$$

Where A is the absorbance.
And then, cytotoxicity% was calculated by:

$$100 - \text{cell viability} \times 100\%$$

Dose-response curves were determined by non-linear regression using a four-parameter logistic Hill equation. The concentration at which 50% inhibition of cell viability occurred (IC_{50}) was estimated for each group using GraphPadPrism 10.

Measurement of BCL2 Concentration

A549 cells were cultured in six flasks and exposed to CP, SITA, and LINA and IC_{50} of CP plus SITA and CP plus LINA IC_{50} for 36 hours (each with triplicate). After treatment, cells were harvested and centrifuged, and the supernatant was removed. The cell pellets were then lysed using a lysis buffer to extract proteins, which were stored in a new 1.5 mL sterile Eppendorf tube and frozen at –20°C until analyzed using a BCL2 ELISA assay kit. An ELISA kit for human BCL-2 obtained from Bioassay Technology Laboratory Company (BT Lab) in Shanghai, China, was used for assessment. The test method was carried out following the company protocol. A microplate reader set at a wavelength of 450 nm was utilized to measure the absorbance value of each well.

Statistical Analysis

The data were collected and examined using GraphPadPrism Edition10 and Microsoft Office Excel 2019. A one-way

ANOVA test and Post hoc (Tukey) were used to assess significant differences among the data means. A *P*-value of 0.05 or less indicates a statistically significant difference.

Results

Cytotoxicity in A549 cells

Cisplatin activity in the A549 cell line

Cisplatin significantly increased the cytotoxic effect on the A549 cell line.

($P < 0.0001$) in all concentrations versus the control group, as listed in Table 1 and Figure 1.

Sitagliptin and linagliptin activity on A549 cell-line

Sitagliptin or linagliptin significantly increased the cytotoxic effect on the A549 cell line ($P < 0.0001$) in all concentrations versus the control group, as listed in Tables 2, 3 and Figures 2, 3.

Effect of cisplatin plus sitagliptin or linagliptin combinations on A549 cell line

In this investigation section, A549 cells were exposed to a fixed ratio (1:1) of cisplatin plus sitagliptin or linagliptin concentrations. The results indicated a substantial cytotoxic effect on A549 for all concentrations in both combinations compared to the control group ($P < 0.0001$), as seen in Table 4 and Figures 4, 5, respectively.

Table 1. **Cytotoxic effect of cisplatin against the A549 cell line**

CP Concentration (µg/ml)	Cytotoxicity % ± SD
Control	0 ± 0
15.625	23.07 ± 1.30
31.25	35.23 ± 3.24
62.5	64.36 ± 2.41
125	77.43 ± 1.27
250	81.39 ± 1.26
500	83.62 ± 0.40

CP: Cisplatin; SD: Standard deviation.

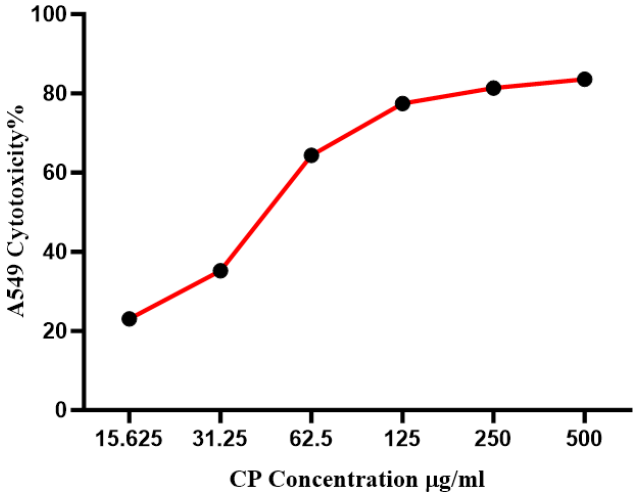

Fig. 1 **The anticancer activity of Cisplatin on the A549 cell line. CP: Cisplatin; A549: lung cancer cell line model.**

Table 2. **The cytotoxic effect of sitagliptin against the A549 cell line**

SITA Concentration (µg/ml)	Cytotoxicity % ± SD
Control	0 ± 0
15.625	9.58 ± 0.550719
31.25	14.34 ± 2.8293
62.5	21.91 ± 2.9226
125	26.70 ± 0.406857
250	38.18 ± 2.18260
500	40.88 ± 0.465

SITA: Sitagliptin; SD: Standard deviation.

