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Effects of FOLFIRINOX Components on Pancreatic Adenocarcinoma Cells

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Abstract

Pancreatic adenocarcinoma is one of the lethal types of cancer worldwide. This study aimed to evaluate the cytotoxic effects of FOLFIRINOX compounds [oxaliplatin (OXA), irinotecan (IRI), fluorouracil (5FU), and leucovorin (LEU)] on a pancreatic adenocarcinoma cell line (PANC-1), both individually and collectively. Cell viability was determined using the colorimetric MTT reagent and apoptosis was assessed using the propidium iodide – Hoechst 33342 staining assay.

Based on cell viability data, 5FU and FOLFIRINOX treatments were more potent against PANC-1 than the other test groups. The apoptotic trend, nevertheless, was not different between them and the control group. Moreover, OXA, LEU, and IRI significantly increase apoptotic cell death. Our findings indicate that 5FU and FOLFIRINOX are comparably effective in reducing the PANC-1 cell viability. In the future, 5FU and FOLFIRINOX may be adapted to local pancreatic adenocarcinoma treatments, but the development of these localized drug release platforms requires further attention as the microenvironment of pancreatic adenocarcinoma inherently contains many unknowns.

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Keywords: FOLFIRINOX; PANC-1 cells; Pancreatic Adenocarcinoma; Cytotoxicty; Apoptosis.

FOLFIRINOX Bileşenlerinin Pankreas Adenokarsinom Hücreleri Üzerindeki Etkileri

Öz

Pankreatik adenokarsinoma tüm dünyada en ölümcül kanser türlerinden biridir. Bu çalışma, FOLFIRINOX bileşiklerinin (oksaliplatin (OXA), irinotekan (IRI), fluorourasil (5FU) ve leucovorin (LEU)) tekli ve toplu olarak pankreas adenokarsinom hücre hattı (PANC-1) üzerindeki etkilerini hücre canlılığı ve apoptoz için araştırmayı amaçlamıştır. Hücre canlılığı, MTT reaktifi kullanılarak belirlendi ve apoptoz, propidium iyodür – Hoechst 33342 boyama testi kullanılarak değerlendirildi.

Hücre canlılığı sonuçları, FOLFIRINOX uygulamasının diğer test gruplarına kıyasla en yüksek toksisite oranına sahip olduğunu göstermiştir. Ancak apoptotik eğilim kontrol grubundan farklı bulunmadı. Ek olarak, 5FU tekil uygulamada diğer tekil ilaç uygulamalarına nazaran en yüksek toksisiteyi göstermiştir, fakat apoptotik indeksi kontrol grubundan ve FOLFIRINOX tedavisinden farklı bulunmamıştır. Genel olarak, çalışma sonuçları, tedavi rejiminin pankreas adenokarsinom hücre dizileri üzerinde etkili olduğunu gösterdi. Gelecekte, FOLFIRINOX ve 5FU tedavisi lokal pankreas adenokarsinomu tedavilerine uyarlanabilir, ancak pankreas adenokarsinomunun mikroçevresi, doğası gereği pek çok bilinmeyeni barındırdığından, bu lokalize ilaç salım platformlarının geliştirilmesi daha fazla dikkat gerektirmektedir.

Anahtar Kelimeler: FOLFIRINOX; PANC-1 hücreleri; Pankreatik Adenokarsinoma; Sitotoksisite; Apoptoz.

1. Introduction

Pancreatic adenocarcinoma (PA) accounts for roughly 2.5% of all malignancies diagnosed globally and is a fatal disease with a rising frequency. The incidence rate and age-standardized rate of pancreatic cancer are 6.4% and 4.9%, respectively. Poor dietary habits are a major cause of disease occurrence [1]. Although the disease has a high prevalence and lethal effect, surgeons and oncologists consider that surgical resection is the only potentially curative treatment for PA in the light of what is known about the disease [2]. Drug screening and drug repurposing studies, therefore, are too critical for PA.

FOLFIRINOX drug combination is one of the PA treatment strategies that has shown promising results when compared to conventional drugs [3]. FOLFIRINOX is known as the abbreviation of the combination chemotherapy regimen consisting of oxaliplatin (OXA), irinotecan (IRI), fluorouracil (5FU), and leucovorin (LEU). In this treatment, OXA targets guanine and cytosine moieties of DNA,

and 5FU blocks DNA synthesis, while IRI is responsible for inhibiting DNA topoisomerase enzymes. One of the vitamin B family members, LEU increases 5FU activity [4].

