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Research Article
Griffipavixanthone Enhances Chemosensitivity to Cisplatin in Human Non-Small Lung Cancer A549 and H157 Cells

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Abstract

Background and Objective: Griffipavixanthone (GPX), a bioactive compound isolated from *Garcinia oblongifolia* Champ, induced apoptosis in cancer cells. Here, we aimed at investigating the effects of GPX on cisplatin-treated Non-Small Cell Lung Cancer (NSCLC) A549 and H157 cells. **Materials and Methods:** The A549 and H157 cells were treated with GPX or in combination with cisplatin and CCK8 assay and Annexin V/Propidium Iodide (PI) assay were conducted to determine the cell proliferation and apoptosis. **Results:** The GPX improved the antiproliferative effects of cisplatin on NSCLC and sensitized NSCLC to cisplatin-induced apoptosis. Finally, the cell cycle was analyzed by flow cytometry. The GPX alone or in combination with cisplatin-induced G0/G1 and G2/M cell-cycle arrest in A549 cells, but GPX in combination with cisplatin only leaded to a G2/M cell-cycle arrest in H157 cells. **Conclusion:** The results indicated that GPX in combination with chemotherapeutic agents could be a therapeutic option for NSCLC.

Key words: Griffipavixanthone, lung cancer, cisplatin, chemosensitivity, apoptosis, cell-cycle arrest, drug combination

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lung cancer is the most frequently diagnosed cancer globally because there are almost 2.09 million new cases and 1.76 million deaths surveyed in 2018[1]. More than one-third of new cases of lung cancer occurred in China and it is the most common cancer in China, which is also the leading cause of cancer-related mortality[2,3]. Cigarette smoking is regarded as the major risk factor of lung cancer and there are many other risk factors, such as lung infections, chronic lung disease, genetics and environmental exposures to ionizing radiation, diesel, asbestos or radon[4,5].

Nearly 84% of lung cancers are NSCLC[6]. Surgery, adjuvant chemotherapy, neoadjuvant chemotherapy, immunotherapy and target therapy have been applied to treat NSCLC[7-9]. Cisplatin, one apoptosis-induction chemotherapy agent, is the first-line drug used for the treatment of NSCLC[10], but patients are easy resistant to cisplatin because of the decreased apoptotic activity, leading to a limited therapeutic efficacy[11]. Therefore, it is imperative to explore more methods of increasing apoptosis to overcome chemoresistance during the treatment of NSCLC.

Griffipavixanthone (GPX), a bioactive compound extracted from *Garcinia oblongifolia* Champ, has been reported to have anti-proliferative effects and induce apoptosis in different cancer cell lines, for example, human colon cancer cells, human prostate cells, human breast cancer cells and human oesophagal cancer cell lines[12-14]. Besides, since natural products have low toxicity, we aim at exploring whether GPX can increase cisplatin-induced apoptosis in NSCLC. In the present study, we found GPX enhances chemosensitivity to cisplatin in NSCLC A549 and H157 cells.

MATERIALS AND METHODS

Study area: This study was carried out at the Lab in the Department of Thoracic Surgery, Binzhou People's Hospital, China from August, 2020 to August, 2021.

Chemical: Griffipavixanthone (GPX) was purchased from Shanghai Chengshao Biotechnology Co., LTD. It was dissolved in DMSO to make a 50 mM stock solution before being used for treatment. Cisplatin (DDP) was purchased from Sigma-Aldrich (Shanghai, China) and dissolved in the medium before being used for treatment.

Cell culture and maintenance: Human lung cancer cells A549 and H157 were obtained from the Institute of Biophysics, Chinese Academy of Sciences. Both A549 and H157 were cultured in RPMI-1640 medium (Thermo Fisher, USA) supplemented with 1% penicillin and streptomycin (Gibco, USA) and 10% FBS (Gibco, USA) at 37°C with 5% CO_2.

Cell viability analysis: According to the manufacturer's instructions, Cell Counting Kit-8 (CCK8) assay (Biyuntian Biological Technology Co., LTD, China) was used to detect cell proliferation. A total of 1000 cells in a volume of 100 µL/well were cultured in a 96-well plate in a medium containing 10% FBS. After treatment with GPX or DDP at various concentrations for the indicated time, 10 µL CCK-8 reagent was added into each well. Then, the optical density (OD) was measured at 450 nm using a multimode plate reader (EnSpire, USA).

