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Research Article

The Role of Folic Acid on PC3 Prostate Cancer Cell Line

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Abstract

Aim: Prostate cancer (PCa), one of the most common malignant solid tumors, has become a significant and rapidly increasing global health concern for men. One of the vitamins in the B group that is essential in decreasing the risk of cancer is folic acid (FA). However, the protective effects of FA against PCa are insufficiently examined, and the underlying mechanism is still unknown. In this study, androgen-nonresponsive (PC3) human PCa was used to get a better understanding of the effect of FA on cell proliferation.
Material and Method: In the present study, the MTT assay was used to assess FA's inhibitory effect on cellular proliferation. Additionally, all groups underwent the TUNEL immunofluorescence staining procedure to identify apoptosis in the PC3 cell line.
Results: The most appropriate cytotoxic dose was determined to be the 24-hour FA values. When apoptotic TUNEL staining was evaluated in the PC3 cell line, FA significantly increased apoptosis. There was not a significant difference observed between the docetaxel (Dtx) and FA groups in terms of TUNEL-positive cell immunoreactivity in the PC3 cell line. There was no apparent distinction in the immunreactivity intensity of TUNEL-positive cells in these groups.
Conclusion: The present study provides a fresh perspective on the fundamental mechanism underlying FA's capability to prevent PC3 cancer cells from proliferating. Our findings suggest that FA effectively inhibits PC3 cell line proliferation through the upregulation of apoptosis. Consequently, FA may be a potential novel cytotoxic and therapeutic strategy in the treatment of PCa disease.

Keywords: Folic acid, prostate cancer, docetaxel, apoptosis

INTRODUCTION

Cancer (an abnormal and uncontrolled proliferation of cells) is one of the world's leading causes of death. The World Health Organization (WHO) predicts that 11 million people receive a cancer diagnosis each year and that 7 million people die due to cancer-related causes (1). As a result, cancer is currently one of the deadliest diseases, and over the past few decades, advances in medical technology have produced a variety of options for treating cancer (2). Traditional chemotherapy has several limitations, including high toxicity, ineffective tumor-specific delivery, and the potential to cause multi-drug resistance (3). Prostate cancer (PCa) continues to be the cancer that is most prevalent in men worldwide and the third-largest cause of cancer-related mortality (4). A PCa survey conducted in 2019 founded that there were about 1,64,690 new cases and 29,430 deaths in the US. However, hormonal therapy, chemotherapy, radiation therapy, and radical prostatectomy (surgery) were the usual methods utilized to treat PCa. Cancer patients experience a variety of side effects from their treatment, including decreased sexual desire, incontinence, infertility, and hormone-based side effects (5). The high mortality rate among PCa patients is the primary cause of the early detection of PCa. Therefore, to increase patient survival rates, it is essential to develop rapid and early diagnostic strategies for PCa.

One crucial water-soluble B vitamin is folate, which can be found in a variety of foods, including legumes, fruit juices, and green leafy vegetables. Naturally occurring and non-immunogenic, folic acid (FA) has a small molecular

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weight. It is transformed into folate within the body, which has a strong affinity for the receptor (6). FA is the synthetic form of this vitamin that is typically found in foods that have been fortified, particularly grains and cereals, as well as supplements. While FA is the oxidized form of folate that contains a single glutamate moiety and is easily bioavailable, folate in the diet is present in a reduced form with side chains that contain multiple glutamate residues and must be absorbed through either oxidation or hydrolysis. Dietary folate has a bioavailability that varies from 10 to 98% (7). Due to its involvement in both nucleotide synthesis and methylation, folate has been the subject of much research (8). FA is a cheaply non-immunogenic chemical that functions as a great targeted ligand in a variety of cancer cells, including those from the brain, ovary, prostate, breast, lung, and colorectal cancer (9). Studies have indicated that FA-targeting nanodrugs have promising potential applications in a range of cancer types. FA is a high-affinity medication that targets the folate receptor (10). It has been found that some cancers, such as neoplasms of the colon, cervix, lung, esophageal, and pancreatic, were positively correlated with lower dietary FA consumption and low circulatory folate status (11).