FOLFIRINOX has been accepted as salvage therapy for PA [5]. The clinical studies demonstrated that FOLFIRINOX treatment for locally advanced or borderline resectable PA seems effective with a manageable toxicity profile [6]. Individually FOLFIRINOX compounds have shown a significantly cellular death profile in independent research studies; however, the cellular level response of the FOLFIRINOX regimen is still unclear and requires more attention. Cytotoxicity profile of FOLFIRINOX may provide an insight for clinicians to low-dose treatment schemes and reduced side effects of the disease [4].

The alterations to the balance between cell proliferation and cell death trigger tumor development with a series of genetic variations during cancer progression. Therefore, monitoring the apoptosis propensity of cell lines is essential to drug applications [7]. The study focused on how the FOLFIRINOX treatment affects the pancreatic adenocarcinoma cell line (PANC-1). The apoptosis trend of the cells was followed after single and multiple drug applications on the cell line in addition to cell viability analyses. We aimed to demonstrate the effects of the FOLFIRINOX compounds individually and collectively on the PANC-1.

2. Material and Methods

2.1. Materials

American Tissue Culture Collection provided a pancreatic ductal adenocarcinoma cell line (PANC1, CRL-1469). Oxaliplatin (O9512), leucovorin (47612), irinotecan (I1406), 5-Fluorouracil (F6627), Dulbecco's Modified Eagle's Medium (DMEM, D3744), fetal bovine serum (FBS, 2327864), trypsin-EDTA (59428C), propidium iodide (PI) (BCC1398), Hoechst bidBenzamide H 33342 trihydro chloride (BCCC5645), and HEPES (H4034) were purchased from Sigma-Aldrich, Germany. L-glutamine (CP21-4047), penicillin-streptomycin (CP21-4079), phosphate-buffered saline (PBS, PBS-1A) were used as received from Capricorn, Germany, and methanol (947.046.2500) was obtained from Isolab, Germany. The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution (NC461720) was obtained from Biobasic, Canada.

2.2. Drug Preparation

OXA, LEU, IRI, and 5FU were prepared according to FOLFIRINOX administration protocol [8]. Two milligrams (mg) of drugs were dissolved in one ml of HEPES, HEPES: methanol (3:2 v/v), methanol, dimethylsulfoxide, respectively. Then the final concentrations of the substances were prepared with serial dilutions (Table 1).

Table 1: The analysis concentrations and incubation time for the combinations of OXA, LEU, IRI, and 5FU

Chemicals	Incubation Time (h)	Concentration (mg/m^2)
OXA	2	85
LEU	2	400
IRI	0.4	180
5FU	48	400
OXA-LEU	4	85 – 400
OXA-LEU-5FU	52	85 – 400 – 400
OXA-5FU	50	85 – 400
FOLFIRINOX	52.4	85 – 400 – 180 – 400

2.3. Cell viability

The PANC-1 cell line was grown in DMEM medium supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin-streptomycin at 37 ℃ in a 5% CO_2 atmosphere. The drugs were applied to the cells in a 96-well plate (10^5 cells/well) at various concentrations (Table 1). After 24 h of treatment, the cell medium was exchanged with MTT solution, and the plates were incubated for 4 hours at 37 ℃. The formed formazan crystals were dissolved with dimethylsulfoxide, and optical densities were measured at 590 nm using a plate reader (Biotek, Synergy). The serum-free medium was used as the negative control.

2.4. Propidium iodide – Hoechst 33342 Staining

Hoechst 33342 (2 µg/mL) and PI (1 µg/mL) were dissolved in PBS. The cells were incubated with the solutions for 30 minutes at 37 ℃, respectively [9]. After PBS washing, well images were captured using the Operetta CLS High Content Analysis System. In the experiment, apoptotic and total cells were counted from 3 different images and analyzed following the formula:

$$\text{Apoptotic index } \% = \frac{\text{number of apoptotic cells}}{\text{number of total cells}} \times 100$$

2.5. Statistical Analyses

This experiment was performed in triplicate, and negative control was used in the experiment. Results are presented as mean (standard deviation) (n=3). Student t-test and the one-way analysis of variance (ANOVA, Microsoft Excel) was used for statistical analyses.

3. Results and Discussion

3.1. Results

In the FOLFIRINOX treatment, OXA, LEU, IRI, and 5FU are intravenously administered for different dosages and application times as shown Table 1, respectively. However, the drug amount and application time have not affected cell death for individual OXA, LEU, and IRI applications. The application time of individual 5FU on the cell line may have increased cellular toxicity. On the other hand, for the combined applications, synergetic effects of the drugs have been observed on the cell line (Figure 1).

