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APR-246 enhances the anticancer effect of doxorubicin against p53-mutant AsPC-1 pancreatic cancer cells

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Abstract

Pancreatic cancer (PC) is a disease with a high mortality rate, and the early diagnosis of PC is still challenging. The 5-year relative survival rate remains below 8%, and therapeutic strategies are ineffective at increasing patient survival rates. In PC cells, resistance to treatment has been associated with genetic changes that initiate aberrant developmental signaling pathways; therefore, new strategies for treating PC are warranted. Here, we sought to explore the small molecule p53 reactivator, APR-246, either as a mono-drug or combined with doxorubicin (Doxo) in the p53 mutant (p53-mut) AsPC-1 PC cell line. Cytotoxicity of APR-246 and Doxo was studied alone or in combination with the AsPC-1 cell line with MTT assay. CalcuSyn software was used to calculate the combination index (CI), which was based on the Chou and Talalay technique. Apoptosis was assessed after staining with Acridine orange/ethidium bromide (AO/EB). Morphological alterations were examined under a 200x magnification by an inverted microscope. Gene expression was quantified using quantitative reverse transcription PCR assay (RT-qPCR). APR-246 alone exhibited modest anti-proliferative effects, while Doxo combined with APR-246 showed synergistic effects associated with morphological change; no more enhanced apoptosis was observed, as supported by *NOXA* gene levels. In conclusion, APR-246, whether monotherapy or combined with Doxo, was hindering the proliferation of p53-mut AsPC-1 PC cells, and further *in vitro* and *in vivo* research is warranted.

Keywords: APR-246, Combination, Doxorubicin, Pancreatic Cancer, p53 reactivator.

Introduction

One of the worst malignancies is pancreatic cancer, the seventh-highest cause of death from cancer globally. The disease has a poor prognosis and is currently in its mature stage [1]. Several reasons contribute to this, including the absence of specific and sensitive tumor markers and non-specific clinical symptoms and, occasionally, negligible. In addition, gene mutations affecting specific genes and some malignant cells serving as cancer stem cells are decreasing the efficacy of chemotherapy and radiation therapy. Human malignancies, such as PC, have been found to harbor mutations in oncogenes and tumor suppressor genes [2]. According to research, pancreatic cancer has been linked to many mutations, including Kirsten rat sarcoma viral oncogene homolog (*KRAS*), tumor protein p53 *(TP53)*, breast cancer gene *(BRCA)*, cyclin-dependent kinase inhibitor 2A *(CDKN2A)*, and

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Mothers against decapentaplegic homolog 4 (*SMAD4)* [3]. About 50% of all malignancies have a *TP53* mutation, while the same mutation is present in about 75% of PC patients [4]. The p53 protein, encoded by the *TP53* gene, binds to specific DNA regions and controls the transcription of downstream molecules. In addition to performing various biological functions, this protein plays a crucial role in maintaining stem cells, the cell cycle, energy metabolism, metabolic activity, and apoptosis [5].

Doxorubicin (also known as Adriamycin) is used to treat patients with various cancers, including breast cancer and leukemia; however, it is not a first-line medication for PC therapy. Doxo is a member of the anthracycline group of chemotherapeutic agents. Anthracyclines disrupt topoisomerase II, which causes double-strand breaks and inhibits DNA and RNA production by intercalating between base pairs of the DNA/RNA strands. Doxo is a potent inducer of p53 and reactive oxygen species (ROS), a topoisomerase inhibitor that intercalates into DNA. However, mutations in *TP53* are associated with resistance against chemotherapies, including Doxo, challenging treatment as a single agent [6].

The innovative therapy protocols for resistant malignancies are promising because they add selective inhibitors to traditional chemotherapies for cancerous cells [7]. Small molecules known as p53 reactivators have been developed, which bind with mutant p53 and partially restore its tumor suppressor function to arrest tumor cells or cause apoptosis. APR-246 is one of these reactivators, also called PRIMA-1^{MET,} and the clinical form is eprenetapopt. APR-246 is a methylated analog of PRIMA-1 that inhibits cellular proliferation in cells with mutant p53 [8]. Several cancer cells, including osteosarcoma, lung cancer, multiple myeloma, colon cancer, breast cancer, and PC, have responded favorably to PRIMA-1 and its methylated version, PRIMA-1^{MET}. In phase I/II clinical trials, APR-246 was used to treat prostate cancer and hematological malignancies, and it has demonstrated efficacy and safety. Recently, a phase I trial was conducted on patients with *TP53* mutation-positive acute myeloid leukemia (AML). The clinical versions of APR-246, eprenetapopt, azacitidine, and venetoclax were administered to the patients, and they demonstrated acceptable safety and encouraging activity [9, 10].

In this work, our objective was to investigate the efficacy of the small molecule APR-246, which reactivates the p53 protein, either as a single medication or in combination with Doxo, in the AsPC-1 PC cell line with a mutant p53 gene. We assessed the effects of the drugs on cell viability, morphology, and apoptosis in vitro.

Materials and methods

Cell lines and drugs

In this investigation, pancreatic adenocarcinoma-derived AsPC-1 cells were employed; they express mutant p53 protein [11]. The AsPC-1 cell line was generously provided by the postgraduate toxicology laboratory affiliated with the College of Pharmacy at Mustansiriyah University. RPMI-1640 medium with L-glutamine (Capricorn, Germany) was used to cultivate AsPC-1 cells. Additionally, 1% penicillin/streptomycin solution (100x) and 10% fetal bovine serum (FBS) were added to the RPMI-1640 medium (Euroclone, Italy). In a humidified (95% humidity) CO2 incubator, cells were incubated at 37 °C. [12, 13]. We bought Doxorubicin (Cat: ab120629) and APR-246, a p53 reactivator (Cat: Ab145974), from Abcam (USA). Dimethyl sulfoxide (DMSO) was used to dissolve the compounds to create a solution with a stock concentration of 10 mM for each inhibitor.