Apoptosis assay: According to the recommended protocol, an annexin V-FITC apoptosis detection kit (Biyuntian Biological Technology Co., LTD, China) was selected to detect apoptosis activity. Cells (2×10^5 each well) were seeded into 6 well plates and then treated with GPX or DDP for the indicated time. The cells were washed once with PBS, which were then stained with 10 µL of propidium iodide and 5 µL Annexin V-FITC in a 195 µL binding buffer for 15 min at RT in the dark. Finally, flow cytometry (BD, USA) and FlowJo software were chosen to analyze the apoptosis rates. Both Annexin V+/PI- cells and Annexin V+/PI+ were considered apoptotic cells.

Cell cycle analyses: The propidium iodide (PI) staining method was used to quantify the cell cycle. The cells (2×10^5 cells/well) were seeded in a six-well plate, which was then cultured in serum-free media for 24 hrs for synchronization. After being treated with different concentrations of GPX or DDP, the cells were washed with 1 mL PBS. Cold 70% ethanol was used to fix the cells overnight at -20 and then ethanol was removed by washing with 1 mL PBS. To ensure only DNA was stained, 50 µg mL^{-1} ribonuclease (Takara, Japan) was used to treat the cells. the cells were stained with 25 µL 1 mg mL^{-1} PI at 37 for 30 min, which was placed in an ice bath for cell cycle analysis. Finally, CytoFLEX flow cytometer (BD, USA) and Flowjo software were used to analyze the cell cycle.

Statistical analysis: GraphPad Prism program was used to determine the statistical significances. The data were presented as Mean±Standard Deviation (SD) from three independent experiments. The student's t-test was chosen to determine the statistical difference between control and treated cells. The value of $p<0.05$ was considered statistically significant.

RESULTS

GPX improved the antiproliferative effects of cisplatin on NSCLC: Human NSCLC cells A549 and H157 were treated with different concentrations of GPX (5, 10, 15, 20, 25 and 30 μM) and incubated for 24 and 48 hrs. Cell proliferation was detected by the CCK-8 assay. The GPX treatment inhibited cell proliferation of both A549 and H157 in a dose-dependent and time-dependent manner (Fig. 1). The IC_{50} of GPX for A549 and H157 at 24 hrs post-incubation were 17.1±1.7 and 16.9±0.6 μM, respectively (Fig. 1a). The IC_{50} of GPX for A549 and H157 at 48 hrs post-incubation were 9.5±0.2 and 9.1±0.1 μM, respectively (Fig. 1b). Besides, 10 μM GPX in the combination with 2 or 10 μM cisplatin at 48 hrs post-incubation significantly elevated the inhibition of A549 proliferation compared with 2 or 10 μM cisplatin alone (Fig. 2a) and the situation is the same to H157 cells (Fig. 2b).

GPX sensitized NSCLC to cisplatin-induced apoptosis: To investigate whether the inhibitory effects of GPX on A549 and H157 cells proliferation was due to the induced apoptosis, apoptotic activities were assessed by Annexin V-FITC and PI staining method. In Fig. 3a, b 10 μM of GPX at 48 hrs post-incubation increased the apoptotic activities