Treatment with 5FU is more toxic towards PANC-1 than OXA, LEU, and IRI ($p<0.0005$). OXA-LEU combination demonstrated a less toxic effect on the cancer cell compared to different combination scenarios. Additionally, the OXA-LEU-5FU combination shows a statistically similar toxic effect with individual 5FU and OXA-5FU combination ($p>0.05$). The FOLFIRINOX application caused the highest toxic profile for the cells.

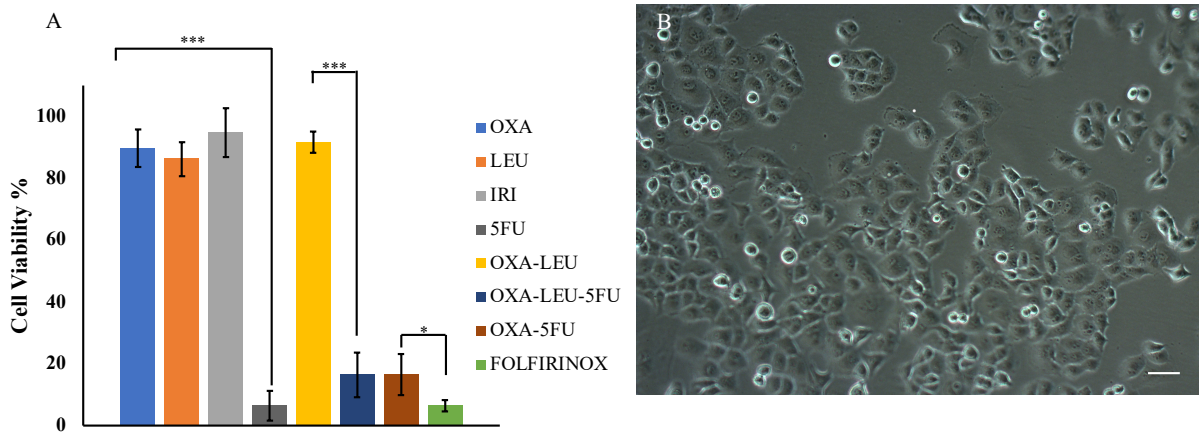

Figure 1: PANC-1 cell line viability analysis for the composition of FOLFIRINOX, (*$p<0.05$, ***$p<0.005$) (A), and optical microscope images of PANC-1 cell line before drug treatments (B)

Since Hoechst 33342 can readily pass cell membranes to dye DNA of living and dead cells, it was used to determine the number of total cells. In contrast, PI was only used to detect dead cells selectively via entering compromised plasma membranes. The analysis demonstrated that cellular

disruption was the main driver for the apoptosis results (Fig. 2). For OXA-treated group, the apoptotic index was consistent with the cell viability percentage (89.72% ± 6.14) which is one of the highest values among the samples, and the apoptosis trend for the sample (83.08% ± 3.71) is statistically different from the control group (67.14% ± 1.90, p=0.026). It is indicated that OXA is not toxic for the scenario but leads to early apoptosis of the cells. The same results were observed in the other individual drug applications except for the 5FU samples since application time (48 h) is significantly higher than the other individual drug applications.

The apoptotic trend was found similar to the control group for the drug combinations (OXA-LEU, OXA-LEU-5FU, OXA-5FU). While, the number of cells was decreased (Fig. 2. E-G) when compared to the control group (Fig. 2. I).

The combinations, with the exception of OXA-LEU, had a negative impact on cellular attachment since they were more cytotoxic. Moreover, FOLFIRINOX combination treatment was the most toxic on PANC-1 cells compared to the other drug combinations (6.53% ± 1.79%), but the same as the programmed cell death in the control group (p = 0.31).