Table 3. **The cytotoxic effect of Linagliptin against the A549 cell line**

LINA Concentration (µg/ml)	Cytotoxicity % ± SD
Control	0 ± 0
15.625	17.156 ± 3.88647
31.25	17.220 ± 6.78542
62.5	34.659 ± 13.89682
125	83.771 ± 9.69750
250	92.851 ± 1.41866
500	92.658 ± 0.46367

LINA: Linagliptin; SD: Standard deviation.

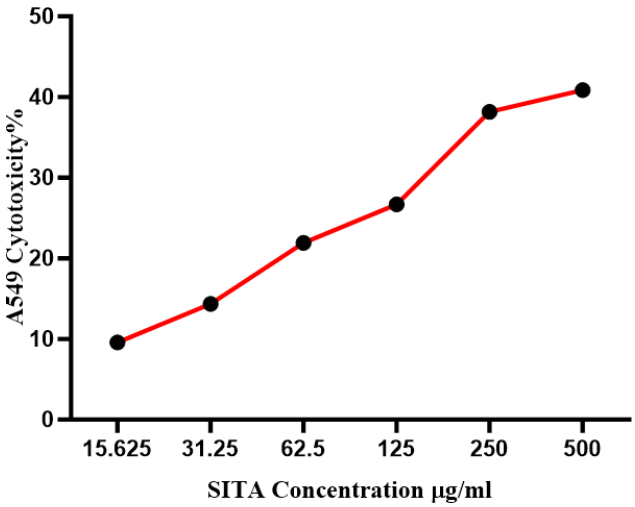

Fig. 2. **Anticancer effect of Sitagliptin on the A549 cell line. SITA: Sitagliptin; A549 = A549: lung cancer cell line model.**

Comparison between the activity of cisplatin alone against cisplatin plus sitagliptin or linagliptin combinations regarding the A549 cell line

Both CP plus SITA or LINA combination demonstrated a higher significant increase in cytotoxicity percent ($P < 0.0001$)

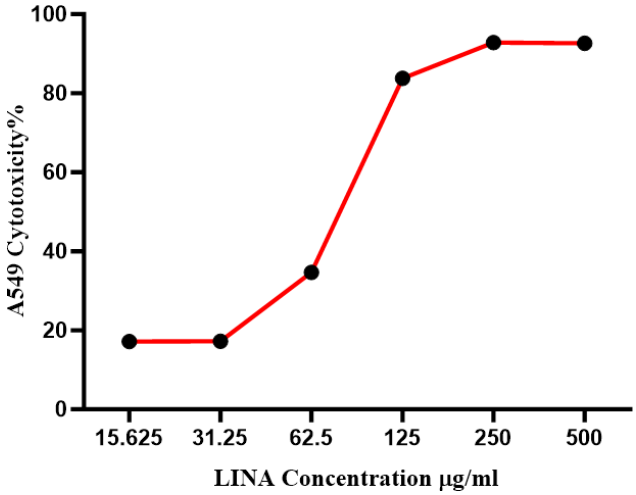

Fig. 3 **Anticancer effect of linagliptin on the A549 cell line. LINA: Linagliptin; A549 = A549: lung cancer cell line model.**

Table 4. **The cytotoxic effect of cisplatin plus sitagliptin and cisplatin plus linagliptin against the A549 cell line**

Combination concentration (µg/ml)	CP+SITA cytotoxicity % ± SD	CP+LINA cytotoxicity % ± SD
Control	0 ± 0	0 ± 0
15.625 + 15.625	37.94 ± 8.54	45.328 ± 8.54
31.25 + 31.25	76.88 ± 1.02	75.098 ± 1.02
62.5 + 62.5	76.66 ± 0.0	75.187 ± 0.00
125 + 125	79.35 ± 2.68	77.478 ± 2.68
250 + 250	82.44 ± 1.16	79.882 ± 1.16
500 + 500	82.55 ± 0.34	83.277 ± 0.34

CP: Cisplatin; SITA: Sitagliptin; LINA: Linagliptin; SD: Standard deviation.