Cell viability assay

Using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, we evaluated the viability of AsPC-1 cells treated with mono-drugs (APR-246, Doxo) and their combinations. In brief, cells were seeded at a density of 7×10^3 cells/well in 96-well plates with a flat bottom, and they were incubated for 24 hours to ensure adhesion. The cells were then treated with APR-246 and Doxo for 72 hours. Once a 20µl MTT solution (5 mg/ml) was applied to each well, it was incubated at 37°C for three to four hours or until a violet precipitate was visible. An ELISA plate reader (BioTek, USA) was used to measure the absorbance of each well at 492 and 620 nm after adding 50µl of DMSO [14]. The viability percentage is calculated as [(A test-A blank)/(A control-A blank)] x100, where absorbance is indicated by A. GraphPad Prism software version 6 (San Diego, CA, USA) was used to normalize the absorbance data as a percentage of

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the control vehicle (CV). GraphPad Prism was used to determine the growth inhibitory concentration at which viability falls to 50% (GI_{50}).

Calculation of the CI values

A combination of the two drugs (APR-246 and Doxo) was studied, and the cells were incubated with doses of each inhibitor in predetermined ratios (0.25x, 0.5x, 1x, 2x, 4x). The CalcuSyn software (Cambridge, UK) was used to calculate the CI using the Chou and Talalay approach. There is an additive effect when the CI is one, a synergistic effect when the CI is below one, and antagonism when the CI is greater than one.

Observation of morphology under an inverted microscope

The cellular morphology of AsPC-1 cells was studied after co-culture with APR-246 alone, Doxo alone, or both agents together. Plates with six wells and a flat bottom were used to plate AsPC-1 cells at a density of 5×10^4 cells per well. The following day, APR-246 and Doxo were applied to the cells separately and together for 72 hours in an environment with 95% humidity, 37°C, and 5% CO2. A 200x inverted microscope (Meiji Techno, Japan) was used to examine the cells. A Canon camera and the EUS utility program were used to take the pictures.

Detecting apoptosis with dual AO/EB staining

Following a prior study [7], AO dye permeates into both live and dead cells and reacts with DNA or RNA, emitting either a green or red color. Only dead cells display red (EB) due to the breakdown of the membrane structure. At a density of 21×10^3 cells per well, AsPC-1 cells were seeded onto 24-well plates with a flat bottom. The next day, AsPC-1 cells were exposed to APR-246 and Doxo for 72 hours, alone and combined. After trypsinization and PBS washing, AsPC-1 cells were collected in Eppendorf tubes. Subsequently, one µl of the dual fluorescent staining solution (100µg/ml AO and 100µg/ml EB) was mixed with nine µl of cell suspension, then a drop of the mixture was placed on a clean slide and covered with a coverslip. The slide was examined using a fluorescence microscope (Humascope, Germany). After counting one hundred cells, their morphology was categorized into four phases. While viable cells exhibit a green color and a universal nucleus (bright green dots), apoptotic cells exhibit a green/yellowish color with chromatin condensation (early apoptosis). An orange or red nucleus with segmented chromatin will also be present in late apoptotic cells. In contrast, dead or necrotic cells will show uniform orange-to-red nuclei without condensed chromatin.

RT-qPCR (real-time PCR)

TRIzol™ Reagent (Invitrogen, USA) was used to extract total RNA from approximately 3 million AsPC-1 PC cells after a 24-hour treatment with CV, Doxo, and APR-246. Based on the manufacturer's instructions, extracted RNA's quantity and purity were determined using the QubitTMRNA HS Assay Kit (ThermoFisher®, USA, Cat: Q32852). This assay can handle contaminants such as salts, free nucleotides, solvents, detergents, or proteins and is highly reliable and selective for quantifying low-abundance RNA samples. Then, the ProtoScript® First Strand cDNA Synthesis Kit (NEB, UK, Cat: E6300S) was used according to the manufacturer's instructions. Total RNA was reverse transcribed to obtain complementary DNA (cDNA). The cDNA product quantification was also done through Qubit™RNA HS Assay Kit and stored until the second step (Relative quantitative PCR) was performed. This assay is highly specific for double-stranded DNA (dsDNA) over RNA. Following that, Luna Universal qPCR Master Mix (NEB, UK, Cat: M3003S) was used. It is an optimized 2X reaction mix designed to detect and quantify target DNA sequences using the SYBR®/FAM channel of most real-time qPCR instruments. Taq DNA polymerase and a passive reference dye are included in the kit, which can be used with various instruments [15]. The $2^{-\Delta\Delta CT}$ method was employed to evaluate gene expression, with *GAPDH* as the reference gene. The primer sequences utilized in this study were as follows: ***NOXA***, **F**: 5′-GAAGGGAGATGACCTGTGATTAG-3′, **R**: 5′-TGCTGAGTTGGCACTGAAA-3′; and ***GAPDH***, **F**: 5′-GGTGTGAACCATGAGAAGTATGA-3′, **R**: 5′-GAGTCCTTCCACGATACCAAAG-3′.