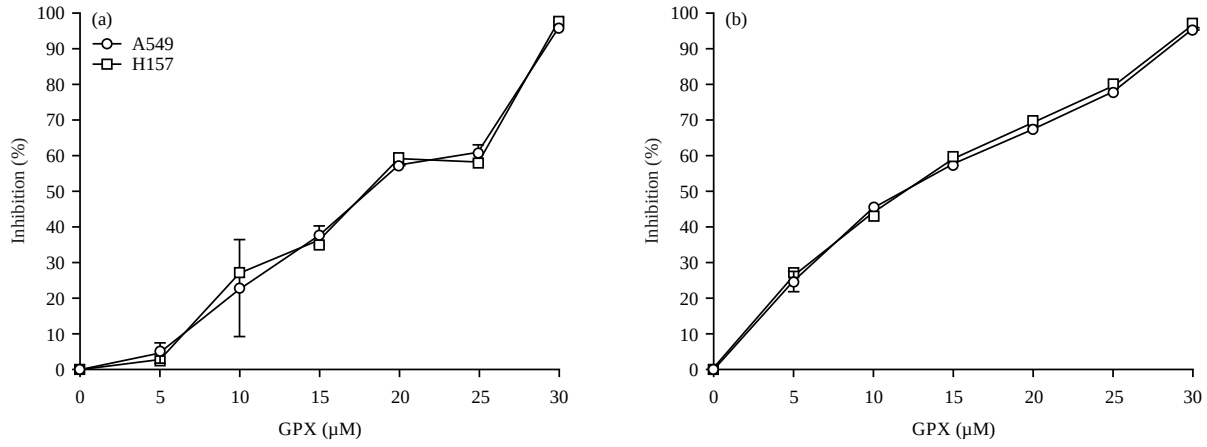

Fig. 1(a-b): GPX inhibited the cell proliferation of human NSCLC A549 and H157 cells in a dose-dependent manner, (a) 24 hrs and (b) 48 hrs post-incubation

Data represented the Mean±SD of triplicates from three independent samples

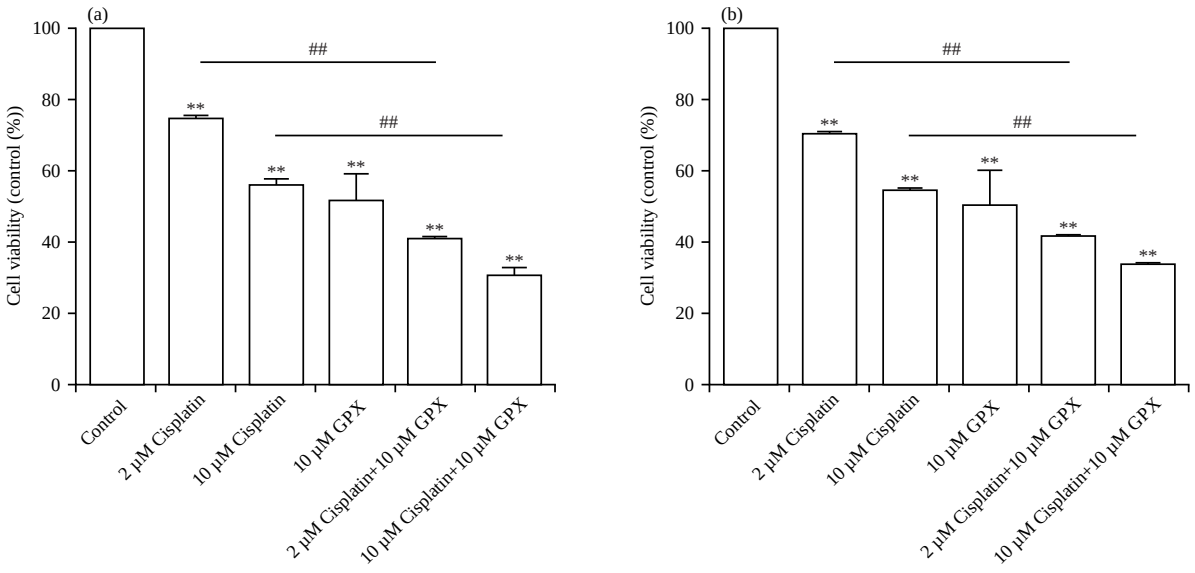

Fig. 2(a-b): GPX increased cisplatin-induced inhibition of, (a) human NSCLC A549 and (b) H157 cell proliferation

Data represented Mean±SD of triplicates from three independent samples, **p<0.01 versus control cells and ##p<0.01 versus cisplatin group