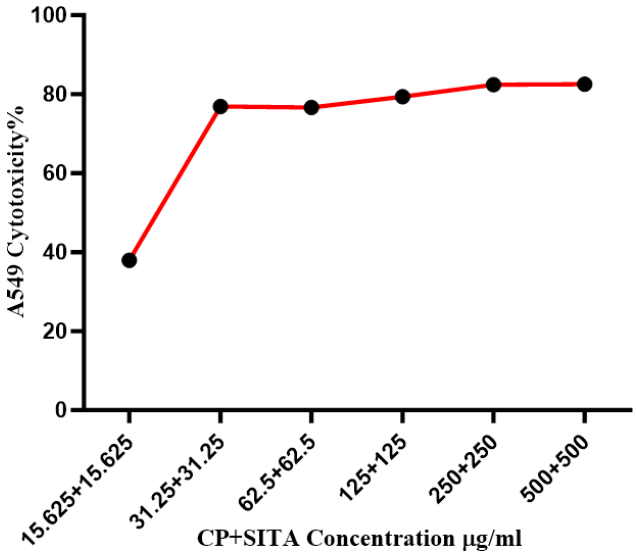

Fig. 4 **Anticancer effect of Cisplatin plus Sitagliptin on the A549 cell line. CP+SITA: Cisplatin + Sitagliptin; A549: lung cancer cell line model.**

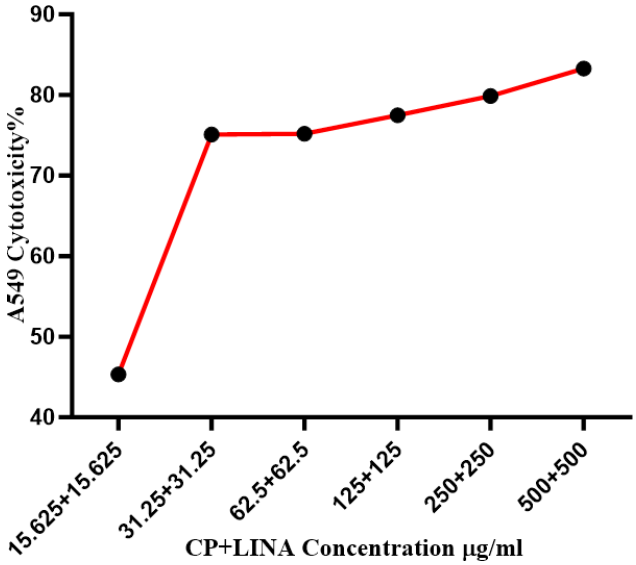

Fig. 5 **Anticancer effect of Cisplatin plus Linagliptin on the A549 cell line. CP+LINA: Cisplatin + Linagliptin; A549: lung cancer cell line model.**

on the corresponding cell line compared to CP alone, particularly at concentrations of 15.625, 31.25, and 62.5% µg/ml, as seen in Table 5 and Figures 6, 7, respectively.

The study compared the effect of CP, SITA, and LINA alone and a combination of CP plus SITA or LINA on IC_{50} in the A549 cell line

The IC_{50} of CP, SITA, and LINA alone was reduced from 49.81,829.5 and 81.1 µg/ml, respectively, to 26.42 µg/ml for a combination of CP plus SITA and 26.47 for a combination of CP plus LINA, as shown in Table 6.

Human BCL-2 Measurement

Cisplatin effect on BCL2 level

The study findings demonstrated a significant elevation in BCL2 concentration ($P < 0.0001$) following treatment of A549 cells with IC_{50} of CP compared to the control group, as seen in Figure 8.

Sitagliptin or linagliptin effect on BCL2 level

The results indicated a significant reduction in the BCL2 level ($P < 0.0001$) and ($P < 0.001$) following treatment of A549 cells with SITA or LINA IC_{50} when compared to the control group. However, no significant difference was detected between SITA and LINA-treated cells, as seen in Figure 8.

Comparison between the effect of cisplatin alone against cisplatin plus sitagliptin or linagliptin combinations on the BCL2 Level

Exposing A549 cells to a combination of CP plus SITA or LINA IC_{50} demonstrated a significant decrease in BCL2 level ($P < 0.0001$) compared to cells treated with CP alone. Furthermore, no significant differences were observed between cells treated with CP plus SITA and those treated with CP plus LINA, as shown in Figure 8.

