Original Article

Effect of Vitamin C Administration 24 Hours After 5-Fluorouracil Versus its Concomitant Administration on Cellular Proliferation, Cell Death and Lipid Peroxidation in WI-38 Human Fibroblast Cell Line

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ABSTRACT

Background: 5-Fluorouracil (5-FU) is a routine anticancer agent for several cancer types, its short half-life requires continuous administration in high doses. This results in toxic side-effects to the normal tissues in the body. Vitamin C is an antioxidant which believed to impart cyto-protection to normal tissues in cancer patients treated with chemotherapeutic drugs.

Aim of the Study: To assess the potential benefit of vitamin C administration 24 hours after 5-FU treatment compared to its immediate supplementation on normal fibroblast cell line.

Materials and Methods: Fibroblast cell line (WI-38) was divided into 5 groups. Group I not treated (control group), group II treated with 5-FU only and group III received vitamin C only. Groups IV and V received 5-FU with vitamin C simultaneously and after 24 hours of 5-FU administration, respectively. The viability of cells in all groups was determined using MTT assay. MDA Quantification Assay was used to determine lipid peroxidation. Flow cytometry was used for evaluation of the cell cycle and cell death. All recorded data was statistically analyzed.

Results: Cells in group IV seems like group II fewer and rounded while in group V some of them adherent spindle and the others rounded non-adherent. The least viability was noted in group II. MDA levels were significantly lower in groups III compared to all the other groups. Cell cycle analysis revealed the presence of statistically significant differences between groups in different phases of the cycle. Flowcytometry analysis showed the highest apoptosis in groups II and IV.

Conclusion: Co-treatment of normal fibroblasts with vitamin C along with 5-FU reduces cell death and lipid peroxidation while enhancing viability and proliferation in treated fibroblasts, indicating healing and repair. Vitamin C administration 24 hours after 5-FU is more effective in reducing cell death and lipid peroxidation compared to its concomitant administration with 5-FU.

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Key Words: 5 fluorouracil, fibroblast cell line, lipid peroxidation, ROS, vitamin C.

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INTRODUCTION

Not long after its primary synthesis in 1957, 5-Fluorouracil (5-FU) became a routine anticancer agent for several cancer types including colorectal and head and neck cancers. 5-FU, is often applied topically for the treatment of skin lesions like keratoacanthomas, actinic keratosis, hypertrophic scars, keloid, skin metastasis from a melanoma and superficial skin squamous cell carcinoma, as well[1].

The short half-life and poor membrane permeability require continuous administration of 5-FU in high doses to sustain the lowest acceptable therapeutic serum concentration. This commonly results in severe toxic side-effects to the normal tissues in the body. The pharmacological action of 5-FU is attributed to it being an antimetabolite[2,3]. Intracellularly 5-FU is converted to a number of active metabolites; fluorouridine triphosphate (FUTP), fluorodeoxyuridine triphosphate (FdUTP) and fluorodeoxyuridine monophosphate (FdUMP), these metabolites disturb RNA and DNA configuration and inhibit Thymidylate synthetase (TS). TS is involved in early stages of DNA biosynthesis during the S phase of the cell cycle. It is unique in the aspect that it's a "bottleneck" enzyme in TS and is the main mean by which a methyl group is inserted at the 5-position of pyrimidine[4]. DNA damage mediated via TS inhibition results in cell cycle arrest followed by signaling either for DNA repair or apoptosis. This protection mechanism to DNA damage is maintained in normal cells but is disrupted in a lot of human cancers through p53mutations[5].

5-FU also triggers oxidative stress and apoptosis in non-tumor tissues of cancer patients[6]. The oxidative status of these patients is associated with higher vitamin C requirement. 5-FU was found to significantly lower vitamin C levels of cancer patients so much so that, in certain cases, patients may complain of scurvy-like symptoms[7].

