AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544. 2024.16 (2), 15-24.

Original Research Paper

Exploring the Anticancer and Antioxidant Potential Effects of Sitagliptin: An In Vitro Study on Lung Cancer Cell Lines

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Article history
Received: 05/03/2024
Revised: 18/05/2024
Accepted: 22/05/2024

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Abstract: Lung cancer possesses the most significant worldwide mortality rate among all types of tumors, and the majority of current treatments exhibit a broad spectrum of adverse reactions. This requires investigating novel drugs that can inhibit the growth of cancer cells while minimizing harm to normal cells. Recent studies have demonstrated that Dipeptidyl peptidase-4 (DPP4) inhibitors can effectively suppress the growth of cancer cells in several types of malignancies, including colorectal, prostate, and kidney cancers. Hence, this study evaluated the effectiveness of sitagliptin in suppressing the proliferation of lung carcinoma cells (A549) alone as well as in conjunction with cisplatin.

A549 cells were divided into four groups: control (untreated cells), cells treated with cisplatin, cells treated with sitagliptin, and cells treated with cisplatin plus sitagliptin combination (ratio 1:1). After incubation for 72 hours, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test was utilized to evaluate cell viability percent and concentration of 50% inhibition for cell viability (IC_{50}) for each group. Then, A549 cells were cultured into four flasks and exposed to the IC_{50} concentration. The resulting cell pellets were gathered and subjected to lysis to assess the malondialdehyde (MDA) level using ELISA kits.

The vitality of A549 cells was significantly reduced by Sitagliptin therapy compared to the control ($P < 0.0001$). When the combination of sitagliptin and cisplatin was administered, it exhibited markedly superior anticancer effectiveness compared to their usage alone. Significantly, sitagliptin reduced MDA levels, whether used individually or in combination with cisplatin.

As a result, sitagliptin exhibited significant anticancer and antioxidant properties against A549 cells. This suggests a possible synergistic anticancer effect with cisplatin.

Keywords: sitagliptin, A549 cell line, cisplatin, antioxidant.

DOI: 10.36320/ajb/v16.i2.16101

1. Introduction: Lung cancer, also called pulmonary cancer, is a malignant tumor that develops in the lungs. It is characterized by the rapid and abnormal development of cells, resulting in tumor formation. These tumors progressively increase in number and size, obstructing the lung's capacity to exchange oxygen efficiently [1]. Lung cancer is a significant cause of morbidity and death globally, accounting for 12% of newly detected cancers and responsible for 18% of yearly cancer-related deaths [2]. It has the highest fatality rate worldwide due to its poor prognosis [3]. In 2020, the Global Cancer Observatory (GLOBOCAN) estimated that there were 2,206,771 new cases of lung cancer diagnosed globally; lung tumors were the leading cause of cancer incidence and mortality in men, with 1,435,943 new cases and 1,188,679 deaths. Among women, it was the third most prevalent cause of cancer, with 770,828 reported cases, and the second leading cause of cancer-related deaths, with 607,465 deaths after breast cancer [4]. Lung cancer typically consists of two main histologic types: small cell lung cancer (SCLC), which accounts for 15% of cases, and non-small cell lung cancer (NSCLC), which makes up 85% of cases [5]. Therapeutic options consist of one or more of the following: surgery, radiotherapy, immunotherapy, and chemotherapy, such as cisplatin (CP) [6]. CP is a platinum-based drug typically given intravenously as the first chemotherapy treatment for patients with different types of cancers [7-9]. Anticancer medications cause oxidative stress in living organisms, which leads to the oxidation of lipids and the creation of different reactive aldehydes. Oxidative stress can augment the efficacy of anticancer therapies by inhibiting the proliferation of malignant cells[10]. Although CP monotherapy has demonstrated favorable clinical outcomes, numerous studies have documented significant adverse effects. Moreover, certain cancer patients have shown considerable drug resistance and toxicity. Therefore, novel formulations and combination therapies involving other medications have been evaluated to improve CP's therapeutic efficacy. Recent research has demonstrated that dipeptidyl peptidase-4 (DPP4) inhibitors, specifically sitagliptin (SITA) [11], have significant anticancer impacts against several types of cancer cells, including colon [12] and ovarian [13] cancer cells. A glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretin hormones that stimulate insulin release and are inactivated via the DPP4 enzyme [14]. The DPP4 enzyme, also known as Cluster of Differentiation 26 (CD26), is a transmembrane protein expressed by many normal cell types, with variable expression in cancers depending on the type [15]. Some cancers, such as breast and endometrial, are associated with decreased DPP4 expression, suggesting its tumor-suppressing activity. Other, such as renal, colon, and lung cancers, are associated with increased DPP4 expression, suggesting its tumor-activating role in these cancers [16]. DPP4 enzyme plays a vital role in cancer biology, progression, and formation of metastases in tumor cells [17,18]. And prognosis of cancer patients,[19] representing a helpful tumor marker and a novel therapeutic target for selected tumors [20]. Although DPP4 inhibitors have been shown to improve the management of particular cancer types, such as colon, breast, prostate, kidneys, and colorectal [21-25], and alter the balance of oxidative stress during chemotherapy [26], Direct comparative studies on the activity of SITA alone or in combination with standard chemotherapy against lung tumor cell lines (A549) are limited Furthermore., the precise molecular mechanisms through which these drugs exert their anticancer effects, particularly in the lung cancer, still need to be fully understood. Investigating this gap can enhance the understanding of the possible involvement of DPP-4 inhibitors in lung cancer treatment. It can help in the creation of novel therapeutic approaches for lung cancer. Therefore, this study aimed to assess SITA's anticancer and antioxidant effects on the lung cancer cell line (A549).