Within the taxoid family, docetaxel (Dtx) is a semisynthetic anticancer mitotic ("antineoplastic" or "cytotoxic") chemotherapy drug. According to Rivero-Buceta (2019) (12), patients with hormone-refractory metastatic PCa are advised to consider Dtx as an optional treatment option. It has been established by many studies that Dtx binds to β-tubulin, interfering with the microtubules' normal polymerization dynamics, dividing cells during mitosis, connecting to microtubules, and inducing apoptosis (13). Drug resistance, however, is defined as a decrease in the effects of chemotherapy drugs such as Dtx. In this context, one of the main obstacles to successful chemotherapy is considered to be drug resistance.

Here are some indications of the cytotoxicity and apoptotic effects of FA. Overall, we hypothesized that treating the PC3-PCa cell line with FA reduces cell proliferation by inhibiting apoptosis. The aim of the current study is to determine whether FA increases the cytotoxicity of the PC3-PCa cell line in order to test the hypothesis. Future patient survival rates would be significantly impacted by the development of efficient Dtx chemotherapy, which would have a considerable impact on patient survival rates in the future.

MATERIAL AND METHOD

The study does not require ethics committee approval.

Cell Line

The androgen-independent human prostate cell line PC3 (ATCC. CRL1435TM) was utilized. RPMI-1640 (Thermo Fisher Scientific, USA) medium was used to grow the cells. To make complete media, 10% fetal bovine serum (Thermo Scientific, USA), 1% L-glutamine (Thermo Scientific, USA), and 1% penicillin and streptomycin (Thermo Scientific, USA) were added to RPMI.

Cell Viability Assay

FA (Merck, Germany) was diluted in dimethyl sulfoxide (DMSO, Sigma, MO, USA) (14). 1 mM stock solution was made. The Dtx (160 mg/8 mL, Koçak Farma, Istanbul) was supplied in liquid form. The MTT assay was used to evaluate the cell proliferation as previously described (15). Following cell culture, PC3 cells were treated with FA at doses of 1, 10, 100 µM and 1 mM or Dtx at doses of 1, 4, 16, and 64 nM. For every concentration, four duplicate samples were analyzed. The IC50 values of FA and Dtx were calculated statistically.

Cell Apoptosis Assay

Cells were seeded in 12-well plates at a density of $5x10^5$ cells per well. Then, cells were incubated with the IC50 dose of FA or Dtx. The ApopTag Fluorescein In Situ Apoptosis Detection Kit (EMD Millipore, Darmstadt, Germany) was used as previously described (16). To observe cells, an Olympus BX51 fluorescent microscope (Tokyo, Japan) was used to evaluate preparations. Ten different fields of view in total were chosen at random for quantitative analysis, and the immunoreactivity of TUNEL-positive cells was measured with Image J software (Bethesda, USA).

Statistical Analysis

The Graph-Pad Prism 9.4.1 program (San Diego, CA, USA) was used for the statistical analysis. The data's conformance to the normal distribution was assessed using the Kolmogorov-Smirnov test. To examine comparisons between two groups, a one-way ANOVA was used, while for comparisons among multiple groups, Bonferroni's multiple comparisons test was used. The data is shown as the mean±standard deviation.

RESULTS

Effects of FA and Dtx on the growth of PC3 human PCa cells: Following treatment for 24, 48, and 72 h, the FA's and Dtx's inhibitory effects on the PC3 PCa cell lines were evaluated by an MTT assay. Cell viability in the PC3 cell lines decreased with dose and the passage of time upon treatment with FA or Dtx. The IC50 values of the effect of FA or Dtx on PC3 cells were 116.8 mM and 3.69 nM at 24 h, respectively (Figures 1 and 2). These FA and Dtx concentrations were used for further investigations based on this dose-effect relationship.