Statistical analysis

Microsoft Excel 2019 and GraphPad Prism were used for the statistical analysis. There were usually one to three biological replicates in most experiments. An unpaired student's t-test was used in this study to compare mean values. A p-value below the threshold of 0.05 is deemed statistically

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significant for all data reported in terms of the standard error of the mean (SEM).

Results

APR-246 and Doxo mono-treatment inhibit proliferation

We examined the effects of increasing concentrations of APR-246 and Doxo on the viability of a mutant p53 (AsPC-1) PC cell line using the MTT assay. After incubation for 72 hours, both single drugs decreased the viability of cells in a dose-dependent manner (Figure 1). AsPC-1 was moderately sensitive to APR-246 (Mean GI_{50} = 79 µM) (Figure 1A); in contrast, it was more sensitive to Doxo (Mean GI_{50} = 1.58 µM) (Figure 1B). Accordingly, individual drugs reduced the proliferation of AsPC-1 but it was more sensitive to Doxo.

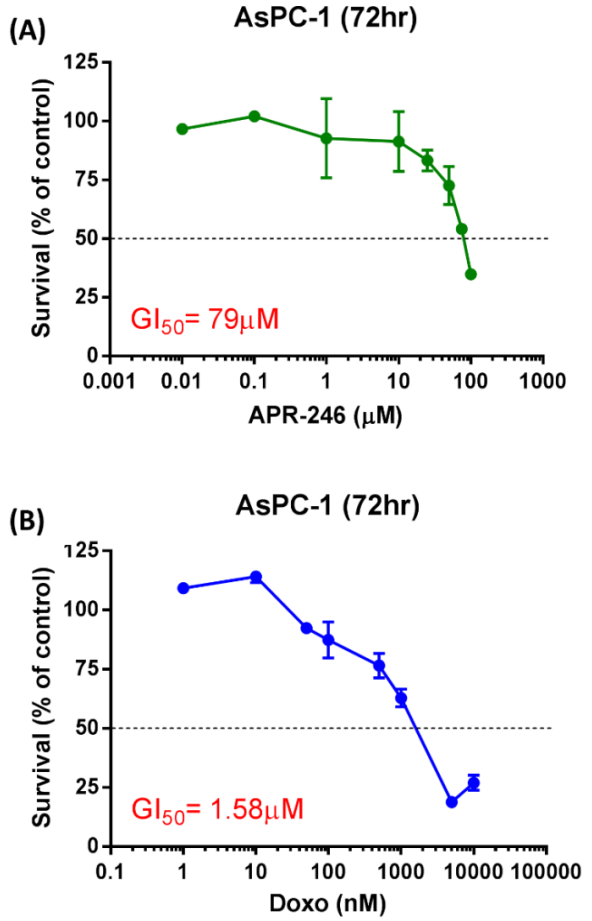

Figure 1. Sensitivity curves for AsPC-1 cells cultured for 72 hours at escalating concentrations of APR-246 (A) and Doxo (B). Utilizing the MTT test, cell viability was evaluated. The data is the mean survival percentage ± standard error of the cells that treated with CV. The GI_{50} value is shown in red.

Co-treatment of APR-246 with Doxo has synergistic effects

The CalcuSyn program was employed to compute CI values to ascertain the synergistic effects of APR-246 and Doxo combination on AsPC-1 cells. AsPC-1 cells were cultured with CV, fractions of GI_{50} of APR-246 alone, Doxo alone, or both combined for a duration of 72 hours, as shown in the viability dose-response curves (Figure 2A). Interestingly, the simultaneous use of the drugs exhibited synergistic effects (CI<0.9) at effective dose 50 (ED50) (Mean CI=0.82 ± 0.09), ED75 (Mean CI=0.83 ± 0.11) and ED90 (Mean CI=0.85 ± 0.12) (Figure 2B).

To confirm the synergistic effect, we also investigated the morphology of AsPC-1 cells under an inverted microscope after exposure to CV, APR-246 (10µM), Doxo (1µM), or both drugs simultaneously for 24 and 72 hours (Figure 2C). After 24 hours, only a slight reduction in cell number was observed in Doxo and dual treatment and the morphology of cells was nearly similar in all treatments (Figure 2C, top panel). However, the cell number was remarkably reduced in dual drug treatment after 72 hours compared to single agents. Moreover, the cells were elongated or floating in Doxo and mixture treatments, and the effect was more potent in combination treatment (Figure 2C, bottom panel). These results indicate a potentially synergistic effect on the viability and morphology of PC cells.

