Medical Journal of Ahl al-Bayt University, Vol.3, No.1, Aug.2024

Evaluation of the effect of valproic acid on 5-fluorouracil cytotoxicity on colon cancer cells. An in vitro study

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ABSTRACT

Background: The disease known as colonic rectal cancer, which only affects the colon or rectum, is brought on by the aberrant proliferation of glandular epithelial cells in the colon. The three primary types of CRC are colitis-associated, hereditary, and sporadic. According to several research, family history, chronic inflammation, and dietary and lifestyle choices are risk factors for colorectal cancer (CRC) [1]. Colorectal cancer (CRC) accounts for 10% of cancer-related deaths, with ~1.4 million new cases and 693,900 deaths reported in 2012.

Materials and methods: colon cancer SW480 cells, Vero cells were incubated with different concentrations of valproic acid combined with IC50 of 5-fluorouracil then MTT assay, TNF *α*, NRF2, and casp.3 were assessed after 48 hours.

Results: valproic acid and 5-fluorouracil combination decreases the viability of SW480 colon cancer cell lines in a dose-dependent way, decreasing TNF-*α* level, NRF2, and casp.3 concentration.

Conclusion: Our results have shown that 5-fluorouracil and valproic acid inhibit the growth of colon cancer SW480 cell lines in a dose-dependent way and their combination induces a more cytotoxic effect decreasing TNF-α, NRF2, and decreasing casp.3.

Keywords: colon cancer, SW480 cells, Vero cells, valproic acid, 5-fluorouracil, TNF-α, NRF2, and casp.3.

Introduction

The disease known as colorectal cancer is limited to the colon or rectum and is brought on by the aberrant proliferation of glandular epithelial cells in the colon. The three primary types of CRC are colitis-associated, hereditary, and sporadic. Every day, there are a growing number of cases with CRC. The risk of developing CRC is influenced by both genetic and environmental factors. Moreover, older people with Crohn's disease and ulcerative colitis have a higher chance of developing CRC. Numerous studies have demonstrated that family history, chronic inflammation, and food and lifestyle are risk factors for colorectal cancer (CRC) [1]. One type of cancer that starts in the colon or rectum—a portion of the large

intestine in the gastrointestinal system—is called colorectal cancer (CRC). The survival rate of people with colorectal cancer has increased dramatically because to recent breakthroughs in diagnosis, chemotherapy, and biological agent-based therapy when combined with liver resection [2]. CRC is defined as a transformation of the normal colonic and rectal epithelium to a precancerous lesion (adenomatous intermediate) and, eventually, to an invasive carcinoma (adenocarcinoma). This can spread to various distant organs and give rise to metastatic lesions, with the liver being the most affected organ. CRC primarily consists of adenocarcinoma of the colon and rectum. Over a period of 10 to 15 years, this process necessitates the accumulation of genetic mutations that are either germline (inherited) or somatic (acquired) [3]. 5-Flurouracil is extensive usage in the treatment of several cancer kinds, such as those of the colon, rectum, anus, esophagus, pancreas, stomach, liver, breast, cervix, head and neck, thymus, and bladder; administered intravenously (IV) into a vein, typically in the arm, wrist, hand, or chest. Although 5-FU increases response rates and survival in head and neck and breast cancers when combined with other chemotherapeutic drugs, 5-FU has shown the biggest effects in colorectal cancer. Patients with stage III colorectal cancer that has been removed have better overall and disease-free survival while receiving 5-FU-based chemotherapy. However, just 10-15% of patients respond to 5-FU-based chemotherapy when it is used as a first line treatment for advanced colorectal cancer.

(Longley, Harkin, and Johnston, 2003). 5-FU is frequently used to treat a variety of malignancies, such as breast, colorectal, and aerodigestive tract tumors. 5-FU has had the biggest effect on colorectal cancer, even though it also increases response rates and survival in head and neck and breast malignancies when combined with other chemotherapeutic drugs[4].

Valproic acid (sodium valproate, VPA) is the most frequently used antiepileptic medicine in the treatment of generalized epilepsy, but it is also useful in partial epilepsy. Because of its broad range of activity against many forms of seizures and status epilepticus, VPA's antiepileptic action is most likely the result of a combination of numerous events in the brain[5] . Because of its ability to inhibit histone deacetylase (HDAC), recent research has investigated its usage as an adjuvant drug in cancer, HIV therapy, and neurological disease [6]. VPA's therapeutic responsibilities have expanded to encompass bipolar disorder and migraine prophylaxis, with more recently proposed applications in cancer, Alzheimer's disease, and HIV treatment[7]. After being exposed to VPA, the colon cancer cell line produced much less vascular endothelial growth factor (VEGF), and the expression of proteins and mRNA that codes for VEGF was also downregulated. After receiving 3 mM VPA for 48–72 hours, the highest possible inhibition rate was attained [8]**.** Through modification of cell cycle regulation, VPA exhibits anti-neoplastic efficacy in both in vitro and in vivo colorectal carcinoma cell lines. By influencing several processes, such as the cell cycle, apoptosis, angiogenesis, metastasis, differentiation, and senescence, VPA is known to have an associated anticancer impact. The degree of differentiation and related underlying genetic changes that vary throughout tumor entities influence these effects to some extent [9].