2. Methodology

Chemicals and cell line

A549 lung cancer cells were obtained from Basrah's Iraq Biotech Cell Bank Unit, passage number 20. Generally, A459 cells were isolated from a human alveolar-cell carcinoma of a White, 58-year-old man in January 1972. They underwent continuous *in vitro* propagation for over three years, resulting in over 1,000 cell generations widely studied as lung cancer models. [27].

Dimethyl sulfoxide (DMSO), MTT (3-(4,5 dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye powder, and RIPA lysis buffer were obtained from Sigma, USA. 10% fetal bovine serum (FBS), phosphate buffer saline (PBS), and Roswell Park

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Memorial Institute-1640 (RPMI-1640) were obtained from Gibco, USA. Trypsin- EDTA was obtained from Capricorn, USA. The trypan-blue stain was obtained from Flow Laboratories, UK. Benzylpenicillin and streptomycin were obtained from Troge, Germany.CP was obtained from Pfizer, USA. SITA was obtained from Anhui Haikang Pharmaceutical CO, China. A human MDA ELISA kit was obtained from the Bioassay Technology Laboratory in China.

Cell culture and MTT assay:

The A549 cell lines were extracted using trypsin-EDTA as a proteolytic protein, PBS for rinsing the media, and FBS to deactivate trypsin, then cultured in a 96-well plate using RPMI-1640 liquid media with 100 units/mL penicillin, and 100 µg/mL streptomycin. The sample was allowed to incubate for 24 hours at 37 °C, 5% CO2, and 95% humidity to encourage the formation of a single layer of cells (80% growth phase). Viable cells were counted using trypan blue dye. The previous medium was then substituted with 200 µL of the medium, including the test medicines and control group.[28] four primary groups were used: control (untreated cells), CP-treated cells, SITA-treated cells, and CP plus SITA-treated cells (ratio 1:1). For each treated group, six concentrations (each with four replicates) were used:500, 250, 125, 62.5, 31.25, and 15.625 µg/mL. A blank (containing only medium) was used to evaluate the non-specific conversion of formazan and the tested drugs. After 72 hours of incubation, the (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2Htetrazolium bromide (MTT) test [29] was used to determine cell viability and dose-effect curves were calculated by non-linear regression based on a four-parameter logistic Hill equation. The concentration of 50% inhibition for cell viability (IC_{50}) was calculated using GraphPadPrism10 for each group. The cell viability percentage was evaluated using the following formula:

$$\boldsymbol{Cell\ viability}\% = \frac{\boldsymbol{As - Ab}}{\boldsymbol{Ac - Ab}} \times \mathbf{100}\%$$