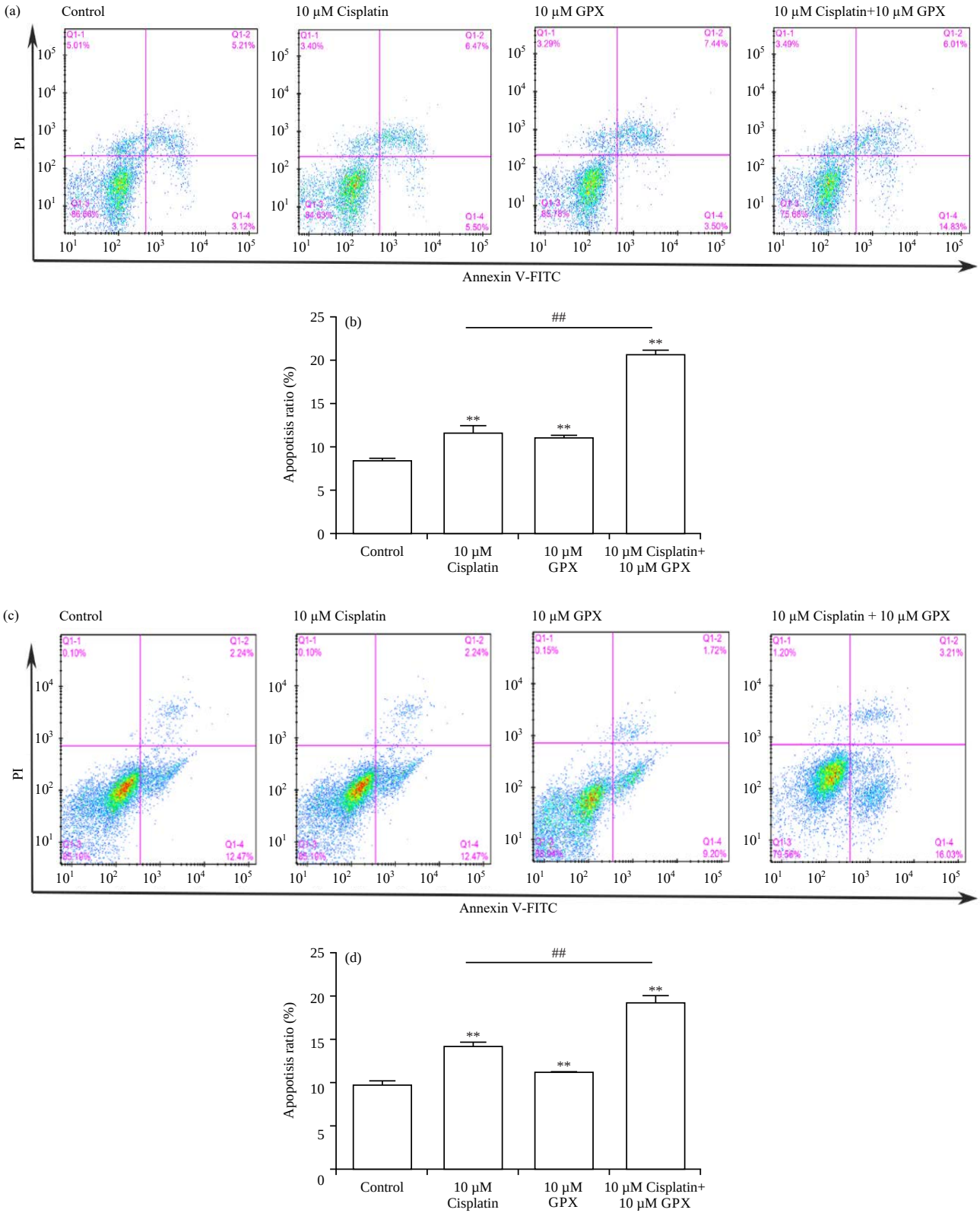

Fig. 3(a-d): GPX sensitizes human NSCLC A549 and H157 to cisplatin-induced apoptosis, (a, c) Representative flow cytometry profiles of A549 and H157 and (b, d) Quantitative analysis of A549 and H157

Data represented the Mean±SD of triplicates from three independent samples, *p<0.05, **p<0.01 versus control cells and ##p<0.01 versus cisplatin group

of both A549 cells as compared to control cells (10.88±0.4 vs. 8.34±0.34%). Moreover, the combination of GPX and cisplatin profoundly enhanced the apoptotic activities of both A549 cells as compared to cisplatin-treated cells (20.53±0.66 vs. 11.45±0.96%). The situation is the same for H157 cells (Fig. 3c, d).