Materials and Methods

Cells:

The human colon cancer cell line (SW480), and Vero cells were acquired from the University of Babylon's College of Medicine's Tissue Culture Laboratory. The cells were cultured in RPMI 1640 medium (Capricorn, Germany) containing 10% (v/v) fetal bovine serum (Capricorn, Germany) and 1% Penicillin-Streptomycin (Capricorn, Germany) at $37C^0$ and passaged with Trypsin-EDTA (US biological, USA) every three days to maintain cells monolayer. The valproic acid solution from (Meryer company, China) and 5-fluorouracil (Pfizer company, USA) were used in this study.

Cytotoxicity assay

Cell Cytotoxicity was evaluated using MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide) (Roth, Germany). SW480 cells were cultured in 96 well plate with RPMI-1640 supplemented with 10 % FBS and 1% Penicillin-Streptomycin and incubated at 37 C^0 for 48 hr. After incubation, the medium was removed, and the cells were treated with different concentrations of valproic acid (31.25,62.5,125,250,500,1000) µg/ml combined with IC50 of 5-fluorouracil (43.87 µg/ml) and incubated for 48 hr. After incubation, 10 µl of MTT solution (5 mg/ml) was added, and the wells were incubated for three hours. The tetrazolium salts were transformed by the mitochondrial reductase enzyme in living cells into a colorful formazan product, which can be detected immediately (at 570 nm) with an absorbance plate reader.

Determination of TNF-α, NRF2, and total CASP.3 capacity

The anti-inflammatory, antioxidant effects and apoptosis were assessed by using TNF-α, NRF2, and CASP.3 capacity Elisa immunoassay kits (Elabscience, USA).

Depending on MTT results, SW480 cells were incubated with different concentrations of valproic acid (31.25,62.5,125,250,500,1000) µg/ml combined with IC50 of 5-fluorouracil (43.87 µg/ml) and incubated for 48hr. After incubation, the levels of TNF-α, NRF2, and CASP.3 capacity in the supernatant were measured by using immunoassay Elisa kits according to manufacture protocol.

Results

Cytotoxicity assays

As illustrated in Figure (1), Sw480 and Vero cells were treated with different concentrations of valproic acid (VAL) (Figure1a and d respectively). Valproic acid caused a significant ($p \leq 0.05$) decrease in cancer cell viability at concentrations ranging from (125 to 1000 µg/ml) compared to the control group. On normal cells valproic acid at high concentrations (1000, 500) µg/ml had significant ($p \leq 0.05$) cytotoxic effects- on normal cells compared to the control group after incubation for 48 hr, whereas at other concentrations there were no significant effects. 5-Fu is a Known anticancer drug that has a severe cytotoxic effect on colon cancer cells, it causes a dose-dependent cytotoxicity. The same, but less severe effect was seen on normal cells, (Figure1b and e respectively). The effect of (the valproic acid and

5-fluorouracil) combination caused a significant (p-value ≤0.05) decrease in the viability of the SW480 cell line at all concentrations used compared to negative control. In contrast, these combinations decreased cells viability at all concentrations, except for 31.125 µg/ml compared to the positive control group. Against Vero cells, these combinations significantly (p-value ≤0.05) decreased the viability of the Vero cell line (had a cytotoxic effect) at all concentrations used compared to the negative control. Only (1000 µg/ml) significantly (p-value ≤0.05) enhances the cytotoxicity of 5-Fu compared to the positive control group, (figure 1c and f respectively).