Where (As) is the sample's absorbance, (Ab) is the blank's absorbance, and (Ac) is the control absorbance. All the determinations were done in four replicates.

Oxidative stress assay: ELISA measurement of MDA concentration

A549 cells were later seeded in six flasks (each with three replicates) and treated with the resulting IC_{50} for 36 hours. Cells were then extracted and centrifuged, and the supernatants were discarded. The pellets were collected and lysed by lysis buffer to obtain proteins and stored in a sterile Eppendorf tube, then frozen at -20°C to be used later for MDA *measurement using the* ELISA assay kit. The human MDA ELISA kit, obtained from Bioassay Technology Laboratory Company (BT Lab) in Shanghai, China, was used to measure the MDA concentration in the samples. The experiment was conducted based on the BT LAB procedure following the company protocol provided (as a manual kit guideline). The standard stock solution was diluted at ratios ranging from 1:2 to 1:16. 50 µL of the standard was added to the standard well, 40 µL of the sample to the sample wells, and 10 µL of anti-MDA antibody and 50 µL of streptavidin-HRP to both the sample and standard wells. A sealant was used to cover the plate, and was incubated at 37°C for one hr. The plate was repeatedly cleaned in the wash buffer. Each wash lasted between 30 seconds and one min. After dispensing 300 µL of wash buffer, each well received 50 µL of substrate solution A, followed by substrate solution B (per the kit manual guideline). A color change occurred upon applying the substrate solutions, directly correlated with the human MDA level. An absorbance reading was taken at 450 nm after the procedure was terminated by adding an acidic stop solution. A microplate reader (Thermo Fisher Scientific, USA) was configured to operate at a wavelength of 450 nm to determine the absorbance value of each well.

Statistical analysis

The data were collected and examined using GraphPad Prism Edition 10 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 indicated a statistically significant difference.

3. Results and discussion

The main difficulties in cancer treatment involve the detrimental impacts of medications and the emergence of resistance to treatments, which are responsible for more than 90% of deaths in patients undergoing chemotherapy [30]. Many therapeutic alternatives, such as combination therapy, may overcome these restrictions, enhancing the drug's therapeutic efficacy and safety and reducing the

DOI: 10.36320/ajb/v16.i2.16101

adverse effects of anticancer treatments by decreasing the dosage [31,32].

MTT cell viability assay

This study aimed to evaluate the anti-cancer effects of SITA on A549 cancer cells, alone and in combination with CP. To accomplish this, the viability of the cancer cells was assessed using the MTT assay.

Cisplatin activity on the viability of the A549 cell line

The findings demonstrated a significant reduction in the viability of cells ($P < 0.0001$) for all concentrations (500, 250, 125, 62.5, 31.25, and 15.625 µg/mL) when compared to the control group, as seen in Figure 1.

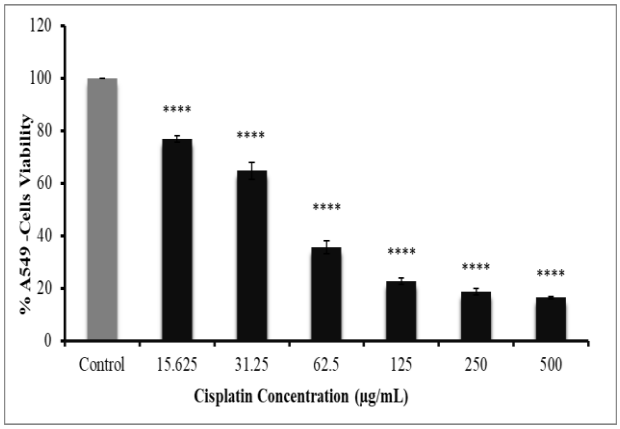

Figure 1: The impact of different cisplatin concentrations on the viability of the A549 cell line.
One-way ANOVA was used for the examination. Data are displayed as mean.
± SD, **** $P < 0.0001$, n= 4, incubation for 72 hours.