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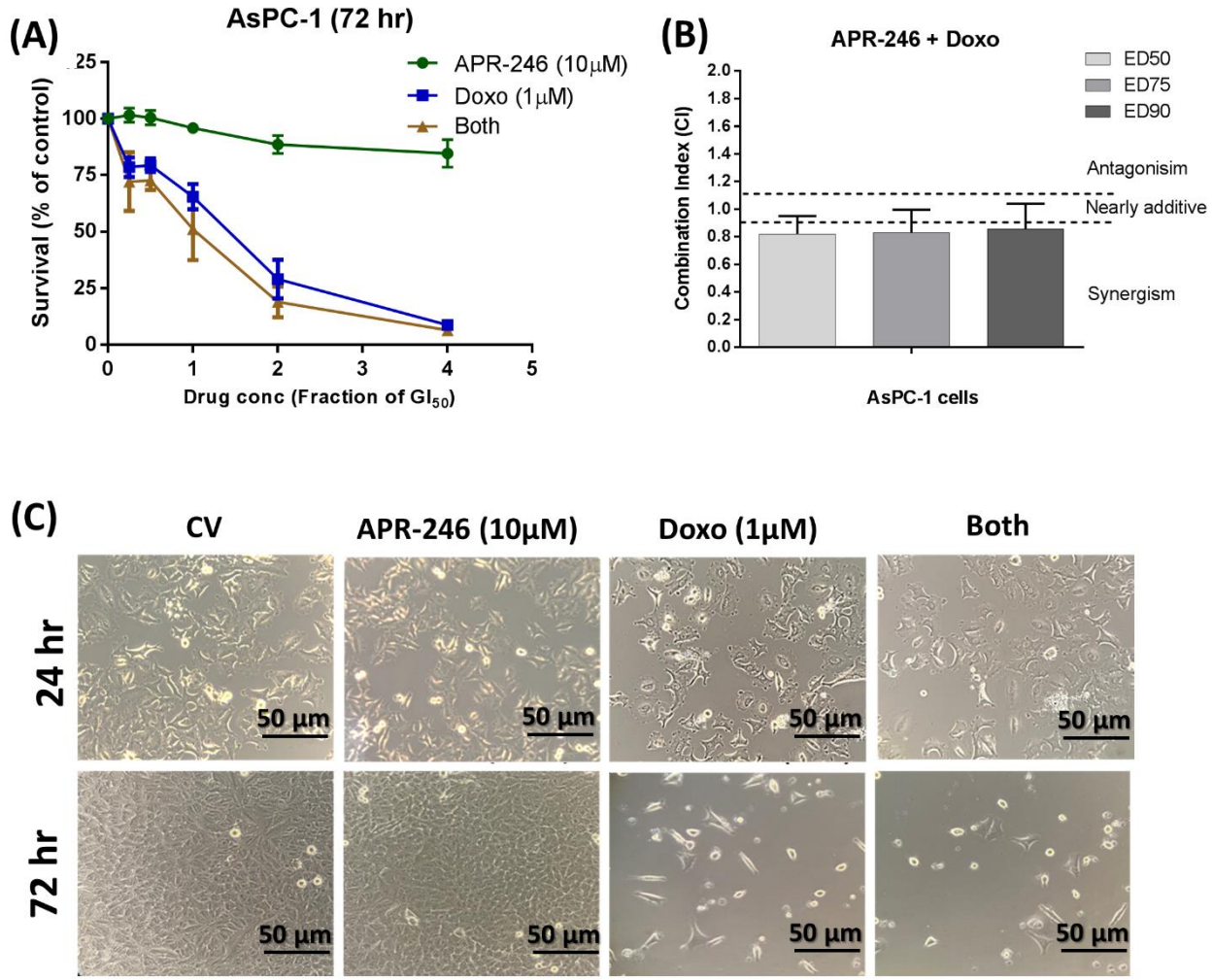

Figure 2. The combination of APR-246 and Doxo is synergistic. (A) AsPC-1 cells were incubated with CV, fractions of GI_{50} APR-246 alone, Doxo alone, or both drugs simultaneously. Cell viability was determined using the MTT assay. (B) Based on CalcuSyn software, CI values were calculated at ED50, ED75, and ED90. (C) Representative images of AsPC-1 exposed to CV, APR-246 (10μM), Doxo (1μM), or a combination of both drugs for 24 (top panel) and 72 hours (bottom panel), an inverted microscope was used. Data represent the average of two biological replicates. Ten and one micromolar were considered the GI_{50} values for APR-246 and Doxo, respectively.

Effect of APR-246 co-treatment with Doxo on apoptosis and *NOXA* gene expression

After staining with AO/EB, fluorescent microscopy was used to assess the percentage of apoptosis induction in the AsPC-1 cells subjected to single and combined drugs. The cells were co-cultured with APR-246 (10μM), Doxo (1μM), or both drugs for 72 hours (Figure 3A, B). Remarkably, AsPC-1 PC cells cultured with Doxo either by itself or in conjunction with APR-246 exhibited a substantial rise in apoptosis (early plus late apoptosis). On the other hand, compared to cells treated with CV, cells treated with APR-246 monotherapy did not exhibit a statistically significant increase in apoptosis. (Figure 3A). Nevertheless, no further increase in the percentage of apoptotic cells was observed in the dual treatment compared to the Doxo treatment. Similarly, no significant increase was seen in the ratio of necrotic cells in different treatments compared to CV. The morphology and coloration of 100 cells were used to classify them into different phases of apoptosis (Figure 3B).