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Vitamin C must be obtained exogenously[8], either via the diet or as a supplement since humans are unable to synthesize it[9]. From a cancer patient standpoint, it is thought to impart cyto-protection to the normal non-tumor cells by preventing cell death and inhibiting mutations induced by oxidative stress while sensitizing cancer cells to the effects of the chemotherapeutic drugs, thus representing a win-win scenario[7]. Furthermore, vitamin C is also a key mediator of epigenetic modulation through DNA demethylation as it is a cofactor for ten eleven translocation (TET) dioxygenases[9], and is a nutritional stimulator of autophagy[10]. It is also notorious for its effect on fibroblast differentiation and function. It upregulates collagen production by fibroblasts and inhibits hyaluronidase function, which aids in healing and walls-off tumor cells thus complicating the latter`s metastasis[11].

Vitamin C also plays a pivotal role in scavenging various reactive oxygen species (ROS), as well as reducing lipid peroxidation and Malondialdehyde (MDA) levels[12]. MDA is believed to be the most mutagenic lipid peroxidation byproduct. Lipid peroxidation within physiological limits, stimulates cellular preservation and viability through antioxidants signaling pathways. In contrast, 5-FU stresses the cells with a lipid peroxidation state that overcomes the oxidative damage repair capacity, leading to DNA mutations followed by cell cycle arrest, cell death or senescence[13].

Several research state that antioxidants like vitamin C, do impart health benefits and protection to the normal non-tumor tissues[14]. Nevertheless, other research argues that the concurrent use of vitamin C with 5-FU may reduce the latter's effectiveness in cancer treatment[15].

Given the existence of conflicting data about the efficacy of vitamin C as a cytoprotective agent, this study was performed to evaluate the effect of vitamin C supplementation with 5-FU on normal fibroblasts cell viability. MDA levels, cellular proliferation and cell death. Moreover, this study assessed the potential added benefit of administering vitamin C 24 hours after the fibroblast were treated with 5-FU over the treatment of fibroblasts with both simultaneously.

MATERIALS AND METHODS

The materials used in this study

Cell line

Normal Fibroblast cell line (WI-38) from American Type Culture Collection (ATCC® CCL-75™) were obtained from VACSERA-EGYPT. WI-38 is a diploid adherent human fibroblasts cell line, derived from the lung tissue of a 3-month-gestation aborted caucasian female fetus[16]. These cells have a finite lifetime and biosafety level 1, based on U.S. Public Health Service Guidelines. Cells were cultured using DMEM (In *vitro* gen/Life Technologies) supplemented with 10% FBS (Hyclone), 10 ug/ml of insulin (Sigma®), and 1% penicillin-streptomycin. All the other chemicals and reagents were from either Sigma® or In *vitro* gen®. Plate cells (cells density 1.2 – 1.8 × 10,000 cells/well) in a volume of 100µl complete growth medium per well in a 96-well plate for 24 hours before testing.

Vitamin C

Cells were treated with different doses of vitamin C (Sigma, Cat, # A4034). The MTT assay was then carried out to assess the effect of vitamin C on cell viability, and IC50 values was calculated[17].

Fluorouracil

A stock solution of 5-fluorouracil (Baird Bull Laboratories, Warwick, United Kingdom) was prepared in phosphate-buffered saline, stored at 4°C, and diluted in phosphate-buffered saline as required for each experiment. Phosphate-buffered saline acted as a vehicle control. MTT assay was then carried out to assess IC50 values[18].

Methods

Grouping

Normal Fibroblast cell line (WI-38) were divided into group I did not receive treatment (control) and group II treated with 5-FU only. Groups III received vitamin C only. Group IV received 5-FU with vitamin C simultaneously and group V received vitamin C after 24 hours of 5-FU administration (Table 1).

Table 1: Experimental groups description

Group I	Fibroblast
Group II	Fibroblast+ fluorouracil
Group III	Fibroblast +vitamin C
Group IV	Fibroblast+ fluorouracil +vitamin C
Group V	Fibroblast+ fluorouracil then vitamin C added after 24 hours of fluorouracil administration

Inverted microscope

The samples were viewed under the Inverted Phase Contrast Microscope (Olympus CKX41)®, for evaluation of confluence of the WI-38 cell line in the studied groups.