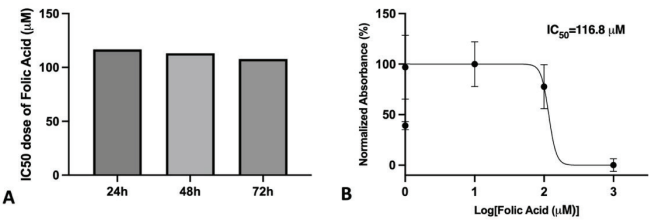

Figure 1. 1A. Change in FA IC50 dose depending on time. **1B.** Dose response curves for FA (24h)

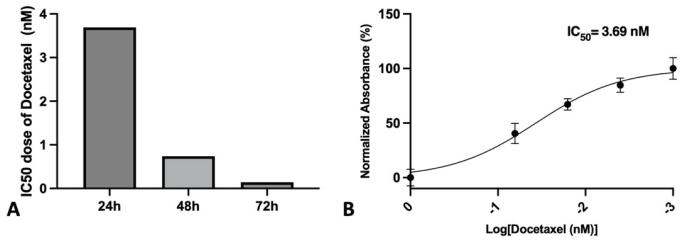

Figure 2. 2A. Change in Dtx IC50 dose depending on time. **2B.** Dose response curves for Dtx (24h)

Effects of FA and Dtx on the apoptosis of PC3 human PCa cells: To examine the mechanism underlying the cell death effects of FA and Dtx, the TUNEL assay was performed. As shown in Figure 3, apoptosis was triggered by FA and Dtx in PC3 cells. After FA and Dtx treatment, nuclear fragmentation was detected in PC3 cells. Compared with the control, treatment with FA significantly increased the proportion of TUNEL-positive apoptotic cells (p=0.0005). There was a noticeable increase in TUNEL-positive apoptotic cells in PC3 cells when comparing the Dtx group to the control group (p<0.001). There was an increase in TUNEL positive apoptotic cells in the Dtx group compared to the FA group. But this was not statistically significant (p<0.001) (Table 1).

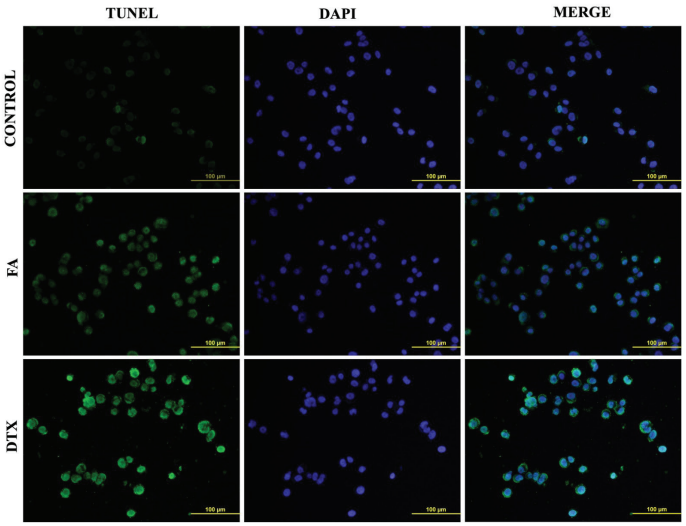

Figure 3. TUNEL images of the PC-3 cell line treated with FA and Dtx. The apoptotic bodies released in the cells were detectable under the fluorescent microscope as green reflections

Table 1. TUNEL statistical analysis results of PC-3 human PCa cell lines treated with FA or Dtx

	Control	FA	Dtx	p
Immunoreactivity intensities of TUNEL	(1.78± 0.38)[a]	(3.35± 0.77)[b]	(3.95± 0.49)[b]	<0.0001

Data are expressed as mean ± st. deviation. p: refers to the significance of the difference between the groups. The same lowercase letters in the same row show similarity between groups, and different lowercase letters indicate differences between groups. P value of <0.05 was used for significance

DISCUSSION

It is especially crucial to consume exogenous folates, such as FA, in order to replenish the intracellular folate pool in rapidly dividing cells, such as cancer cells (17). Thus, it's critical to ascertain whether FA causes or prevents cell damage. This study examined how FA administration's targeted mechanism affected the PC3 PCa cell line's capacity to undergo apoptosis. Few epidemiological studies examined the impact of dietary folic acid or folate on the incidence of prostate cancer. Moreover, taking the inconsistency of study designs into consideration, it comes as no surprise that their conclusions vary so dramatically. Therefore, with growing experimental evidence indicating that folic acid contributes to prostate progression, continued research is required to further delineate these complex relationships (18). We therefore turned to experimental models to glean any clues about the role of folate in prostate carcinogenesis.