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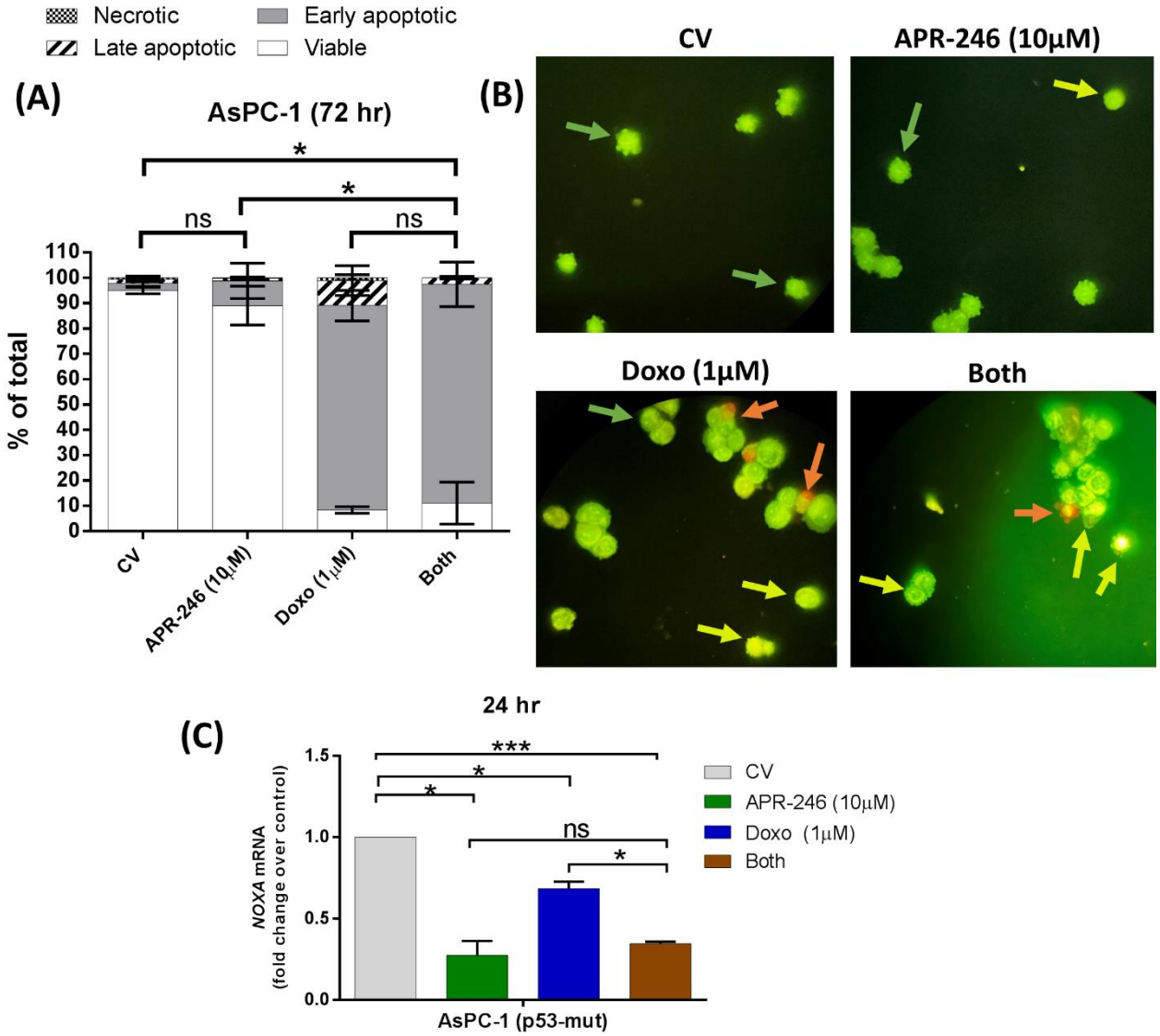

Figure 3. APR-246 and Doxo mixture affect apoptosis and *NOXA* expression in APC-1 cells. (A) AsPC-1 cells were exposed to CV, APR-246 (10μM), Doxo (1μM), or both drugs for 72 hours before apoptosis detection. (B) Representative images of two biological replicates of AsPC-1 stained with AO/EB and examined under a fluorescence microscope at a magnification of 400x. (C) Additionally, after 24 hours of treatment, the expression of *NOXA* gene in AsPC-1 cells was identified. After being incubated with the indicated treatments, the RT-qPCR bars show the fold change over CV; as a reference gene, *GAPDH* was used. Compared to the equivalent control (CV) bar, asterisks indicate significant changes in the displayed bars. The bar graphs (A, C) with error bars represent the mean ± SEM of two replicates, * p<0.05; *** p<0.001; ns=not significant. (B) Green arrows are viable cells, yellow arrows are early apoptotic cells, and orange arrows are late apoptotic cells.

To further understand the mechanism of synergy, we investigated the *NOXA* gene mRNA level by RT–qPCR in AsPC-1 cells incubated with APR-246/Doxo combination for 24 hours (Figure 3C). Noxa is a pro-apoptotic protein that mediates apoptosis induction downstream of the p53 pathway through the mitochondrial route [16]. Interestingly, *NOXA* expression was significantly reduced in cells treated with single and combination treatments compared to cells incubated with CV (Figure 3C). As a result, Doxo alone induced apoptosis; however, when combined with APR-246, no further increase was obtained, which was confirmed at the *NOXA* gene level.

Discussion

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The p53 protein is widely postulated to be responsible for most of the "hallmarks of cancer" while also being implicated in other cellular processes, such as apoptosis and control of the cell cycle [17]. Therefore, restoring p53 activity is a promising therapeutic strategy, primarily focusing on specific disease hallmarks. Interestingly, selective small molecules have gained popularity with recent advancements as a treatment for various cancers, including PC [18]. Additionally, 50–70% of PC patients carry mutations of the *TP53* gene [3], which may make patients more resistant to chemotherapeutics like Doxo. Consequently, the protein encoded by the *TP53* gene, whether in its wild-type or mutant form, represents a promising target for therapeutic intervention. Reactivating p53 has the potential to counteract the development of resistance to chemotherapy [19]. Thus, this study aimed to determine whether the p53 reactivator APR-246 enhances the activity of chemotherapy treatment (doxorubicin) using human-derived PC cells with mutant p53 protein.