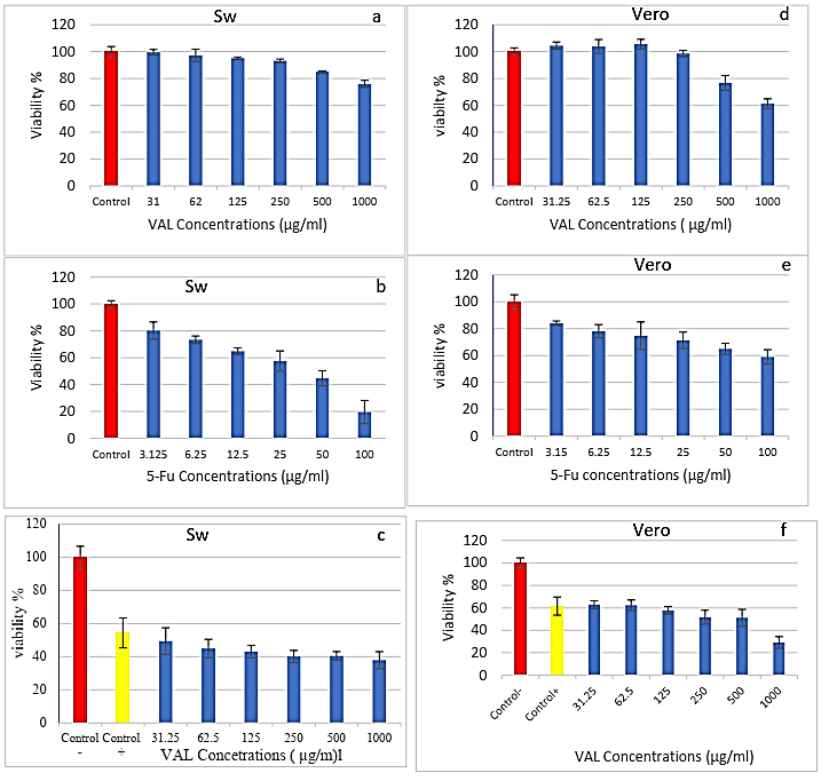

Figure 1: Cytotoxicity of valproic acid (VAL), 5-fluorouracil (5-fu), and Combinations of Valproic acid and 5-fluorouracil on SW480 colon cancer cell line (a, b, c respectively), and on vero cell line (d, e, f respectively). Cell viability was assessed using MTT assay after an incubation period of 48 hours with each material. Control; untreated cells, Control +; cells treated with 5-fu (43.87 µg/ml) only.

Biochemical assays

Effect of Valproic acid,5-Fluorouracil, combination (Valproic acid- 5-Fluorouracil) on TNF-α level.

In this part of our work, TNF-α level in SW480 colon cancer cells and Vero normal cells were estimated after the treatment with VAL, 5-Fu, or combinations of VAL and 5-Fu (figure 2). The results showed that the levels of pro-inflammatory cytokines TNF-α in the colon cancer cell line decreased significantly (p ≤0.05) in all concentrations in comparison to the control group. the levels of TNF-α decreased significantly in all concentrations with p values ≤0.05 in comparison with control in the Vero cells line after incubation of 48hrs.

Treatment with 5-fluorouracil decreased the levels of TNF-*α* level significantly in SW cells at all concentrations in comparison with the control group. Regarding Vero cells, the results showed that the levels of TNF-α did not change significantly ($p \leq 0.05$) at any concentration used in comparison with the control group.

Treatment of SW cells with a combination of (VAL-5-Fu) showed no significant effect on TNF-*α* levels at all concentrations in comparison with negative control. Compared to the positive control group, only (1000 µg/ml) showed a significant ($p \leq 0.05$) decrease in TNF-α level. Regarding Vero cells, a combination of (VAL-5-fu) showed no significant alteration in TNF-α levels compared to the untreated control group. On the other hand, this combination caused a significant ($P \leq 0.05$) decrease at the concentrations (500 and 1000 µg/ml) compared to the positive control group.