In vitro toxicology (MTT) assay

MTT(3-[4,5-dimethylthiazol-2-yl]-2.5-diphenyl tetrazolium bromide) in *vitro* toxicology assay kit (Trevigen SIGMA, Saint Louis, Missouri, USA) was used for the determination of cell viability and IC-50 in all groups, as per manufacturer's recommendations. Cells were briefly plated in a 96-well tissue culture plates,103–105 cells/well, in a final volume of 100uL of the medium and was left to overnight. 10ul per well MTT reagent was added, then the plate was incubated for 12 hours for intracellular reduction of the soluble yellow MTT to the insoluble purple formazan dye. The detergent reagent was added to each well to solubilize the formazan dye. The absorbance of each sample was then measured in a ROBONIK P2000 Spectrophotometer, wavelength:450-560 nm.

Lipid Peroxidation (MDA) Assay Kit

Lipid Peroxidation Assay Kit (Catalog # K739-100)/ BioVision Incorporated, USA, provides a convenient tool for sensitive detection of MDA. MDA in the sample was reacted with Thiobarbituric Acid (TBA) to generate the MDA-TBA adduct which was easily quantified colorimetrically (OD 532 nm). This assay detects MDA levels as low as 1 nmol/well colorimetrically. Cells were homogenized on ice in 300 µl of MDA Lysis Buffer, then centrifuged to remove insoluble material. 200 µl of the supernatant from each sample were placed into a microcentrifuge tube and 0, 2, 4, 6, 8, 10 µl of 2 mM MDA were adjusted to 200 µl with dd H_2O and used to generate five standards. 600 µl of TBA reagent were added into each vial. It was then incubated at 95°C for 60 min., and then cooled to room temperature in an ice bath for 10 min. 200 µl were Pipette into a 96-well microplate for analysis, absorbance read at 532 nm. MDA Standard Curve was plotted and MDA amount in the test sample in nmol was determined by interpolation from the standard curve.

Flowcytometry

Cell Cycle Analysis using Flow cytometry

The effect of 5-FU treatment, and combination of 5-FU and vitamin C were tested by the cell cycle analysis using the flow cytometry kit (BioVision®). Cells were incubated and trypsinized and rinsed with PBS then suspended in 75% ice cold ethanol. Sample was stained using staining solution containing 50 µg/mL PI, 100 µg/mL RNase and 0.1% Triton X-100. The test determined DNA content, from which the cell cycle phase was defined.

Apoptosis assessment

For detection the apoptotic cells, Annexin V Apoptosis Detection Kit was used (Propidium Iodide (PI) ab139418) (BioVision®). Cells were washed with cold PBS and the pellet was re-suspended in 100 µL Annexin V Binding buffer. After mixing, 5 µL of Annexin V-FITC and 5 µL of propidium iodide were added and each sample was incubated for 15 min in the dark at room temperature. The samples were afterwards centrifuged and resuspended in 500 µL of cell wash solution and analyzed, using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector.

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. A one-way analysis of variance (ANOVA) was used for comparing means among all groups. Post Hoc test: Least Significant Difference (LSD) was used for multiple comparisons between different variables.

Using: One Way Analysis of Variance; *p-value* <0.05 was considered significant. For Post HOC test, a: indicated significant difference with control WI-38; b: significant difference with WI-38+5FU, c: significant difference with WI-38+Vit C and d: indicated significant difference with WI-38+Vit C+5FU. Pearson's correlation coefficient (r) test was used to assess the degree of association between two sets of variables. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, *P-value* <0.05 was considered significant.

RESULTS

Inverted microscopic results

After examination with an inverted microscope group I showed cells are relatively small and fusiform in shape, with clear and continuous edges (Figure 1). The cells in group II are relatively fewer and larger than group I. Several cells exhibiting round morphology with nuclear fragmentation and chromatin margination are noted, consistent with apoptosis (Figure 2). The cells of group III are mostly similar to cells in group I. They are fusiform in shape with clear and continuous edges. Some of them non-adherent cells that are round in morphology (Figure 3). Group IV showed cells are relatively fewer and larger than group I yet are seemingly more than those in group II. Few cells exhibiting round morphology with chromatin margination are noted (Figure 4). While group V showed adherent spindle fusiform cells and the round non-adherent cells exhibited somewhat similar shapes and numbers to those of group IV (Figure 5).

