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Original paper

The Cytotoxic Effects of 5FU on Hek293 and HeLa Cells *in vitro*

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Abstract

Background and aim: 5-Fluorouracil (5FU) is one of the most commonly used drugs in chemotherapy for cancer treatment. In combination with other cancer drugs, it is used to treat many types of cancer including breast, colon, stomach and cervix cancer. However, treatment with 5FU is followed by serious side effects on healthy non-cancerous cells. The aim of this study was to investigate the cytotoxic effects of 5FU on cervical cancer (Hela) cells compared to non-cancerous (Hek293) cells.

Materials and methods: In this experimental-laboratory study, HeLa and Hek293 cells were divided into control group (no- treated) and groups treated with 3.90625, 7.8125, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml of 5FU. MTT assay method was used to evaluate the cell viability.

Results: The findings showed that 3.90625, 7.8125 and 15.625 µg/ml of 5FU did not significantly change cell viability, however, 31.25, 62.5, 125, 250 and 500 µg/ml of 5FU significantly decreased the Hek293 cell viability. Treatment of HeLa cells with 3.90625 and 7.8125 µg/ml of 5FU did not significantly change the cell viability, while HeLa cells treated with 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml of 5FU showed a significant decrease in cell viability.

Conclusion: In conclusion, high doses of 5FU has the same cytotoxic effects on HeLa and Hek293 cells, however, there was a narrow difference between 5FU cytotoxic effects on HeLa cancer cells compared to non-cancerous in lower doses indicating that 5FU concentration play a significant role in its cytotoxic action on healthy cells.

Keywords: *5FU, Cytotoxic effect, HeLa, Hek293*

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Introduction

Cervical cancer, known as the fourth most common cancer in women, is a malignant tumor that develops in the cervix. In 2018, approximately 569,000 new cases of cervical cancer were diagnosed worldwide, and 311,000 deaths were attributed to cervical cancer. Of these, between 84 and 90 percent occurred in low- and middle-income countries such as South Africa, India, China, and Brazil. The most common cause of cervical cancer is persistent infection caused by human papillomavirus, which is sexually transmitted. Other factors that contribute to the incidence of cervical cancer include geography, traditional practices and beliefs, levels of screening, socioeconomic status, access to health care, public awareness, use of oral contraceptives, smoking, dysregulation [1], [2], [3]. One of the ways to prevent cervical cancer is the use of prophylactic vaccination [4].

The treatment of cervical cancer depends on several factors, including the type and stage of cancer, possible side effects, and the patient's preferences and overall health. Different methods have been investigated to treat cervical cancer including radiotherapy, surgery [5], [6], [7], taking melatonin [8], [9], and using bile acid complexes with metals such as platinum, zinc, nickel and copper [10]. However, the use of chemotherapy with 5FU is one of the most common methods of treating cervical cancer.

5FU is a key anticancer drug that has been used to treat a variety of malignancies due to its broad antitumor activity as well as in combination with other anticancer drugs [11]. Cancer stem cells (CSCs) are a small population of cancer cells that undergo continuous self-renewal, differentiate into heterogeneous cells, and develop tumor-initiating potential. For this reason, the presence of CSCs allows the recurrence of tumors treated with chemotherapy. 5FU is an approach to overcome chemotherapy failure by preventing tumor recurrence [12]. 5FU is also used in the treatment of many types of cancer, including breast, liver, and colon cancer [13], [14]. Chemotherapy with 5FU has complications such as heart failure and neutropenia, intestinal mucositis, renal, hepatotoxicity, and reproductive organs [15], [16], [17], [18].

Considering that the use of chemotherapy can damage healthy and non-cancerous cells, the present study examines the effects of different doses of 5FU on cervical cancer cells compared to healthy non-cancerous cells.

Material and Methods

Drug and Cells Preparation

Fresh solution of 5FU was prepared by dissolving 5FU powder in DMSO and different concentrations (3.90625, 7.8125, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml) were prepared. Hek293 and HeLa cells were purchased from Pasteur Institute (Tehran, Iran).