One of the numerous cancers associated with chromosome rearrangement, DNA uracil mismatch, and epigenetic modification is PCa (19). Even though chemotherapy is frequently used to treat cancer, most chemotherapy drugs have harmful side effects and organ toxicity. The medications' nonselective distribution as well as poor pharmaco-bioavailability are the main causes of these issues. In order to overcome these limitations, it is essential to develop novel therapeutic systems that specifically target abnormal tissues while avoiding normal ones. Fruits, dairy products, and green leafy vegetables are common sources of FA, an essential B vitamin that plays a major role in immune response (20). Currently, the exact mechanism by which FA inhibits the proliferation of tumors is still unknown.

FA is a ligand that specifically targets cancer cells and has a strong affinity for the overexpressed folate receptors found in the epithelium of cancer cells. On the other hand, overexpression of different molecular receptors is typically associated with cancer cells (21). There is an overexpression of folate receptors in certain tumors, such as cancers of the colon, breast, ovary, lung, and prostate. Because non-cancerous cells lack folate receptors, FA is an incredibly attractive ligand (22). After attaching to the folate receptor, FA and folate conjugates form endosomes that allow them to enter cells (23). Recent research has confirmed that both folate receptors (FRα and FRβ) are expressed in pancreatic cancer (24). Additionally, reports have stated that PCa cells overexpress folate receptors (25).

It has been suggested that FA acts through the transfer of one-carbon units for methylation reactions and nucleotide biosynthesis through folate one-carbon metabolism. Consistent with structural and clinical observations, FA requires long-term supplementation to have a significant effect because nucleotide biosynthesis, DNA methylation, gene transcription, and expression all require sufficient reaction timescales in organisms (26).

Drug treatments for diseases will become possible once the mechanisms of apoptosis' cellular death are understood (27). A few research investigations have been carried out on the use of folate overexpression in treating PCa, despite the fact that it has been used to treat other forms of cancer (28). It was found that FA supplementation induced apoptosis in sentinel breast cancer cells by upregulating the expression of proapoptotic proteins (BAX, PARP). Nevertheless, FA supplementation was found to have no discernible impact on the expressions of PCNA, BCL-2, BCL-XL, and caspase-3 in the same study (29). When applied to MCF 7 and MDA-MB 23 lines, ß-lactoglobulin nanoparticles and FA loaded with doxorubicin significantly inhibited cell proliferation and induced apoptosis (30). Additionally, it examined how FA affected the up- and down-regulation of Bak1 and Bclx expression in MCF-7 cells. The results showed that a cisplatinum complex containing FA can considerably increase Bak1/Bclx ratios in contrast to cisplatin alone (31). Based on all of these findings, FA could be crucial in promoting apoptosis. Studies on the Bcl-2 protein family can provide a comprehensive understanding of the mitochondria-mediated apoptotic pathway, as they are the key regulators of apoptosis (32). Previous studies have shown that high-dose FA has an important effect on preventing gastric cancer (33). There have been no reports of FA's inhibitory effects on the PC3 PCa cell line as of yet. This is the first study that demonstrates that FA can effectively reduce proliferation, possibly by preventing the apoptotic pathway from being activated. When we evaluated our research in terms of apoptosis, we found that in PC3 cell lines, the FA group increased TUNEL-positive apoptotic cell numbers significantly more than the control group. Thus, it can be said that one useful step in the treatment of PCa may be the inclusion of targeting factors, including FA, in the complex structure.

CONCLUSION

Taken together, the study's findings showed that FA causes cytotoxicity in the PC3 cell line based on the MTT assay results. In PC3 PCa models, the FA group successfully increased the number of TUNEL-positive cells. Furthermore, the TUNEL assay clearly indicates that FA could potentially use in vitro apoptosis. In conclusion, despite being a necessary treatment option for PCa, chemotherapy presents challenges with regard to cost, efficacy, toxicity, and resistance. Additionally, the FA may be suggested as a promising addition to Dtx therapy, prolonging survival times in PCa models. Consequently, the findings indicate that the previously mentioned FA could prove beneficial for PCa treatment and monitoring with fewer adverse effects. Examining the mechanism underlying its impact on various tumor cells is suggested. The safety and efficacy of FA in PCa need to be determined through pre-clinical research in the field.

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Ethical approval: *The study does not require ethics committee approval.*

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