In vitro toxicology (MTT) assay

One Way Analysis of Variants (ANOVA) of the in *vitro* toxicology (MTT) assay detected statistically significant differences between the studied groups. MTT assay evaluation demonstrated that the least viability was noted in group II followed by groups IV and V, the differences in mean cell viability between these groups was found to be non-significant and all three groups exhibited significantly lower cell viability when compared to group I. (Table 2, Figure 6)

Lipid Peroxidation (MDA) Colorimetric Assay

MDA assessment revealed highly statistically significant differences between groups. The MDA levels were found to be significantly lower in group IV and V compared to group II. The difference between groups IV and V was found to be significant (Table 3, Figure 7).

Flow Cytometry (FCM)

Cell Cycle Analysis

Cell cycle analysis (FCM) revealed the presence of highly statistically significant differences between groups in different phases of the cycle. For all groups, the highest number of cells were in the G0-G1 phase. Groups V showed significantly more cells in the S phase than all the other groups and significantly less cells in G2-M phase compared to the other groups. (Table 4, Figures 8,9).

Annexin V/PI FCM results (cell death)

The results of the FCM regarding cell death showed that the highest apoptosis was noted in group II followed by group IV. The differences between the groups were found to always be statistically significant using ANOVA (Table 5, Figures 10,11).

Correlations

Pearson Correlation Coefficient in groups I and III exhibited similar correlations between the assessed variables. It was found that for these groups, there was a correlation (r=1 and *p-value*=0.000) between the total cell death and the number of cells in G0-G1 phase, S-phase, G2-M and PRE-Restriction of the cell cycle. While for Group II, there was a correlation between the cells in the S phase and the pre-restriction phase of the cell cycle (r=1 and *p-value*=0.000).

Group IV exhibited a correlation (r=1 and *p-value*=0.000) between the cell death and the number of cells in the G0-G1, the S and PRE-Restriction phases of the cell cycle. Lastly, it was noted that there was a correlation (r=1 and *p-value*=0.000) between the cells in G0-G1 and G2-M phases of the cell cycle as well as between the MTT assay and the cells in the S phase of the cell cycle in Group V.

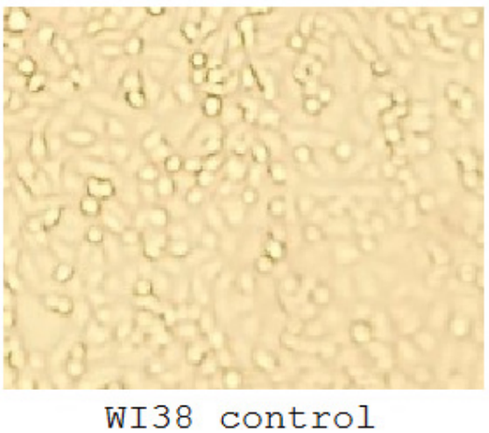

Fig. 1: photomicrograph of group I showed cells are relatively small and fusiform in shape, with clear and continuous edges (blue arrow) (orig. mag. X10).

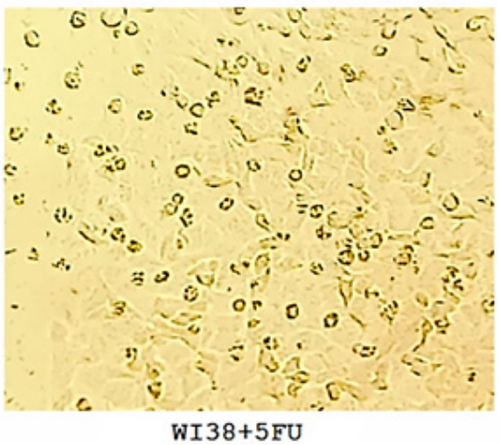

Fig. 2: photomicrograph of group II showed cells are relatively fewer (blue arrow) and others are round morphology with nuclear fragmentation and chromatin margination. (red arrow) (orig. mag. X10).