Cell Culture

Cell culture was performed using routine cell culture method. In brief, the medium was removed from a confluent cell culture. 10 mL of PBS was added to wash off any additional medium or non-adherent cells. The PBS was substituted with 4 mL of 0.25% Trypsin-EDTA 1x solution and incubate at 37 ^{o}C and 5% CO2 for 5 min. 8 mL of DMEM medium was supplemented with 1% (w/v) streptomycin, 1% (w/v) penicillin and 10% (w/v) FBS and aspirate multiple times to resuspend all the trypsinized cells in the medium and avoid cell agglomeration. 10 uL of the culture was mixed with 10 uL of Trypan Blue 0.4% dye and add 10 uL of the mixture into a cell-counter slide.The living cell concentration was measured. 10^6 of living cells were transferred from the old culture into a 50 mL cell culture flask. DMEM medium was supplemented with 1% (w/v) streptomycin, 1% (w/v) penicillin and 10% (w/v) FBS, up to a final volume of 25 mL and was

incubated at incubate at 37 ºC and 5% CO2 until the cells become confluent.

MTT Assay

MTT assay was used to measure the cytotoxic effects of 5FU on Hek293 and HeLa cells. Briefly, media was discarded from cell cultures. 50 µL of serum-free media and 50 µL of MTT solution were added into each well. The plate was incubated at 37°C for 3 hours. After incubation, 150 µL of MTT solvent was added into each well. Plate was wrapped in foil and shaken on an orbital shaker for 15 minutes. The absorbance was read at OD=590 nm.

Statistical Analysis

SPSS20 software was used for statistical analysis. Kolmogorov-Smirnov test was performed to ensure the normal distribution of data, which was followed by one-way analysis of variance (ANOVA) and Tukey post hoc test. P values less than 0.05 were considered statistically significant.

Results

The results of MTT test showed that viability of Hek293 cell cells did not significantly change when treated with 3.90625, 7.8125 and 15.625 µg/ml of 5FU compared to control group. However, treatment of Hek293 cells with 31.25, 62.5, 125, 250 and 500 µg/ml of 5FU resulted in significant decrease in cell viability ($p<0.05$, $p<0.01$, $p<0.001$, $p<0.001$ and $p<0.001$, respectively (Figure 1).

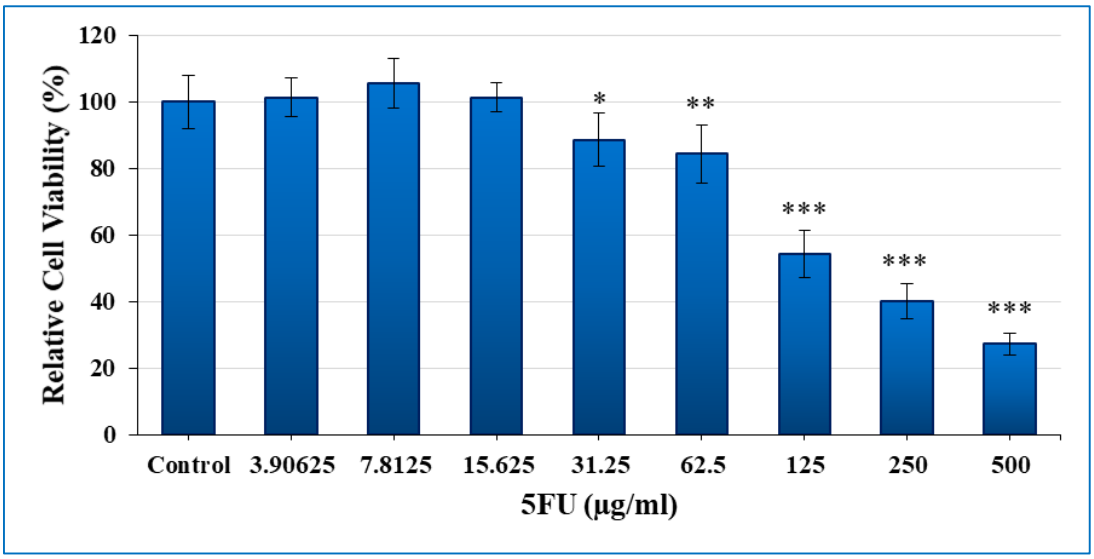

Figure 1. Viability of Hek293 cells treated with different concentrations of 5FU (µg/ml). * indicates significant difference compared with control group (*:$p<0.05$, **:$p<0.01$, ***:$p<0.001$).