CP anticancer activity has already been approved, and this chemotherapy is one of the standard drugs widely used for lung cancer treatment [7]. DNA is the main biological target of CP. [33] It causes cell cycle arrest at S, G1, or G2-M, inhibiting cell growth and subsequently inducing apoptosis [34].

Activity of sitagliptin on the viability of the A549 cell line

SITA demonstrated a significant decrease in the cell viability ($P< 0.0001$) for all concentrations (500, 250, 125, 62.5, 31.25, and 15.625 µg/mL) compared with the control group, as seen in Figure 2.

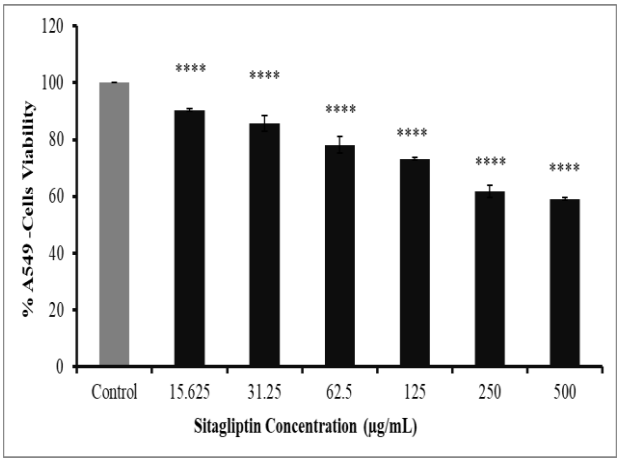

Figure 2: The impact of different concentrations of sitagliptin on the viability of A549 cell line.
One-way ANOVA was used for the examination. Data are displayed as mean ± SD, **** $P < 0.0001$, n= 4, incubation for 72 hours.

A previous investigation showed a notable increase in the expression of the DPP4 enzyme in lung cancer compared to normal lung tissue. These findings indicate that using DPP4 inhibitors to block an enzyme's activity can effectively prevent lung cancer growth [35]. According to the current study findings, SITA has shown anticancer activity (significantly reducing the viability of A549 cells compared with the control using the MTT assay. These results align with those published by Amritha *et al*. [21], who used the MTT assay to determine the anti-cancer activity of DPP4 inhibitors SITA and vildagliptin (VILDA) on colorectal cell lines (HT-29). They found that both drugs had significant anti-cancer effects compared to the control, which acted as a cytotoxic agent in cancer cells. SITA was more effective than VILDA on colon cancer cell lines. Similarly, a recent study found that diabetic people who took the DPP4 inhibitor SITA had enhanced overall survival following surgery for colorectal or lung cancer compared to patients who were given alternative diabetic medications [36].

Activity of a combination of cisplatin plus sitagliptin on the viability of the A549 cell line

In this part of the study, A549 cells were tested with a constant ratio (1:1) of CP plus SITA concentrations. The results showed that the combination significantly reduced the cell's viability ($P < 0.0001$) for all concentrations compared to a control group, as seen in Figure 3.