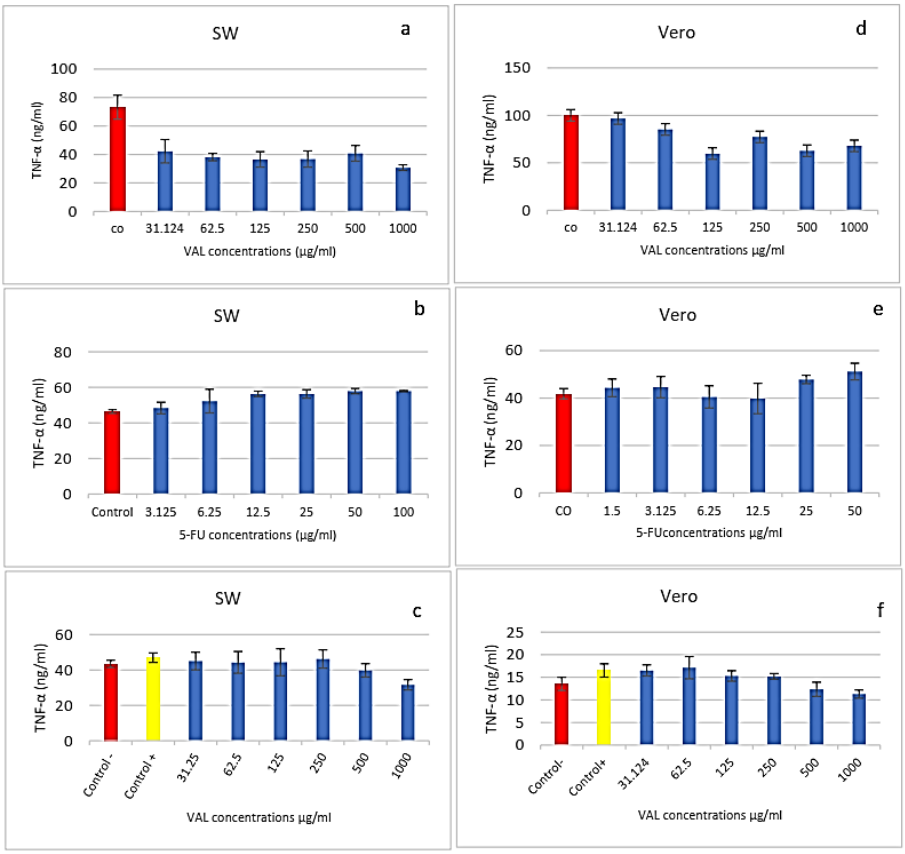

Figure 2: Effect of Valproic acid (VAl) on TNF-α, Effect of 5-Flurouracil(5-FU) on TNF-α, Effect of combination (VAL-5-FU) on TNF-α level in SW480 colon cancer cell line (a,b,c respectively), and on Vero cell line (d,e,f respectively). Cells pellets and supernatants were withdrawn after an incubation period of 48 hours with each material.

Effect of Valproic acid,5-Fluorouracil, combination (Valproic acid- 5-Fluorouracil) on NRF2 level

In this part of our work, NRF2 level in SW480 colon cancer cells and Vero normal cells were estimated after the treatment with VAL, 5-Fu, or combinations of VAL and 5-Fu (figure 3). The results showed a significant ($P \leq 0.05$) increase in NRF2 levels at (62.5 µg/ml) and a significant decrease at (1000 µg/ml) compared to the untreated control group. Regarding

Vero cells, treatment of Vero cells with valproic acid significantly (P≤0.05) increased NRF2 levels at concentrations of (31,62,125 µg/ml) compared to the untreated control group.

Treatment with 5-fluorouracil decreased the level of NRF2 level significantly in SW cells at all concentrations in comparison with the control group. Regarding Vero cells, caused a significant decrease in NRF2 levels only at the concentrations of (100µg/ml) compared to untreated cells.

Treatment of SW cells with a combination of (VAL-5-Fu) caused a significant (P≤0.05) decrease in NRF2 levels at all concentrations used in comparison to untreated cells. Compared to positive control, it caused a significant (P≤0.05) decrease in NRF2 levels at concentrations of (250,500,1000 µg/ml). Regarding Vero cells, treating the normal cell line with a combination of VA and 5-Fu significantly reduced NRF2 levels at all concentrations used when compared with untreated cells, but compared to the positive control group, only 500 and 1000 µg/ml caused significant (P≤0.05) reduction in NRF2 levels.