Table 5. **Comparison between the effect of cisplatin alone against cisplatin plus sitagliptin or linagliptin combinations on the A549 cell line**

Concentration (µg/ml)	CP Cytotoxicity% ± SD	CP+SITA Cytotoxicity % ± SD	CP+LINA Cytotoxicity % ±SD
Control	0 ± 0	0 ± 0	0 ± 0
15.625 + 15.625	23.07 ± 1.30	37.94 ± 8.54	45.328 ± 8.54
31.25 + 31.25	35.23 ± 3.24	76.88 ± 1.02	75.098 ± 1.02
62.5 + 62.5	64.36 ± 2.41	76.66 ± 0.0	75.187 ± 0.00
125 + 125	77.43 ± 1.27	79.35 ± 2.68	77.478 ± 2.68
250 + 250	81.39 ± 1.26	82.44 ± 1.16	79.882 ± 1.16
500 + 500	83.62 ± 0.40	82.55 ± 0.34	83.277 ± 0.34

CP: Cisplatin; SITA: Sitagliptin; LINA: Linagliptin; SD: Standard deviation.

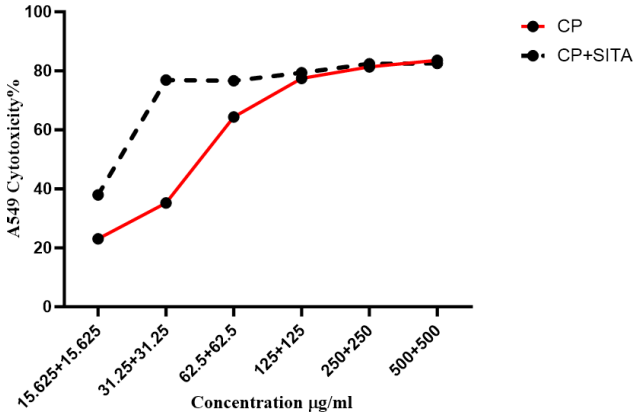

Fig. 6 **Comparison between the Anticancer effect of cisplatin alone and a combination of cisplatin plus Sitagliptin on the A549 cell line. CP: Cisplatin; CP+SITA: Cisplatin +Sitagliptin; A549: lung cancer cell line model.**

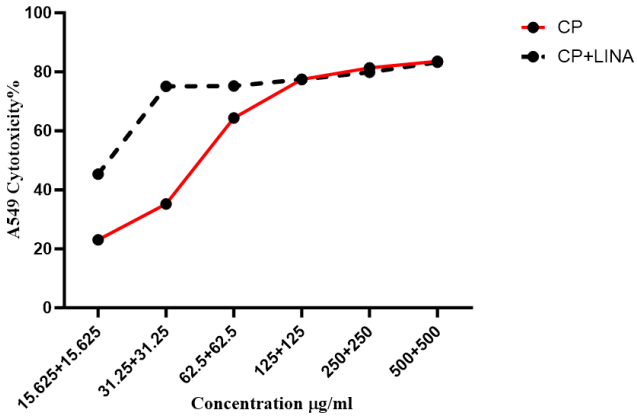

Fig. 7 **Comparison between the Anticancer effect of cisplatin alone and a combination of cisplatin plus linagliptin on the A549 cell line. CP: Cisplatin; CP+SITA: Cisplatin + Linagliptin; A549: lung cancer cell line model.**

Table 6. **Comparison between the effect of cisplatin, sitagliptin, and linagliptin alone and their combinations on IC50 in the A549 cell line**

Parameter	Cp	LINA	SITA	CP+SITA	CP+LINA
IC_{50} µg/ml	49.81	81.1	829.5	26.42	26.47

CP: Cisplatin; SITA: Sitagliptin; LINA: Linagliptin; IC_{50}: The concentration at which 50% inhibition of cell viability occurred.