Treatment of HeLa cells with 3.90625 and 7.8125 µg/ml of 5FU did not significantly effect on cell viability compared to control group; However, the viability in HeLA cells significantly decreased when treated with 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml of 5FU compared with control group ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively) (Figure 2).

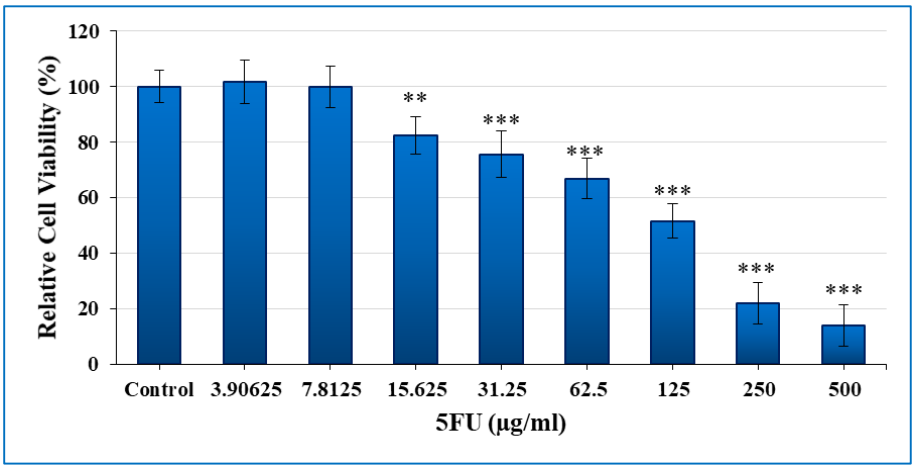

Figure 2. Viability of HeLa cells treated with different concentrations of 5FU (µg/ml). * indicates significant difference compared with control group (**:p<0.01 , ***:p<0.001).

Discussion

Although many studies have shown that 5FU is used as a drug in chemotherapy for cancer treatment, the effect of 5FU on cancer cells and its side effects on normal body cells is still a challenging issue. The results of this study show that 5FU in lower doses did not have a cytotoxic effect on non-cancerous cells, however can destroy cervical cancer cells. High doses of 5FU can have a cytotoxic effect on non-cancerous cells in addition to cervical cancer cells.

Studies show that 5FU interferes with the function of RNA and DNA in two ways, which are 1- the combination of active metabolites directly in nucleic acids, 2- inhibition of thymidylate synthase, which both mechanisms lead to a profound effect on cell metabolism [17]. In addition to these mechanisms, 5FU causes cell death by producing mitochondrial ROS in the P53-dependent pathway and apoptosis through the activation of caspases 1, 3, and 8 [19]. In liver and colon cancer which are incurable, the combination of 5FU with topoisomerase inhibitor induces apoptosis and causes cell death [14]. 5FU increases superoxide production and caspase 3 activation in cervical cancer cells, which results in apoptosis and cell death [19]. In fact, clinical studies have shown that high doses of 5FU can have destructive effects on normal cells of the body, especially reproductive cells, and for this reason, during chemotherapy, certain drugs are used in combination with 5FU, which reduce the destructive effect of 5FU on normal cells to protect the reproductive system in men and women [20], [17].

Conclusion

Overall, the results of this study show that 5FU in lower doses has a cytotoxic effect on cancer cells, but does not have cytotoxic effects on non-cancerous cells. In contrast, high doses of 5FU can have a cytotoxic effect not only on cancer cells but also on non-cancerous cells.

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Conflict of interests

The authors have no conflicts of interests to declare.

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