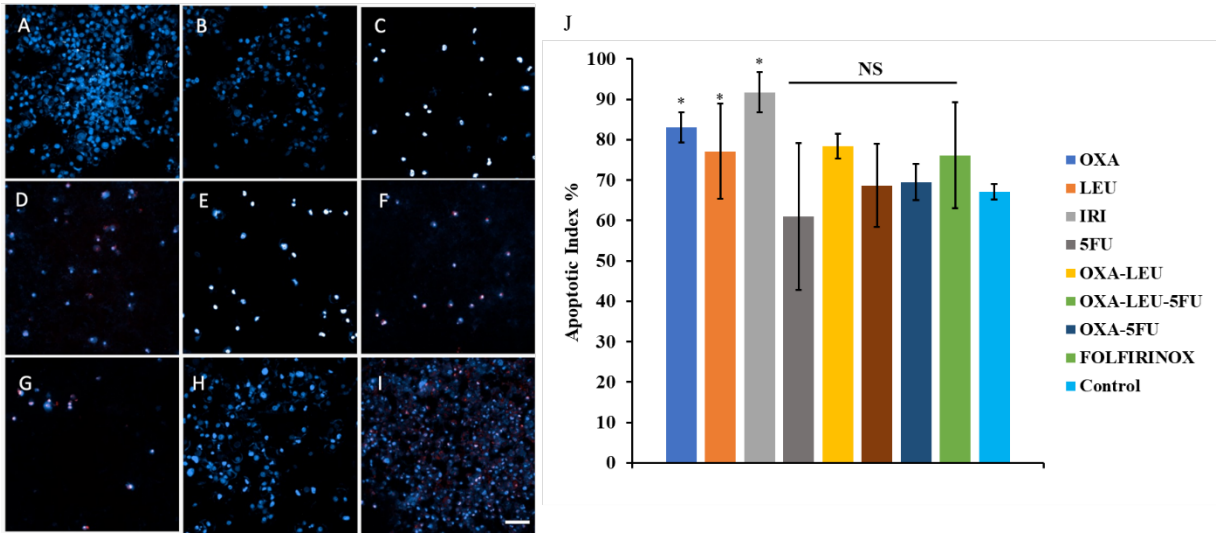

Figure 2: PI - Hoechst 33342 staining images of OXA (A), LEU (B), IRI (C), 5FU (D), OXA-LEU (E), OXA-LEU-5FU (F), OXA-5FU (G), FOLFIRINOX (H), and control (I) groups. Blue indicates Hoechst 33342 staining to determine the number of total cells, while red-pink color, sourced from PI, is selectively detected in apoptotic cells. (J) Apoptotic index values of each drug treatment (*p < 0.05, NS: Not Significant)

3.2. Discussion

Although, FOLFIRINOX therapy is currently in the clinical stage for pancreatic cancer, this study found that 5FU alone was comparable to FOLFIRINOX, based on the cell viability analysis. Our cell viability results indicated that 5FU treated samples have substantial cytotoxic effects compared to other 5FU-free treatments. But, the combination of all drugs caused the highest cellular death. It could be due to the synergetic interaction between the drugs, which has previously been described in the treatment of colorectal cancer [4]. However, the controllable cell death profiles of the samples

demonstrated that there is no significant difference between the 5FU-treated samples and FOLFIRINOX-treated samples. The results also support that localized 5FU treatment can be an option for nonresectable tumors.

The PANC-1 cell line was widely used as a PA model in many studies to investigate drug cytotoxicity and disease pathogenesis. In fact, Ma et al. reported that FOLFIRINOX treatment has no significant effect on PANC-1 migration, while gemcitabine exerts anti-proliferative effect on the cell line [10]. It shows that although FOLFIRINOX treatment appears to improve the survival rate of patients with PA, it still requires some tweaking. In our study, the apoptotic index results also supported the findings in the literature since there is no significant difference between the controllable death profile of FOLFIRINOX applied and PBS applied tumor cells.

FOLFIRINOX treatment in a real scenario could be used in localized therapies and with targeted carrier technologies to reduce the intense side effects of the treatment and increase efficiency on PA lesions. For instance, Byrne et al. have used the iontophoretic device model to explain the effectiveness of the local FOLFIRINOX treatment on a mouse model. The study results demonstrated iontophoretic release has promising results for reducing tumor volume compared to standard FOLFIRINOX treatment. However, cell selectivity remains an unanswered question for this study [11]. Alternatively, liposome-based solutions may help to target and encapsulate the drugs since the drugs are mainly water-soluble; however, further experiments are needed to combine the FOLFIRINOX and localized drug carriers.

Although the lipid-based systems have various drawbacks concerning stability and prolonged circulation time in the bloodstream, the systems can be adapted to the localized tumor treatment with a cancer-specific recognition layer. In this way, systemic toxicity can be controlled, and the localized chemotherapy applications may prevent adversely remodeling of the tumor microenvironment, which can cause chemoresistance. Therefore, the local response of the drugs used is important at the cellular level. At the same time, tumor microenvironment designs and personalized drug applications are the main indicators of such designs.

4. Conclusion

Our findings indicate that 5FU and FOLFIRINOX are comparably effective in reducing the PANC-1 cell viability. Moreover, OXA, LEU, and IRI significantly increase apoptotic cell death, whereas 5FU and FOLFIRINOX do not. The findings suggest that the drug combinations can be an option for unresectable nonmetastatic diseases. The local delivery application can be transformed into a locally targeted therapy. The model can also be adaptable for other metastatic tumors.

5. Ethical Declarations

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the paper's design, execution, and analysis and that they have approved the final version.

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