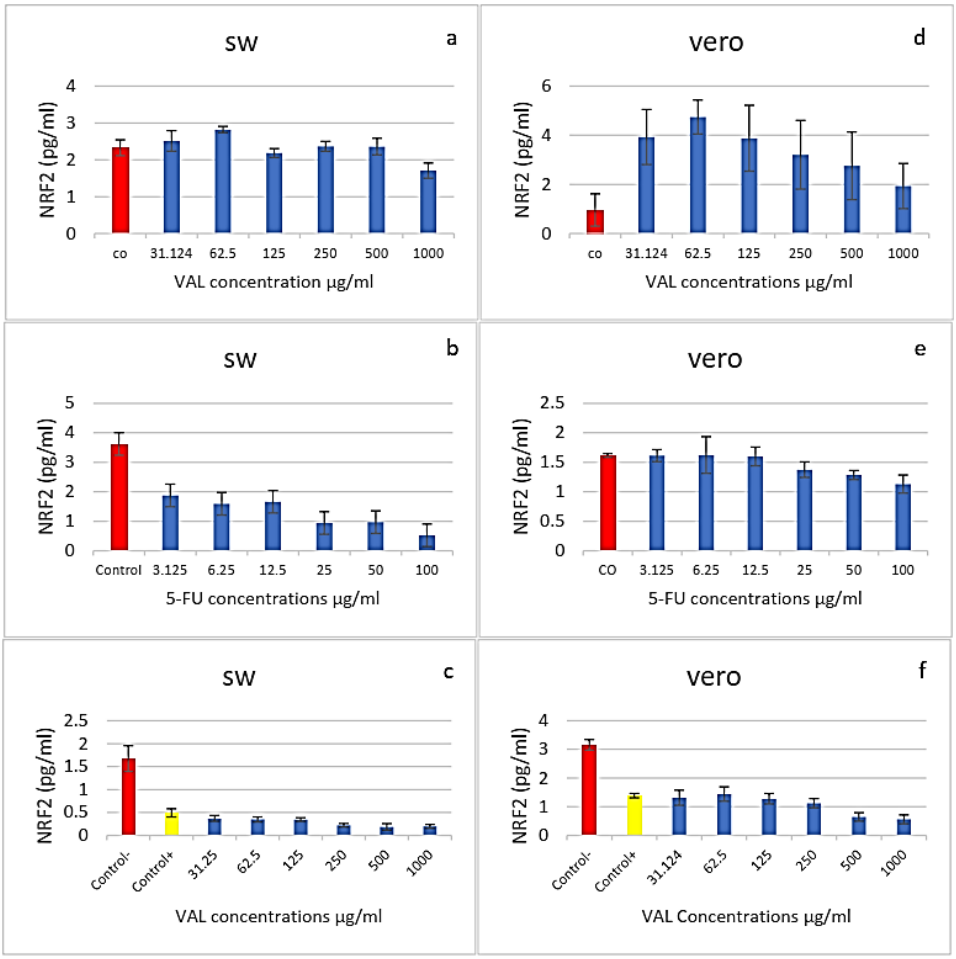

Figure 3: Effect of VAL on NRF2 level, Effect of 5-FU on NRF2 level, Effect of combination (VAL-5-FU) on NRF2 level in SW480 colon cancer cell line (a, b, c respectively), and on Vero cell line (d, e, f respectively). Cell pellets and supernatants were withdrawn after an incubation period of 48 hours with each material.

Effect of Valproic acid,5-Fluorouracil, combination (Valproic acid- 5-Fluorouracil) on CASP.3 level

In this part of our work, NRF2 level in SW480 colon cancer cells and Vero normal cells were estimated after the treatment with VAL, 5-Fu, or combinations of VAL and 5-Fu (figure 4). Colon cancer cells treated with valproic acid showed no significant alteration in Casp-3 levels at all concentrations evaluated compared to the control group. Regarding Vero cells, Statistically, there was no significant change in Casp-3 levels compared to untreated cells.

Treatment with 5-fluorouracil increased Casp-3 levels significantly ($P \leq 0.05$) at concentrations of 50 and 100 µg/ml compared to the control group. Regarding Vero cells, Statistically, there was no significant change in Casp-3 levels compared to untreated cells.

Treatment of SW cells with a combination of (VAL-5-Fu) caused a significant increase in Casp.3 levels after the treatment with 250 and 500 µg/ml compared to untreated cells. Also, there was a significant increase in Casp.3 levels after the treatment with 250,500 and 1000 µg/ml compared to the positive control group. Regarding Vero cells, Statistically, there was no significant change in Casp-3 levels compared to untreated cells.