DOI: 10.36320/ajb/v16.i2.16101

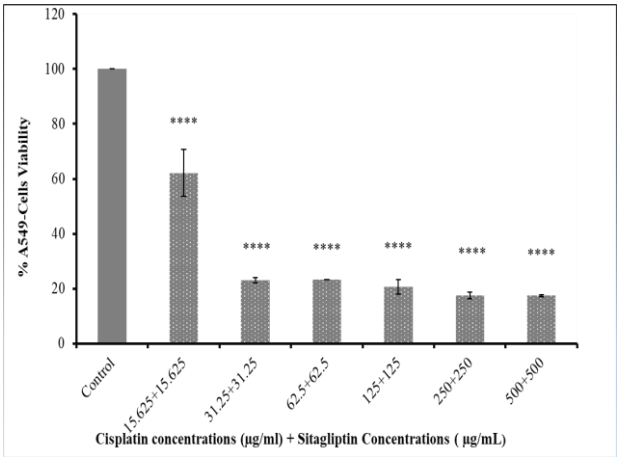

Figure 3: The effect of different concentrations of cisplatin plus sitagliptin on the viability of the A549 cell line.
One-way ANOVA was used for the examination. Data are displayed as mean ± SD, **** P < 0.0001, n= 4, incubation for 72 hours.

Comparison between the activity of cisplatin alone against cisplatin plus sitagliptin combination on the viability of the A549 cell line

As shown in Figure 4, the CP plus SITA combination reduced the viability of A549 cells at a high level of significance (P < 0.0001) at concentrations of 15.625, 31.25, and 62.5 µg/mL in comparison with cells exposed to CP alone. However, compared to CP alone, the combination did not significantly alter cell viability (P > 0.05) at 125, 250, or 500 µg/mL concentrations.

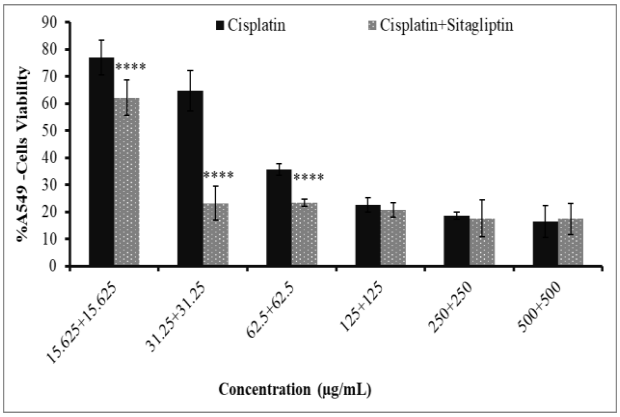

Figure 4: Comparison between the activity of cisplatin alone against cisplatin and sitagliptin combination on the viability of the A549 cell line.
One-way ANOVA was used for the examination. Data are displayed as mean ± SD, **** P< 0.0001, n= 4, incubation for 72 hours.

Combining SITA with CP significantly decreased the viability of A549 cells at low concentrations (15.625, 31.25, and 62.5 ug/mL) when compared with CP alone. This indicates a synergistic effect between CP and SITA that increases cytotoxicity against cancer cells compared to that obtained when using CP alone. Previous studies have shown the synergism between CP and DPP4 inhibitors, such as one that evaluated the impact of SITA alone or in combination with paclitaxel on the growth of ovarian cancer cells and the process of metastasis [37].

Measurement of human MDA levels

The oxidative stress levels of the investigated drugs and combinations in this study were assessed utilizing the MDA test in the tumor microenvironment.
Overproduction of reactive oxygen species (ROS) interrupts the antioxidant defense mechanisms and results in cellular oxidative stress [38], which induces mitochondrial dysfunction, DNA damage, and cell death [39]. Oxidative stress has been associated with tumors' survival, development, propagation, and angiogenesis [40].

Cisplatin effects on MDA concentration

Compared to the control group, the findings demonstrated a substantial elevation in MDA concentration (P < 0.0001) following treating A549 cells with an IC_{50} of CP. As shown in **Figure 5**.
Many studies have demonstrated that oxidative stress is one of the most critical mechanisms of CP cytotoxicity by elevating ROS directly or indirectly, resulting in lipid peroxidation, increased MDA production, decreased activity glutathione (GSH), and eventually inducing apoptosis [41]. The current study estimated the MDA concentration to assess oxidative stress levels. The results demonstrated a substantial elevation in MDA concentration (P < 0.0001) after treatment with the IC_{50} of CP alone, compared to the control group, Indicating CP can cause oxidative stress. The results align with previous studies demonstrating that CP caused lipid peroxidation and resulted in an elevated level of MDA in

DOI: 10.36320/ajb/v16.i2.16101

human leukemia (HL60) cells across all concentrations compared to the control group [42].