PRIMA-1^{MET}, also known as APR-246, is a compound that functions as a reactivator of the p53 protein; it restores the wild-type functionality of mutant *TP53*-encoded proteins. Interestingly, APR-246 causes cell cycle arrest and apoptosis in several cancer types; moreover, it has shown clinical efficacy in treating AML and Myelodysplastic syndromes (MDS) [8]. Given this encouraging result, we examined the effects of APR-246 monotherapy and Doxo monotherapy on PC cells (AsPC-1) with mutant p53 status. Viability results indicated a mild response after mono-treatment of AsPC-1 cells with APR-246. A possible explanation for the mild effect is an increase in the expression of the p53 inhibitor MDM2 in response to a remarkable drug-mediated restoration of the activity of mutated p53 [20]. In addition, APR-246 treatment (10μM) can not significantly alter the morphology of AsPC-1 cells. In contrast, the data reported by Yoshikawa et al. [21] indicated that the use of elevated doses of APR-246 alone (50-100μM) in epithelial ovarian cancer cells was associated with significant morphological changes. Interestingly, the GI_{50} value for Doxo in AsPC-1 cells was comparable to that shown in another study [22].

Interestingly, several preclinical studies showed a synergistic effect after adding the p53 reactivator (APR-246, PRIMA-1^{MET}, PRIMA-1) to Doxo in several cancer types like multiple myeloma [23], ovarian cancer [24], and Neuroblastoma [25]. Moreover, the APR-246 plus liposomal pegylated doxorubicin (LPD) regimen implemented in phase 1b clinical study showed encouraging efficacy in high grade serious ovarian cancer (HGSOC) patients [26]. Therefore, we investigated the potential synergistic impact after combining APR-246 with Doxo on the viability of p53-mut AsPC-1 PC cells, and a synergistic effect was obtained, in addition to changes in cellular morphology. Interestingly, Mohell and co-authors [24] found that adding APR-246 to Doxo reverted resistance and re-sensitized (reduced the GI_{50} of Doxo) Doxo-resistant p53 mutant ovarian cancer cell lines (A2780ADR and the parental cell line A2780) to Doxo. Another study speculated two potential scenarios behind the enhanced effect of the APR-246/Doxo combination. The first mechanism is via reactivation and the refolding process of mutant p53 protein by APR-246, thereby improving the impact of chemotherapeutic agent (Doxo) or by the accumulation of altered p53 protein by Doxo (DNA-damaging agent), hence, potentiates the effect of the p53 reactivator compound (APR-246) [27].

To further understand the synergistic effect seen in AsPC-1 cells, the impact of APR-246 plus Doxo combination was also investigated on apoptosis induction and *NOXA* gene level, a component of the p53-dependent apoptosis pathway [16]. In line with our previous studies, APR-246 monotherapy did not induce significant apoptosis as supported by the *NOXA* gene level [7]. The suppression of *NOXA* might be attributed to mitogen-activated protein kinase (MEK) inhibition by APR-246, as indicated by prior reports [28, 29]. However, significant apoptosis accompanied by a reduction in *NOXA* gene expression was observed in AsPC-1 cells treated with Doxo; this may indicate that Doxo-induced apoptosis is mediated via p53-independent pathways [30]. Although significant apoptosis was seen in both Doxo and combination treatments, the results implicated nearly similar percentages of apoptotic cells (at an early stage). Hence, the synergistic effect of dual therapy can be ascribed to cell cycle arrest besides apoptosis. Cell cycle arrest and apoptosis are the primary consequences of p53 activation; therefore, the reactivation of mutant p53 by APR-246 has been established by eliciting these effects, as observed in wild-type p53 [11, 31]. To this end, a recent study found that APR-246 and Doxo dual drugs are

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synergistic in p53-mut neuroblastoma cells, and the synergy was associated with the activation of the cell cycle inhibitor CDKN1A [25]. As a direct target of p53 after p53 activation, transcription of p21/CDKN1A is significantly increased, leading to the induction of many target genes, which regulate cell cycle progression and eventually induce cell cycle arrest [5]. Consequently, further studies on p53-mut and p53 wild-type PC cells are necessary to confirm the synergism obtained in this combination. Unfortunately, the biggest obstacle in this study was the lack of financial support.

Conclusion

In conclusion, about two-thirds of PC patients have mutant p53 proteins and are less likely to respond to chemotherapy. APR-246 benefits various cancer types by restoring p53 activity and reverting chemo-resistance. Our data showed modest anti-proliferative activity of APR-246 as a monotherapy; however, when combined with Doxo, a synergistic effect associated with morphological changes but no further induction of apoptosis was observed in p53 mutant PC cells. This may indicate that this combination is synergistic via induction of cell cycle arrest. Hence, further preclinical and clinical studies are warranted to determine the efficacy of APR-246 and Doxo as a potential targeted cancer therapy in PC patients with mutant p53.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Al-Nahrain.

Authors' Contribution Statement

AA, designed the study. All authors performed material preparation and data collection. AA, analyzed Figures 1-3b, and SA, analyzed Figure 3c. AA, and SA, wrote the main manuscript text, and all authors reviewed the final draft.