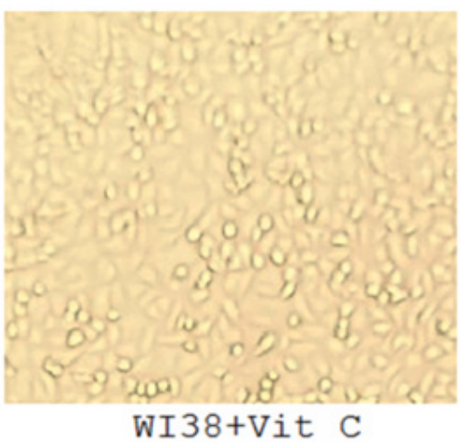

Fig. 3: photomicrograph of group III showed cells are fusiform in shape, with clear and continuous edges (blue arrow) and some non-adherent cells that are round in morphology (red arrow). (orig. mag. X10).

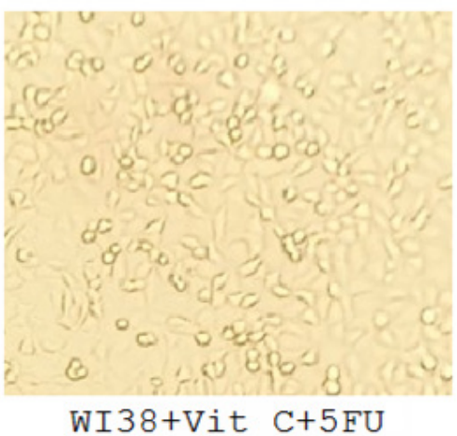

Fig. 4: photomicrograph of group IV showed cells are relatively fewer and larger (blue arrow). Few cells exhibiting round morphology with chromatin margination are noted. (red arrow) (orig. mag. X10).

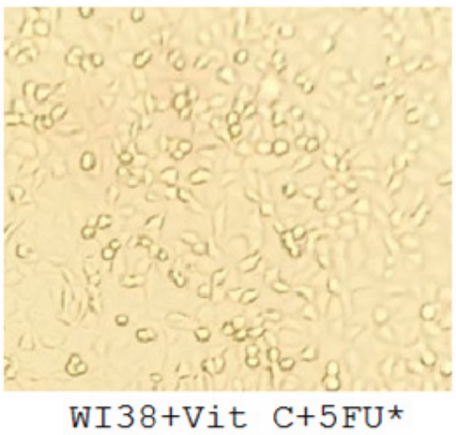

Fig. 5: photomicrograph of group V showed adherent spindle fusiform cells (blue arrow) and the round non-adherent cells (red arrow) (orig. mag. X10).

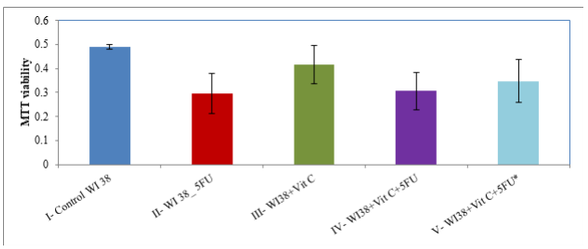

Fig. 6: Column chart showing the mean values of the In *vitro* toxicology (MTT) assay results for all groups.

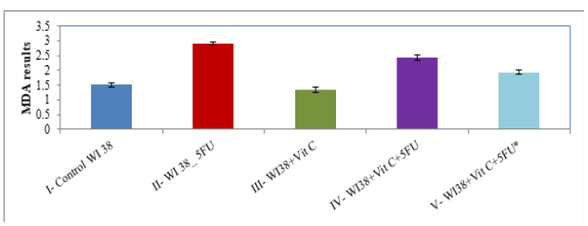

Fig. 7: Column chart showing the mean values of lipid peroxidation/ MDA colorimetric assay results for all groups.

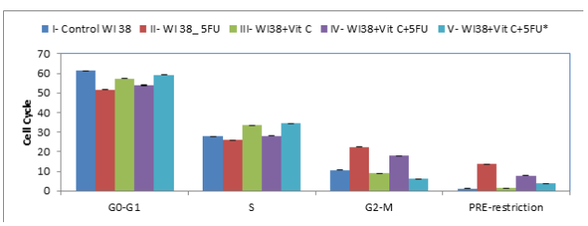

Fig. 8: Column chart showing the mean number of cells in each phase of the cell cycle assessed by FCM for all groups.