Sitagliptin effects on MDA concentration

The findings indicated a notable reduction in MDA concentration ($P < 0.0001$) following treatment of A549 cells with IC_{50} of SITA compared to the control group, as seen in Figure 5.

CP, when administered with other drugs possessing antioxidant properties, has decreased its toxicity. For instance, DPP4 inhibitors (SITA) can stimulate the effect of nuclear factor erythroid 2-related factor 2 (Nrf2), a regulator for many antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), which may be responsible for its antioxidant properties. [24,43]

SITA displayed a notable reduction in the MDA level of the A549 cells relative to the control group; these results were consistent with previous research, which showed the antioxidant properties of SITA in different tissues and situations, including human mononuclear blood cells, Parkinson's disease, diabetic nephropathy, and atherosclerosis [43-46].

Comparison between the activity of cisplatin alone against cisplatin plus sitagliptin combination on the MDA concentration

When A549 cells were treated with IC_{50} of CP plus SITA combination, the concentration of MDA in the cells was dramatically reduced ($P<0.0001$) compared to cells treated with CP alone, as seen in **Figure 5**.

The effect of the combination of CP plus SITA showed a notable reduction in the MDA concentration compared to the control and CP-treated groups. This demonstrated the capacity to reduce oxidative stress through DPP4 inhibitors, consequently decreasing CP toxicity and adverse effects. The results reported by Salama *et al*. [26] and Alameen *et al* [47] showed that the combination of SITA with doxorubicin or CP led to a significant decrease in MDA levels compared to both the control group and the groups treated with doxorubicin or CP alone, which is consistent with the present research results.

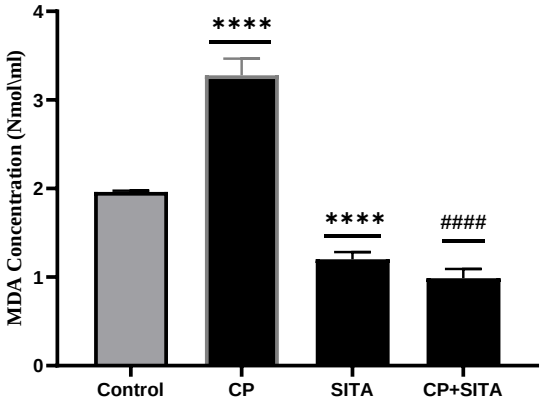

Figure 5: Cisplatin, Sitagliptin, and their combination activity on MDA concentration in the A549 cell line.
One-way ANOVA was used for the examination. Data are displayed as mean ± SD.
******** $P < 0.0001$ compared to control. $P < 0.0001$ compared to Cisplatin.

Conclusion

DPP4 inhibitors showed anticancer activity against A549 cells based on MTT assay results at concentrations of 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL and, in combination with CP, synergistically inhibited the viability of A549 cells at low concentrations of 62.5, 31.25, and 15.625 µg/mL. This may help to decrease the required CP dosage and attenuate its adverse effects on humans during cancer chemotherapy protocols. Furthermore, based on MDA measurements, the antioxidant effect of DPP4 inhibitors on A549 cells indicated partial protection against oxidative stress for healthy cells.

Acknowledgment

The authors express their sincere gratitude to all individuals who contributed to this study through their invaluable support, expertise, and dedication.

Authors' Declaration

The authors, at this moment, declare that the work presented in this article is original, that they will bear any liability for claims relating to the content of this article, and the study was done under the supervision of the Faculty of Pharmacy, University of Kufa, without any external funding.

Conflict of interest: The authors declare no conflict of interest.

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