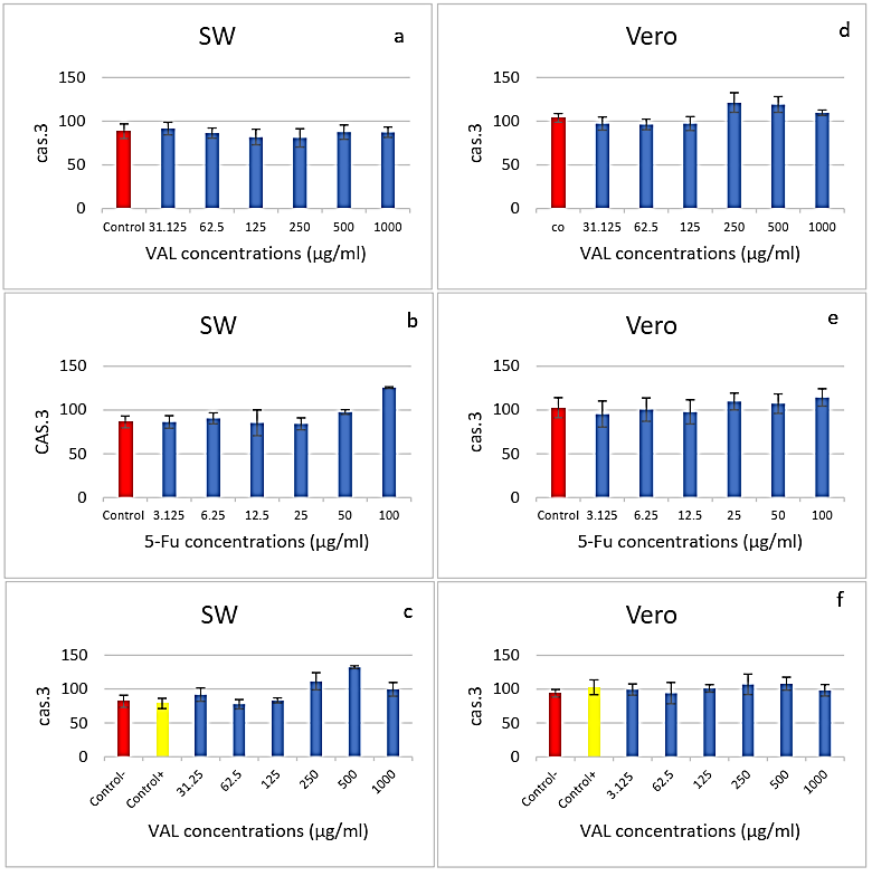

Figure 4: Effect of VAL on CASP.3 level, Effect of 5-FU on CASP.3 level, Effect of combination (VAL-5-FU) on CASP.3 level in SW480 colon cancer cell line (a,b,c respectively), and on Vero cell line (d,e,f respectively). Cell pellets and supernatants were withdrawn after an incubation period of 48 hours with each material.

Discussion

Cytotoxicity assays

Effects of valproic acid on the Viability of Colon Cancer SW480 and Vero Cells Line

In this study colon cancer sw480 Cells were treated with different concentrations of valproic acid. As a histone deacetylase inhibitor, valproic acid has been shown to mediate the cytotoxic effects against tumor cells. Heme deacetylase (HDAC) activity that is out of balance is a feature common to tumor cells.

Cancer cell growth inhibition and death are usually linked to suppression of HDAC activity. Interestingly, VPA exhibits both anticancer and HDAC inhibitory effects.10 Given its safe application as a long-term treatment for epileptic conditions, it may be a suitable option to incorporate into innovative anticancer regimens. Additionally, VPA has better pharmacokinetics for therapeutic application than other HDACi, with a significantly longer plasma half-life (7–9 hours in humans)[10]. VPA caused growth inhibition and programmed cell death that correlated with histone hyperacetylation. VPA modulated the expression of various factors involved in cell cycle control and apoptosis and induced caspase activation[11]. VPA was demonstrated to exert antitumor activity as an HDAC inhibitor [12]. Histone deacetylase inhibitors (HDACIs) have demonstrated in recent years to be potent inducers of drug-resistant subtypes of cancer cell growth arrest, differentiation, and apoptotic cell death of transformed cells. When used in conjunction with other anticancer drugs, they also prevent angiogenesis and make cancer cells more susceptible to overcoming drug resistance. [13]. HDACi have not demonstrated significant anticancer activity when used alone in solid tumors, such as colorectal cancer (CRC), but they do so more effectively when combined with biological agents, chemotherapy, or radiation [14].

Regarding Vero cells, in this study, Vero Cells were treated with different concentrations of valproic acid Valproic acid decreased Vero cells viability at high concentrations (500,1000) µg/ml.

Effects of 5-fluorouracil on the Viability of Colon Cancer SW480 and Vero Cell Line

In this study colon cancer sw480 Cells were treated with different concentrations of 5-fluorouracil.5-Fu decreased cancer cells viability at all concentrations compared with the control group. This agreement with the study that said 5-Fu has the ability to cause apoptosis, promote G0/G1 phase arrest, raise ROS levels, lower the potential of the mitochondrial membrane, and promote endoplasmic reticulum expansion[15]. To cause cytotoxicity in tumor cells, 5-Fu is changed into fluorodeoxyuridine monophosphate (FdUMP). FdUMP has the ability to miss-incorporate into DNA or RNA, suppress TS activity, and ultimately induce cell death [16]. this result agreed with previous studies which indicated that 5-Fu significantly inhibited the growth of SW480 and cells in a dose-dependent manner.

Regarding Vero cell, in this study, Vero Cells were treated with different concentrations of 5-FU.5-FU decreased Vero cells viability at all concentrations.

The Effects of (valproic acid - 5-fluorouracil) combination on the Viability of Colon Cancer SW48 Cell Line

The co-treatment of SW480 cells with valproic acid and 5-fluorouracil ameliorates the viability of cells compared with cells treated with 5-fluorouracil only. in their study, Venkataramani *et al.* find that VPA is a histone deacetylase (HDAC) inhibitor and affects cell growth in different types of cancer in vitro and in vivo[17].

In vitro and in vivo studies have demonstrated that inhibitors of histone deacetylase (HDAC) enhance the effectiveness of anticancer medications [18]. Iwahashi *et al.* found in their study that HDAC inhibitors are useful in cancer treatment when used in combination with current chemotherapeutic drugs, especially in combination with 5-FU, HDAC inhibitors enhance the effect of 5-FU in colorectal cancer cells and enhance the effect of 5-FU in non-small cell lung cancer [19].