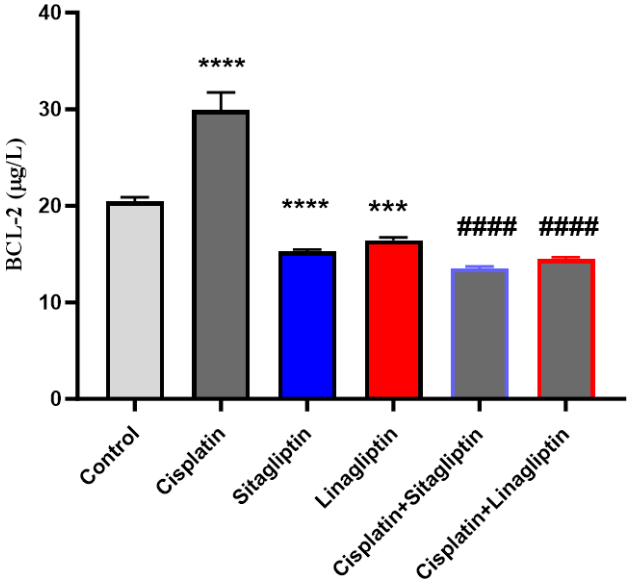

Fig. 8 **The effect of cisplatin, sitagliptin, linagliptin, and their combinations on BCL2 level in the A549 cell line. ****: $P < 0.0001$ compared to control; ***: $P < 0.001$ compared to control; ####: $P < 0.0001$ compared to CP-treated cells; BCL-2: B-cell lymphoma type 2.**

Discussion

The biggest challenges in cancer therapy include the detrimental consequences of medications and an increase in resistance to treatment, which accounts for more than 90% of fatalities in cancer patients undergoing conventional chemotherapy.[31] Researchers attempt to resolve these challenges by employing a variety of treatments.[32] Combination therapy has the potential to enhance the therapeutic effects of anticancer treatments while simultaneously reducing their adverse effects by decreasing the dosage of the drugs.[33,34]

The study was designed to evaluate the potential cytotoxicity and apoptotic effect of SITA and LINA on A549 cells alone and in combination with CP. To achieve these objectives, the toxicity of the tested cancer cells was evaluated using the MTT assay. Furthermore, the apoptotic role of the drugs and combinations under investigation was assessed using the BCL2 assay in the cancer microenvironment. A previous investigation discovered a notable increase in the expression of the DPP4 enzyme in lung adenocarcinoma compared to normal lung tissue. This indicates that the use of DPP4 inhibitors that

block the activity of this enzyme has the potential to limit the progression of lung cancer.[35]

The present study results show that SITA and LINA have demonstrated anticancer effects and cytotoxicity toward A549 cells compared with the control using MTT assay. These findings align with the results reported by Amritha et al.,[24] in which The MTT assay was employed to assess the anticancer efficacy of DPP4 inhibitors, SITA, and Vildagliptin (VILDA) on colorectal cell lines (HT-29). The results demonstrated that both medicines had substantial anticancer properties compared to the control, acting as cytotoxic agents against colorectal tumor cells. Additionally, it was observed that SITA exhibited more potency; the IC50 of SITA is (32.1 µg/ml) compared to the IC50 of VILDA (125 µg/ml) in colon cancer cell lines.

Likewise, in a recent study, it was shown that individuals with diabetes who were administered the DPP4 inhibitor SITA exhibited improved overall survival rates following undergoing surgery for colorectal or lung cancer in comparison to patients who were receiving alternative treatments for diabetes.[36] Additionally, subsequent research demonstrated that LINA has an effective cytotoxic activity against HCT116 (colorectal) cancer cells by the induction of cell cycle arrest, specifically at the G2/M and S phases, hence restricting cell proliferation.[37] More evidence was noticed with recent research indicating that GLP-1 plays a beneficial and defensive function in transplanted tumors and colon cancer cell lines, suggesting that inhibiting the DPP-4 enzyme by DPP4 inhibitors could enhance the efficacy of GLP-1.[38]