Effects of GPX on cell-cycle distribution in cisplatin-treated NSCLC: To investigate if cell cycle arrest contributes to GPX-induced apoptosis, A549 and H157 cells were treated with 10 μM of cisplatin alone,10 μM GPX alone or 10 μM GPX in the combination with 10 μM cisplatin for 48 hrs. The propidium iodide (PI) staining method was used to determine the cell cycle distribution. In Fig. 4a, b, incubation with 10 μM of cisplatin alone, 10 μM GPX alone or 10 μM of GPX in combination with 10 μM of cisplatin led to a G0/G1 and G2/M cell-cycle arrest in A549 cells, as it increased the percentage of G0/G1 and G2/M phase and reduced the

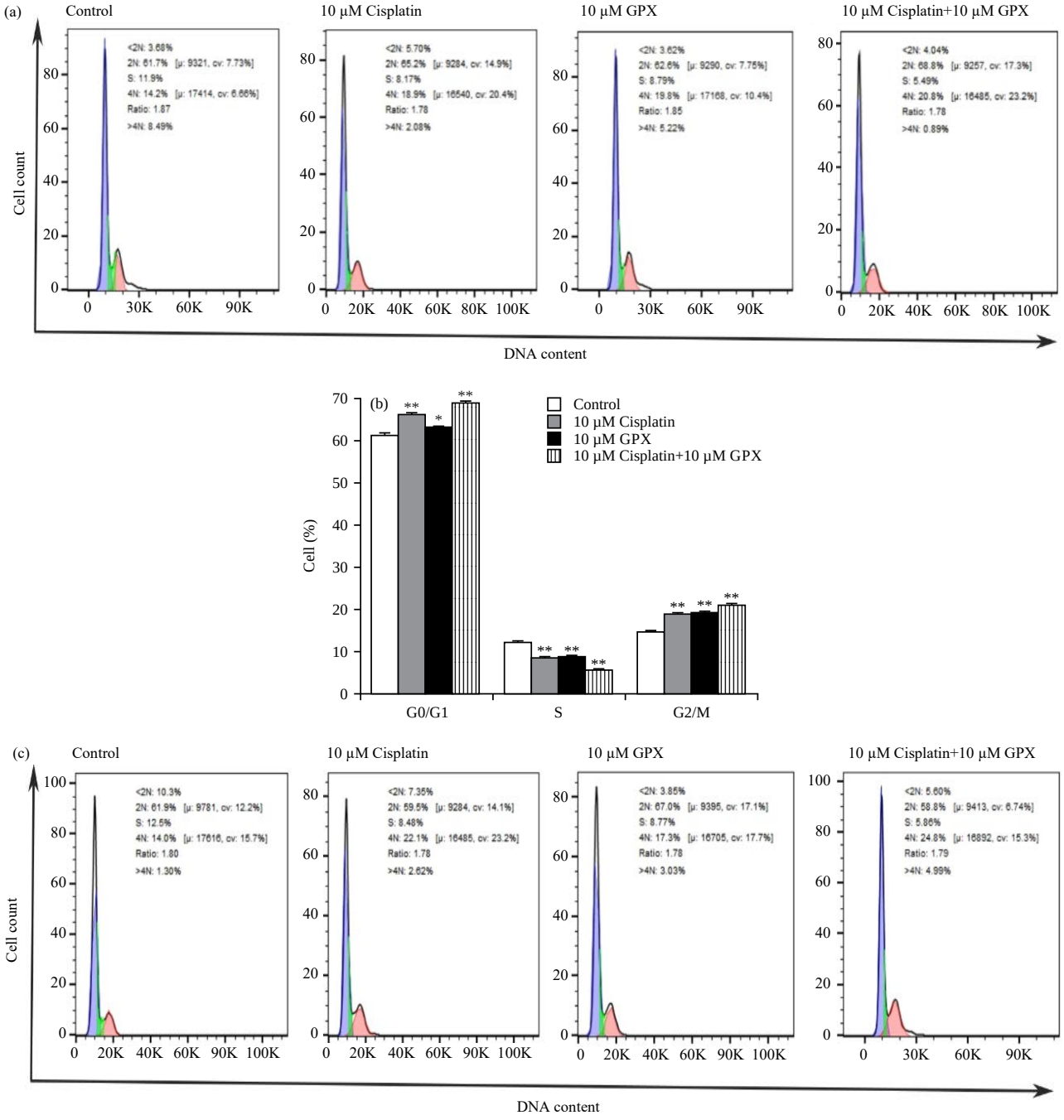

Fig. 4(a-d): Continue

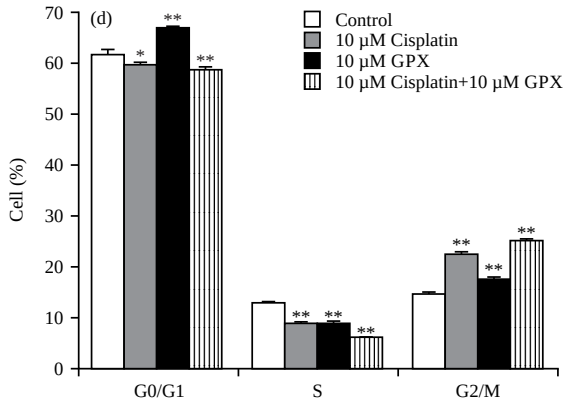

Fig. 4(a-d): Effect of GPX on the cell cycle in human NSCLC A549 and H157 cells, (a, c) Representative analysis of the cell-cycle distribution of A549 and H157 and (b, d) Quantitative analysis of A549 and H157
Data represented the Mean±SD of triplicates from three independent samples, *p<0.05 and **p<0.01 versus control cells

percentage of the S phases. However, in Fig. 4c, d, incubation with 10 μM of GPX in combination with 10 μM of cisplatin only leaded to a G2/M cell-cycle arrest in H157 cells, as it increased the percentage of the G2/M phase and reduced the percentage of the G0/G1 and S phases.

DISCUSSION

In this study, we explore the anti-cancer effect of GPX in combination with cisplatin on NSCLC. A549 and H157 cells were treated with GPX alone or in combination with cisplatin. Cell proliferation was detected by CCK8 assay and GPX significantly improved the antiproliferative effects of cisplatin on NSCLC. Apoptosis was determined by annexin V/propidium iodide (PI) assay and GPX profoundly sensitized NSCLC to cisplatin-induced apoptosis. Finally, we also investigated the possible mechanisms of cell apoptosis by analyzing cell cycle arrest using flow cytometry. The GPX alone or in combination with cisplatin remarkably induced G0/G1 and G2/M cell-cycle arrest in A549 cells, but GPX together with cisplatin only led to a G2/M cell-cycle arrest in H157 cells.