Regarding Vero cell, we exposed vero cells to different concentrations of valproic acid combined with 5-fluorouracil IC50 and incubated for 48 hrs. After incubation, the effects of this combination in addition to 5-fluorouracil alone (positive control) on Vero cells were determined. The results showed a significant decrease in the viability of Vero cells at high concentrations (500,1000) µg/ml compared to the positive control group.

Immunological assay on SW480 cancer and Vero cells line

Effect of valproic acid on TNF-α, NRF2, and CASP.3 capacity in SW480 cancer and Vero cells line

Hoşgörler showed that in the irradiated tissue, VAP dramatically reduced apoptosis and insignificantly decreased the levels of TNF-α and IL-1β proteins. Inhibitors of matrix metalloproteinase (MMP) reduce inflammation in healthy tissues and slow the spread of cancer in healthy tissues. One MMP inhibitor that has radioprotective properties is valproic acid (VAP). Their ability to inhibit MMPs in irradiated tissue is unknown.VAP has anti-inflammatory effects, mediated by MMP-9 inhibition. VAP has anti-cancer effects, mediated by inhibition of MMP-2 and MMP-9[20]. this study agrees with our results that showed a decrease in TNF-α. while in Vero cells the effects of different concentrations of valproic acid on TNF-α in Vero cells at 48 hr of incubation shows a significant increase in TNF-α in concentrations (62.5- 1000) µg/ml used.

Regarding NRF2, the effects of different concentrations of valproic acid on NRF2 in SW480 cancer cells at 48 hr of incubation show a significant decrease in NRF2 in high concentrations used. A developing class of drugs known as histone deacetylase inhibitors (HDACi) targets the enzyme's role in chromatin structure, which controls gene expression. When compared to other HDAC inhibitors, valproic acid (VPA), an anti-epileptic medication with HDAC inhibitory activity, has a far superior safety record. HDACs have recently been found to participate in the DNA damage response and their down-regulation has been associated with impaired DNA repair[21].while in vero cells the effects of different concentrations of valproic acid on NRF2 in Vero cells at 48 hr of incubation shows valproic acid significant increase in NRF2 at all concentrations used.

Regarding CASP.3, the effects of different concentrations of valproic acid on casp.3 in SW480 cancer cells at 48 hr of incubation shows a significant increase in casps.3 in high concentrations used. Unbalanced histone deacetylase (HDAC) hyperactivity is a common feature of tumor cells. Inhibition of HDAC activity is often associated with cancer cell growth impairment and death. Valproic acid (VPA) is an HDAC inhibitor used for the treatment of epilepsy [22]. Histone deacetylase inhibitors (HDACIs) have been shown to have antiproliferative activity through cell-cycle arrest, differentiation, and apoptosis in colorectal cancer (CRC) cells. Application of VPA to the colon adenocarcinoma cell line not only led to a significant reduction of vascular endothelial growth factor (VEGF) secretion but also to a down-regulation of protein expression as well as VEGF coding mRNA[23]. While in Vero cells, the effects of different concentrations of valproic acid on CASP.3 in Vero cells at 48 hr of incubation shows a significant valproic acid increase in CASP.3 at concentrations (250,500,1000) µg/ml used.

Effect of 5-flurouracil on TNF-α, NRF2, and CASP.3 capacity

The effects of different concentrations of 5-FU on TNF-α in SW480 cancer cells at 48 hr of incubation shows a significant increase in TNF-α in all concentrations used. a study by Song and his colleagues in 2013 showed that TNF-α showed a 98% increased expression in the group treated with 200 µg/ml 5-FU compared to the control group(Song *et al.* 2013). While in Vero cell the results showed that different concentrations of 5-FU on TNF-α in Vero cells at 48 hr of incubation shows a significant increase in TNF-α in concentrations (50,100) µg/ml used.

Regarding NRF2, the effects of different concentrations of 5-FU on NRF2 in SW480 cancer cells at 48 hr of incubation show a significant decrease in NRF2 in all concentrations used. The NRF transcription factors control the functionality of the respiratory chain, the main pathway responsible for producing reactive oxygen species (ROS). ROS are extremely harmful to cellular micro molecules because they induce tumorigenesis through non-specific reactions with nucleic acids, proteins, and lipids. Furthermore, NRF2 protects tumor cells from oxidative stress, chemotherapeutic agents, and radiotherapy, promoting tumor genesis and progression, and also metabolic reprogramming to anabolic pathways[24]. Overexpression of NRF2 has been shown to accelerate the growth of colorectal cancers and raise the level of treatment resistance to chemotherapeutic medications like oxaliplatin and 5-fluorouracil. Thus, NRF2 suppression may also be beneficial for the treatment of colorectal cancer[25].these studies agree with our result. overexpression of NRF2 may also have negative effects, by creating the best environment for cell growth to promote the survival of normal cells and cancer cells, protect tumor cells from oxidative stress, chemotherapeutic drugs, and radiotherapy, and promote tumor development[26].while in Vero cells, the effects of different concentrations of 5-FU on NRF2 in Vero cells at 48 hr of incubation shows a significant 5-FU decrease in NRF2 at concentrations (25,50,100) µg/ml used.