Regarding CP, the cytotoxic impact of CP on cancer cells is mainly attributed to its ability to induce apoptosis and cell cycle arrest, as previously reported.[39] Based on the present study results, it was found that CP exhibited cytotoxic effects on A549 cells in a dose-dependent manner, consistent with those reported by Liu et al.,[40] who demonstrated that CP inhibits the proliferation of lung cancer cell lines. In the current study, combining CP with SITA or LINA dramatically increased cytotoxicity in A549 cells at low concentrations (15.625, 31.25 and 62.5) µg/mL Compared to CP alone, which may suggest the presence of synergism between CP and SITA or LINA. This synergism could explain the enhanced cytotoxicity against lung cancer cells. Similar findings were reported in a study conducted to evaluate the impact of SITA, alone or in conjunction with paclitaxel, on ovarian cancer cells and metastatic progression.[41] Furthermore, the antitumor efficacy of SITA, alone in treatment or in conjunction with doxorubicin, has been observed in mice cancer models. Interestingly, concurrent administration of SITA plus doxorubicin significantly reduced tumor size compared to the use of either medicine alone.[26] Induction of apoptosis is an essential approach to cancer treatment. However, cancer cells have evolved diverse mechanisms to evade apoptosis-induced cell death.[42] One of these mechanisms is increasing the production of inhibitors of apoptosis proteins (IAP) related to the BCl-2 family, resulting in resistance to mortality and limiting the effectiveness of treatment; on the other hand, the down-regulation of these proteins can lead to a significant cytotoxic response in cancer cells.[43] The present study showed a relative resistance of A549 cells to CP treatment, which was explained by the increased level of BCL2 in A459 cells treated with CP IC_{50} compared to the control group. These findings are aligned with those of Losert et al.,[44], who found that CP increased BCL2 expression in lung cancer cells and inhibited the phosphorylation of Bcl-2. Furthermore, the current study showed that lung cancer cells treated with IC_{50} of SITA or LINA plus CP significantly lowered the level of BCL2 in the corresponding cells compared to those detected in cells treated with CP IC_{50} alone. Consistently with these results, previous studies by You et al.[45] demonstrated that SITA inhibited cell proliferation and induced apoptosis in the immortalized and primary glioblastoma cells. Similarly, the study reported by Mani et al.[46] showed that the primary mechanism by which LINA exerts its inhibitory effects on colorectal cancer cell growth facilitates cell apoptosis by cell cycle arrest and inhibits BCL2 expression. Furthermore, Alameen et al.[47] indicated that when SITA is combined with CP, the BCL2 level significantly decreases compared to the control and CP-treated groups. Based on the above, combining SITA or LINA plus CP may demonstrate a synergistic apoptotic effect on lung cancer cells.

Conclusion

DPP4 inhibitors, SITA or LINA, revealed antitumor activity against A549 cells based on MTT assay at 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL concentrations. In combination with CP, both these medications synergistically increased the cytotoxicity towards A549 cells at low concentrations of 62.5, 31.25, and 15.625 µg/mL, which can help reduce the dose and, subsequently, cisplatin's side effects in cancer treatment protocols. Moreover, SITA or LINA showed an apoptotic effect against the treated A549 cells based on the BCL2 measurement, which may help augment the apoptotic efficacy of CP on cancer cells.

Acknowledgment

The authors sincerely thank the Faculty of the Pharmacy/ University of Kufa and all individuals who contributed to this study through their invaluable support, expertise, and dedication.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Al-Tariahi KMJ, Hameed WS, Abdul DH, Saheb AM. Evaluation of CA125 as A Marker in Patients with Lung Carcinoma. 2015;
2. Riihimäki M, Hemminki A, Fallah M, Thomsen H, Sundquist K, Sundquist J, et al. Metastatic sites and survival in lung cancer. Lung cancer. 2014;86(1):78–84.
3. Benitez Majano S, Ellis L, Rachet B. Epidemiology of Lung Cancer. Encycl Respir Med Second Ed. 2022 Jan 1;4:663–72.
4. Chaitanya Thandra K, Barsouk A, Saginala K, Sukumar Aluru J, Barsouk A. Epidemiology of lung cancer. Współczesna Onkol [Internet]. 2021;25(1):45–52. Available from: https://www.termedia.pl/doi/10.5114/wo.2021.103829
5. Schabath MB, Cote ML. Cancer progress and priorities: lung cancer. Cancer Epidemiol Biomarkers Prev. 2019;28(10):1563–79.
6. Reck M, Rabe KF. Precision diagnosis and treatment for advanced non–small-cell lung cancer. N Engl J Med. 2017;377(9):849–61.