References

1. Rawla P, Sunkara T, Gaduputi V. Epidemiology of pancreatic cancer: global trends, etiology and risk factors. World J Oncol. 2019; 10(1): 10. https://doi.org/10.14740/wjon1166
2. Blandino G, Di Agostino S. New therapeutic strategies to treat human cancers expressing mutant p53 proteins. J Exp Clin Cancer Res. 2018; 37: 1-13.https://doi.org/10.1186/s13046-018-0705-7
3. Qian Y, Gong Y, Fan Z, Luo G, Huang Q, Deng S, et al. Molecular alterations and targeted therapy in pancreatic ductal adenocarcinoma. J Hematol Oncol. 2020; 13(1): 1-20.https://doi.org/10.1186/s13045-020-00958-3
4. Du J, Gong H, Xiao H. Somatic TP53 mutations and comparison of different TP53 functional domains in human cancers: Data analysis from the IARC TP53 database and the National Cancer Institute GDC data portal. Med Data Min. 2021; 4(3): 1-10.https://doi.org/10.12032/MDM2021033003
5. Engeland K. Cell cycle regulation: p53-p21-RB signaling. Cell Death Differ. 2022; 29(5): 946-60.https://doi.org/10.1038/s41418-022-00988-z
6. Peter S, Alven S, Maseko RB, Aderibigbe BA. Doxorubicin-Based Hybrid Compounds as Potential Anticancer Agents: A Review. Molecules (Basel, Switzerland). 2022; 27(14): 4478.https://doi.org/10.3390/molecules27144478
7. Mohammed I, Alhammer AH, Arif IS. The p53 reactivator PRIMA-1(MET) synergises with 5-fluorouracil to induce apoptosis in pancreatic cancer cells. Invest New Drugs. 2023; 41(4): 587-95.https://doi.org/10.1007/s10637-023-01380-5
8. Sallman DA, DeZern AE, Garcia-Manero G, Steensma DP, Roboz GJ, Sekeres MA, et al. Eprenetapopt (APR-246) and Azacitidine in TP53-Mutant Myelodysplastic Syndromes. J Clin Oncol. 2021; 39(14): 1584-94.https://doi.org/10.1200/jco.20.02341

Published Online First: January, 2024
https://doi.org/10.21123/bsj.2024.10135
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

9. Menichini P, Monti P, Speciale A, Cutrona G, Matis S, Fais F, et al. Antitumor Effects of PRIMA-1 and PRIMA-1Met (APR246) in Hematological Malignancies: Still A Mutant P53-Dependent Affair?. Cells. 2021; 10(1): 98.https://doi.org/10.3390/cells10010098
10. Garcia-Manero G, Goldberg AD, Winer ES, Altman JK, Fathi AT, Odenike O, et al. Eprenetapopt combined with venetoclax and azacitidine in TP53-mutated acute myeloid leukaemia: a phase 1, dose-finding and expansion study. Lancet Haematol. 2023; 10(4): e272-e83.https://doi.org/10.1016/S2352-3026(22)00403-3
11. Izetti P, Hautefeuille A, Abujamra AL, de Farias CB, Giacomazzi J, Alemar B, et al. PRIMA-1, a mutant p53 reactivator, induces apoptosis and enhances chemotherapeutic cytotoxicity in pancreatic cancer cell lines. Investig New Drugs. 2014; 32: 783-94.https://doi.org/10.1007/s10637-014-0090-9.
12. Ali SM, Al-Karam LQ, Alhammer AJN. In Vitro Cancer Cells Therapy with Nano-gold Depending on its Optical Properties. Neuroquantology 2022; 20(2): 57.https://doi.org/10.14704/nq.2022.20.2.NQ22025
13. Alfardan RJBSJ. Small Nuclear RNA 64 (snoRNA64): A novel Tumor Biomarker for Pancreatic Cancer. Baghdad Sci J. 2023; 20((5Suppl.)): 1873-81.https://doi.org/10.21123/bsj.2023.7135
14. Nashaan FA, Al-Rawi MS, Alhammer AH, Rabie AM, Tomma JH. Synthesis, characterization, and cytotoxic activity of some imides from galloyl hydrazide. Eurasian Chem Commun. 2022; 4(10): 966-75.https://doi.org/10.22034/ecc.2022.340135.1453
15. Al-Juboori SKE, Al-Juboori SIK. Hepatocellular Carcinoma Prediction and early Diagnosis of Hepatitis B and C viral infection using miR-122 and miR-223 in a sample of Iraqi patients. Baghdad Sci J. 2023; 20(4(SI)): 1520.https://doi.org/10.21123/bsj.2023.9065
16. Ploner C, Kofler R, Villunger A. Noxa: at the tip of the balance between life and death. Oncogene. 2008; 27 Suppl 1(Suppl 1): S84-92.https://doi.org/10.1038/onc.2009.46
17. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022; 12(1): 31-46.https://doi.org/10.1158/2159-8290.cd-21-1059
18. Voutsadakis IA. Mutations of p53 associated with pancreatic cancer and therapeutic implications. Ann Hepatobiliary Pancreat Surg. 2021; 25(3): 315-27.https://doi.org/10.14701/ahbps.2021.25.3.315
19. Guo Y, Tang Y, Lu G, Gu J. p53 at the Crossroads between Doxorubicin-Induced Cardiotoxicity and Resistance: A Nutritional Balancing Act. Nutrients. 2023; 15(10): 2259-82.https://doi.org/10.3390/nu15102259
20. Bykov VJ, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. Nat Rev Cancer. 2018; 18(2): 89-102.https://doi.org/10.1038/nrc.2017.109.
21. Yoshikawa N, Kajiyama H, Nakamura K, Utsumi F, Niimi K, Mitsui H, et al. PRIMA-1MET induces apoptosis through accumulation of intracellular reactive oxygen species irrespective of p53 status and chemo-sensitivity in epithelial ovarian cancer cells. Oncol Rep. 2016; 35(5): 2543-52.https://doi.org/10.3892/or.2016.4653
22. Liu J, Huang Y, Liu Y, Chen Y. Irisin enhances doxorubicin-induced cell apoptosis in pancreatic cancer by inhibiting the PI3K/AKT/NF-κB pathway. Med Sci Monit. 2019; 25: 6085.https://doi.org/10.12659/MSM.917625
23. Saha MN, Jiang H, Yang Y, Reece D, Chang H. PRIMA-1Met/APR-246 Displays High Antitumor Activity in Multiple Myeloma By Induction of p73 and NoxaAntimyeloma Activity of PRIMA-1Met. Mol Cancer Ther. 2013; 12(11): 2331-41.https://doi.org/10.1158/1535-7163.MCT-12-1166.
24. Mohell N, Alfredsson J, Fransson Å, Uustalu M, Byström S, Gullbo J, et al. APR-246 overcomes resistance to cisplatin and doxorubicin in ovarian cancer cells. Cell Death Dis. 2015; 6(6): e1794.https://doi.org/10.1038/cddis.2015.143
25. Müller M, Rösch L, Najafi S, Gatzweiler C, Ridinger J, Gerloff XF, et al. Combining APR-246 and HDAC-Inhibitors: A Novel Targeted Treatment Option for Neuroblastoma. Cancers. 2021; 13(17): 4476-99.https://doi.org/10.3390/cancers13174476
26. Basu B, Gourley C, Gabra H, Vergote IB, Brenton JD, Abrahmsen L, et al. PISARRO: A EUTROC phase 1b study of APR-246 with carboplatin (C) and pegylated liposomal doxorubicin (PLD) in relapsed platinum-sensitive high grade serous ovarian cancer (HGSOC). Ann Oncol. 2016; 27: vi123.https://doi.org/10.1093/annonc/mdw368.29
27. Bykov VJ, Zhang Q, Zhang M, Ceder S, Abrahmsen L, Wiman KG. Targeting of mutant p53 and the cellular redox balance by APR-246 as a strategy for efficient cancer therapy. Front Oncol. 2016; 6: 21.https://doi.org/10.3389/fonc.2016.00021.
28. Basile KJ, Aplin AE. Downregulation of Noxa by RAF/MEK inhibition counteracts cell death response in mutant B-RAF melanoma cells. Am J Cancer Res. 2012; 2(6): 726-35
29. Lu T, Zou Y, Xu G, Potter JA, Taylor GL, Duan Q, et al. PRIMA-1Met suppresses colorectal cancer independent of p53 by targeting MEK. Oncotarget. 2016; 7(50): 83017.https://doi.org/10.18632/oncotarget.12940