Regarding CASP.3, the effects of different concentrations of 5-FU on casp.3 in SW480 cancer cells at 48 hr of incubation shows asignificant increase in casps.3 in high concentrations used. 5-Fluorouracil (5FU)-based chemotherapy (CT) remains the mainstay

treatment of CRC and activates executioner caspases in target cells. Executioner caspases are key proteins involved in cell disassembly during apoptosis. Caspase-3 targets structural substrates leading to cell disassembly and DNA fragmentation[27].while in Vero cells, the effects of different concentrations of 5-FU on CASP.3 in Vero cells at 48 hr of incubation shows a significant 5-FU increase in CASP.3 at concentrations (100) µg/ml used.

Effect of combination (5-flurouracil – valproic acid) on TNF-α, NRF2, and CASP.3 capacity in SW480 colonic cancer and Vero cells

In a current study, we exposed sw480 cancer cells to different concentrations of valproic acid combined with 5-fluorouracil IC50 and incubated for 48 hrs. After incubation, the effects of this combination in addition to 5-fluorouracil alone (positive control) on TNF level were determined. The results showed a significant decrease in TNF-alpha at all concentrations compared to the positive control group fig (3.13).TNF is a pro-inflammatory cytokine produced by numerous cell types [24]. While in Vero, exposed vero cells to different concentrations of valproic acid combined with 5-fluorouracil IC50 and incubated for 48 hrs. After incubation, the effects of this combination in addition to 5-fluorouracil alone (positive control) on TNF-α in Vero cells were determined. The results showed a significant decrease in TNF-α in Vero cells at concentrations (125,250,500,1000) µg/ml compared to the positive control group.

Regarding NRF2, in a current study, we exposed sw480 cancer cells to different concentrations of valproic acid combined with 5-fluorouracil IC50 and incubated for 48 hrs. After incubation, the effects of this combination in addition to 5-fluorouracil alone (positive control) on NRF2 level were determined. The results showed a highly significant decrease in NRF2 at all concentrations compared to the positive control group. the nuclear factor erythroid 2-related factor 2 (NRF2) plays a pivotal role in safeguarding cells against oxidative stress. This transcription factor regulates the expression of numerous genes, including antioxidant enzymes responsible for shielding cells from various oxidative alterations[28]. While in Vero cells exposed vero cells to different concentrations of valproic acid combined with 5-fluorouracil IC50 and incubated for 48 hrs. After incubation, the effects of this combination in addition to 5-fluorouracil alone (positive control) on NRF2 in Vero cells were determined. The results showed a significant decrease in NRF2 in Vero cells at concentrations (250,500,1000) µg/ml compared to the positive control group.

Regarding CASP.3, in a current study, we exposed sw480 cancer cells to different concentrations of valproic acid combined with 5-fluorouracil IC50 and incubated for 48 hrs. After incubation, the effects of this combination in addition to 5-fluorouracil alone (positive control) on Casp.3 level were determined. The results showed a highly significant decrease in Casp.3 at all concentrations compared to the positive control group.Valproic acid has been demonstrated to mediate cytotoxic effects against tumor cells by acting as a histone-deacetylase inhibitor. HDACs is associated with transcriptional repression. Consequently, HDAC inhibitors such as valproic acid and a number of other chemicals inhibit the respective transcriptional activities. [29]. By preventing colony formation or cellular viability, an HDAC inhibitor enhanced the anticancer effect of 5-fluorouracil (5-FU) in human colon cancer cell

SW48 cells. this mixture causes an increase in caspase-3/7 activation.[30].while in Vero cells, we exposed vero cells to different concentrations of valproic acid combined with 5-fluorouracil IC50 and incubated for 48 hrs. After incubation, the effects of this combination in addition to 5-fluorouracil alone (positive control) on casp.3 in Vero cells were determined. The results showed a significant increase in casp.3 in Vero cells at concentrations (250,1000) µg/ml used compared to the positive control group.

Conclusion: These results have shown that 5-fluorouracil and valproic acid inhibit the growth of colon cancer SW480 cell lines in a dose-dependent manner and their combination induces a more cytotoxic effect decreasing TNF-α, NRF2, and increase CASP.3 concentrations making valproic acid good candidate as an adjuvant for 5-flurouracil chemotherapy.

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