7. Brown A, Kumar S, Tchounwou PB. Cisplatin-Based Chemotherapy of Human Cancers. [Internet]. Vol. 11, Journal of cancer science & therapy. 2019. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32148661%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC7059781
8. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. J Exp Pharmacol. 2021;303–28.
9. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014;740:364–78.
10. Aldossary SA. Review on the pharmacology of cisplatin: clinical use, toxicity and mechanism of cisplatin resistance. Biomed Pharmacol J. 2019;12(1):7–15.
11. Singh L, Aldosary S, Saeedan AS, Ansari MN, Kaithwas G. Prolyl hydroxylase 2: a promising target to inhibit hypoxia-induced cellular metabolism in cancer cells. Drug Discov Today. 2018;23(11):1873–82.
12. Gilbert MP, Pratley RE. GLP-1 Analogs and DPP-4 Inhibitors in Type 2 Diabetes Therapy: Review of Head-to-Head Clinical Trials. Vol. 11, Frontiers in Endocrinology. 2020.
13. Holst JJ, Gasbjerg LS, Rosenkilde MM. The role of incretins on insulin function and glucose homeostasis. Endocrinology. 2021;162(7):bqab065.
14. Raz I, Hanefeld M, Xu L, Caria C, Williams-Herman D, Khatami H. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy in patients with type 2 diabetes mellitus. Vol. 49, Diabetologia. 2006. p. 2564–71.
15. Pro B, Dang NH. CD26/dipeptidyl peptidase IV and its role in cancer. Histol Histopathol. 2004 Oct;19(4):1345–51.
16. Havre PA, Abe M, Urasaki Y, Ohnuma K, Morimoto C, Dang NH. The role of CD26/dipeptidyl peptidase IV in cancer. Front Biosci. 2008;13(1634):45.
17. Femia A Pietro, Raimondi L, Maglieri G, Lodovici M, Mannucci E, Caderni G. Long-term treatment with Sitagliptin, a dipeptidyl peptidase-4 inhibitor, reduces colon carcinogenesis and reactive oxygen species in 1, 2-dimethylhydrazine-induced rats. Int J cancer. 2013;133(10):2498–503.
18. Lam CSC, Cheung AHK, Wong SKM, Wan TMH, Ng L, Chow AKM, et al. Prognostic significance of CD26 in patients with colorectal cancer. PLoS One. 2014;9(5):e98582.
19. Javidroozi M, Zucker S, Chen WT. Plasma seprase and DPP4 levels as markers of disease and prognosis in cancer. Dis Markers. 2012;32(5):309–20.
20. Boccardi V, Marano L, Rossetti RRA, Rizzo MR, di Martino N, Paolisso G. Serum CD26 levels in patients with gastric cancer: a novel potential diagnostic marker. BMC Cancer. 2015;15(1):1–6.
21. Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes Dev. 1999;13(15):1899–911.
22. Gao Q, Yang S, Kang MQ. Influence of survivin and Bcl-2 expression on the biological behavior of non-small cell lung cancer. Mol Med Rep [Internet]. 2012;5(6):1409–14. Available from: https://doi.org/10.3892/mmr.2012.840
23. Yang Y, Zhu J, Gou H, Cao D, Jiang M, Hou M. Clinical significance of Cox-2, Survivin and Bcl-2 expression in hepatocellular carcinoma (HCC). Med Oncol. 2011;28:796–803.
24. Amritha CA, Kumaravelu P, Chellathai DD. Evaluation of Anti Cancer Effects of DPP-4 Inhibitors in Colon Cancer- An Invitro Study. J Clin Diagn Res. 2015 Dec;9(12): FC14-6.
25. Tseng CH. Sitagliptin may reduce breast cancer risk in women with type 2 diabetes. Clin Breast Cancer. 2017;17(3):211–8.
26. Tseng CH. Sitagliptin may reduce prostate cancer risk in male patients with type 2 diabetes. Oncotarget. 2017;8(12):19057.
27. Kabel AM, Atef A, Estfanous RS. Ameliorative potential of sitagliptin and/or resveratrol on experimentally-induced clear cell renal cell carcinoma. Biomed Pharmacother Biomed Pharmacother. 2017;97:667–74.
28. Bishnoi R, Hong Y, Shah C, Ali A, Skelton IV WP, Huo J, et al. Dipeptidyl peptidase 4 inhibitors as novel agents in improving survival in diabetic patients with colorectal and lung cancer: A Surveillance Epidemiology and Endpoint Research Medicare study. Cancer Med. 2019;8(8):3918–27.
29. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res. 1987;47(4):936–42.