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30. Yang MC, Lin RW, Huang SB, Huang SY, Chen WJ, Wang S, et al. Bim directly antagonizes Bcl-xl in doxorubicin-induced prostate cancer cell apoptosis independently of p53. Cell Cycle. 2016; 15(3): 394-402.https://doi.org/10.1080/15384101.2015.1127470

31. Almohaidi AMS, Hassan FM, Rothan H. Editorial: Current advances in anti-infective strategies. Baghdad Sci. J. 2023;20(5):1570. https://doi.org/10.21123/bsj.2023.9731

APR-246 يعزز التأثير المضاد للسرطان لعقار الدوكسوروبيسين ضد خلايا سرطان البنكرياس AsPC-1 المتحولة p53

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الخلاصة

سرطان البنكرياس هو مرض ذو معدل وفيات مرتفع، ولا يزال التشخيص المبكر لسرطان البنكرياس يمثل تحديًا. يظل معدل البقاء النسبي لمدة 5 سنوات أقل من 8%، والاستراتيجيات العلاجية غير فعالة في زيادة معدلات بقاء المريض على قيد الحياة. في خلايا سرطان البنكرياس، ارتبطت مقاومة العلاج بالتغيرات الجينية التي تؤدي إلى ظهور مسارات خلوية شاذة؛ ولذلك، هناك ما يبرر ايجاد استراتيجيات جديدة لعلاج هذا المرض. هنا، سعينا لاستكشاف مُنشط p53، APR-246، إما كدواء أحادي أو مدمج مع دوكسوروبيسين في خط خلايا AsPC-1 ذات بروتين p53 المتحول (p53-mut). تمت دراسة السمية الخلوية لـدوائي APR-246 ودوكوروبيسين اما منفردة أو مجتمعة في خط خلايا AsPC-1 مع اختبار MTT. تم حساب مؤشر معامل قياس التآزر (Combination Index) بناءً على نهج Chou و Talalay باستخدام برنامج CalcuSyn. تم تقييم موت الخلايا المبرمج بعد تصبيغها باستخداممادة الاكريدين اورنج/ايثيديوم بروماید (AO/EB). تم فحص التغيرات المورفولوجية تحت المجهر المقلوب بقوة تكبير 200 مرة تم قياس كمية التعبير الجيني باستخدام فحص النسخ العكسي الكمي PCR . أظهر APR-246 وحده تأثيرات متواضعة مضادة للتكاثر، في حين أظهر دوكسوروبيسين مع APR-246 تأثيرات تآزرية مرتبطة بالتغير المورفولوجي؛ لم يلاحظ أزدياد في نسبة موت الخلايا المبرمج في الخلايا المعاملة بكلا العقارين و هذا ما اكدته مستويات جين ال *NOXA*. في الختام، APR-246، سواء كان العلاج وحيدًا أو مقترنًا بـدوكسوروبيسين، كان يعيق تكاثر خلايا AsPC-1 ذات p53-mut، وهناك ما يبرر إجراء المزيد من الأبحاث في المختبر وفي الجسم الحي.

الكلمات المفتاحية: APR-246، علاج تآزري، دوكسوروبيسين، سرطان البنكرياس، منشط p53.