Among various cancers, lung cancer ranks first in mortality in China[15]. The NSCLC is the main type of lung cancer, which accounts for ~85% of lung cancer cases[16]. Cisplatin, together with another anticancer drug, for example, taxanes, gemcitabine or vinorelbine, is the standard regimen to treat advanced NSCLC. Through phase III trials, cisplatin has been proved to be more efficient than other regimens[10]. However, NSCLC easily develops resistance to cisplatin, because the reduced apoptotic activity is regarded as the most important reason for cisplatin resistance in tumour cells[11]. Arctigenin, produced by *Ipomea cairica*, *Torreya nucifera*, *Saussurea medusa*, *Arctium lappa* L. and *Bardanae fructus*, has also been demonstrated to enhance cisplatin-mediated cell apoptosis in cancer cells[17-19]. To be specific, 10 μM of arctigenin dramatically increased cisplatin-mediated inhibition of proliferation by inducing apoptosis and G0/G1 cell-cycle arrest in NSCLC H460 cells, which is similar to these results in this study[20].

The limitation of this study is that we didn't explore the further mechanisms of GPX in combination with cisplatin in the treatment of NSCLC cell lines and the anticancer activity of GPX in combination with cisplatin in animal models need further exploration as well.

CONCLUSION

In conclusion, It is studied the synergistic effect of CPX and cisplatin in the treatment of NSCLC cell lines and its mechanisms were also explored. The data showed that GPX improved the antiproliferative effects of cisplatin on NSCLC via enhancing apoptosis. Further mechanistic studies indicated that GPX induced cell-cycle arrest in NSCLC. This study showed GPX in combination with cisplatin might have therapeutic implications against NSCLC.

SIGNIFICANCE STATEMENT

This study discovers GPX improves cisplatin-mediated cytotoxicity in NSCLC, which implies GPX can be beneficial to overcoming cisplatin chemoresistance in NSCLC. This study will help the researcher to uncover the synergistic effect of CPX and cisplatin that many researchers were not able to explore before. Thus, a new therapeutic option for NSCLC may be arrived at.

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