30. Van Meerloo J, Kaspers GJL, Cloos J. Cell sensitivity assays: the MTT assay. Cancer cell Cult methods Protoc. 2011;237–45.
31. Bukowski K, Kciuk M, Kontek R. Mechanisms of multidrug resistance in cancer chemotherapy. Int J Mol Sci. 2020;21(9):3233.
32. Kim K, Kim S, Yu S, Park S, Choi H, Yu H, et al. Salinomycin enhances doxorubicin-induced cytotoxicity in multidrug-resistant MCF-7/MDR human breast cancer cells via decreased doxorubicin efflux. Mol Med Rep. 2015;12(2):1898–904.
33. Nakashima T, Nagano S, Setoguchi T, Sasaki H, Saitoh Y, Maeda S, et al. Tranilast enhances the effect of anticancer agents in osteosarcoma. Oncol Rep. 2019;42(1):176–88.
34. Jin X, Wei Y, Liu Y, Lu X, Ding F, Wang J, et al. Resveratrol promotes sensitization to Doxorubicin by inhibiting epithelial-mesenchymal transition and modulating the SIRT1/β-catenin signaling pathway in breast cancer. Cancer Med. 2019;8(3):1246–57.
35. Jang JH, Janker F, De Meester I, Arni S, Borgeaud N, Yamada Y, et al. The CD26/DPP4-inhibitor vildagliptin suppresses lung cancer growth via macrophage-mediated NK cell activity. Carcinogenesis. 2019;40(2):324–34.
36. Varela-Calviño R, Rodríguez-Quiroga M, Dias Carvalho P, Martins F, Serra-Roma A, Vázquez-Iglesias L, et al. The mechanism of sitagliptin inhibition of colorectal cancer cell lines' metastatic functionalities. IUBMB Life. 2021;73(5):761–73.
37. Li Y, Li Y, Li D, Li K, Quan Z, Wang Z, et al. Repositioning of Hypoglycemic Drug Linagliptin for Cancer Treatment. Vol. 11, Frontiers in Pharmacology. 2020.
38. Koehler JA, Kain T, Drucker DJ. Glucagon-like peptide-1 receptor activation inhibits growth and augments apoptosis in murine CT26 colon cancer cells. Endocrinology. 2011;152(9):3362–72.
39. Wang G, Reed E, Li QQ. Molecular basis of cellular response to cisplatin chemotherapy in non-small cell lung cancer. Oncol Rep. 2004;12(5):955–65.
40. Liu H, Yin J, Wang C, Gu Y, Deng M, He Z. FOXO3a mediates the cytotoxic effects of cisplatin in lung cancer cells. Anticancer Drugs [Internet]. 2014;25(8). Available from: https://journals.lww.com/anti-cancerdrugs/fulltext/2014/09000/foxo3a_mediates_the_cytotoxic_effects_of_cisplatin.5.aspx
41. Kosowska A, Garczorz W, Kłych-Ratuszny A, Aghdam MRF, Kimsa-Furdzik M, Simka-Lampa K, et al. Sitagliptin modulates the response of ovarian cancer cells to chemotherapeutic agents. Int J Mol Sci. 2020;21(23):1–13.
42. Nachmias B, Ashhab Y, Ben-Yehuda D. The inhibitor of apoptosis protein family (IAPs): an emerging therapeutic target in cancer. In: Seminars in cancer biology. Elsevier; 2004. p. 231–43.
43. Letai A. Pharmacological manipulation of Bcl-2 family members to control cell death. J Clin Invest. 2005;115(10):2648–55.
44. Losert D, Pratscher B, Soutschek J, Geick A, Vornlocher HP, Müller M, et al. Bcl-2 downregulation sensitizes nonsmall cell lung cancer cells to cisplatin but not to docetaxel. Anticancer Drugs [Internet]. 2007;18(7). Available from: https://journals.lww.com/anti-cancerdrugs/fulltext/2007/08000/bcl_2_downregulation_sensitizes_nonsmall_cell_lung.2.aspx
45. You F, Li C, Zhang S, Zhang Q, Hu Z, Wang Y, et al. Sitagliptin inhibits the survival, stemness, and autophagy of glioma cells and enhances temozolomide cytotoxicity. Biomed Pharmacother. 2023;162:114555.
46. Mani RJ, Anand M, Agarwal K, Tiwari A, Amanur Rahman Hashmi Q, Vikram Singh T, et al. A Systematic Review of Molecular Pathway Analysis of Drugs for Potential Use in Liver Cancer Treatment. Drugs Drug Candidates [Internet]. 2023;2(2):210–31. Available from: https://www.mdpi.com/2813-2998/2/2/13
47. Alameen R, Bairam A, Al-Haddad M. Antioxidant and apoptotic activities of sitagliptin against hepatocellular carcinoma: An *in vitro* study [version 1; peer review: 1 approved with reservations]. F1000Research. 2023;12(962).