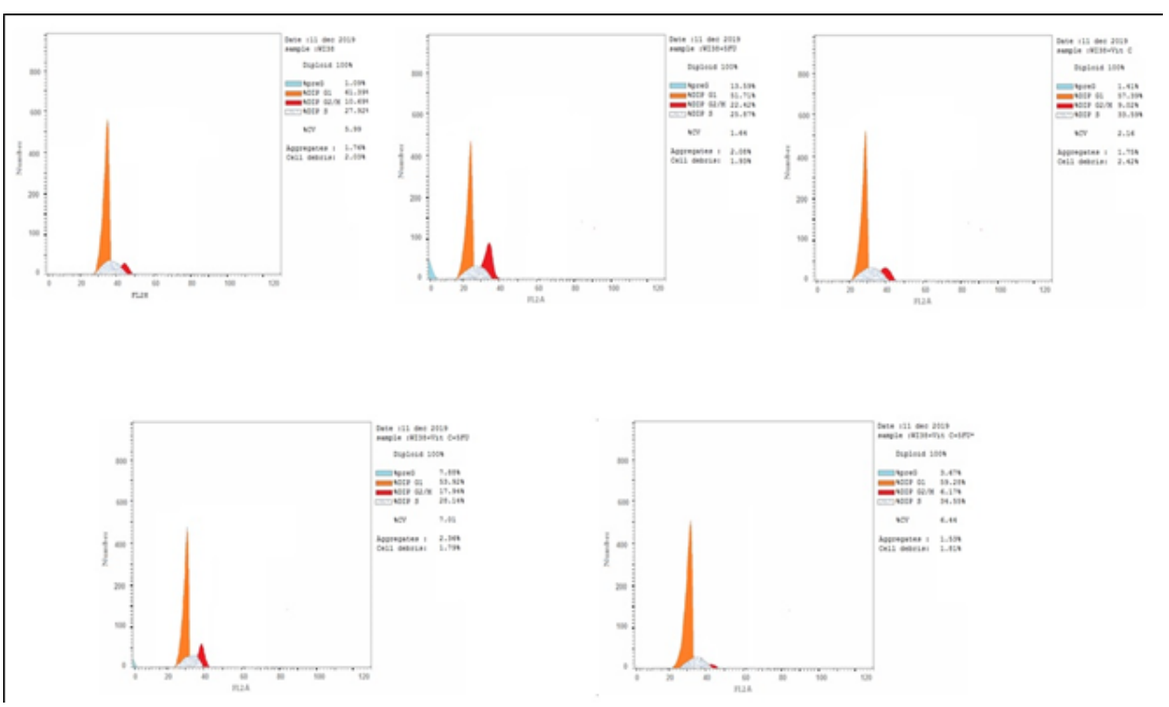

Fig. 9: Representative figures of the cell cycle analysis performed with FCM for all groups

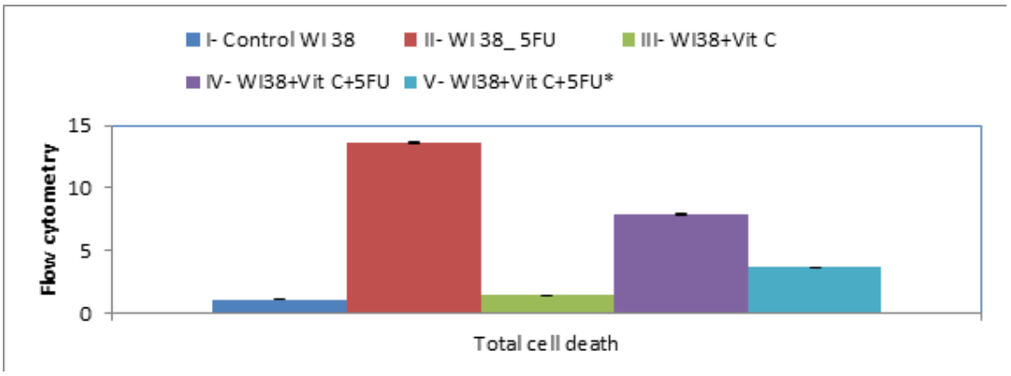

Fig. 10: Column chart showing the mean number Annexin V-positive cells by FCM for all groups

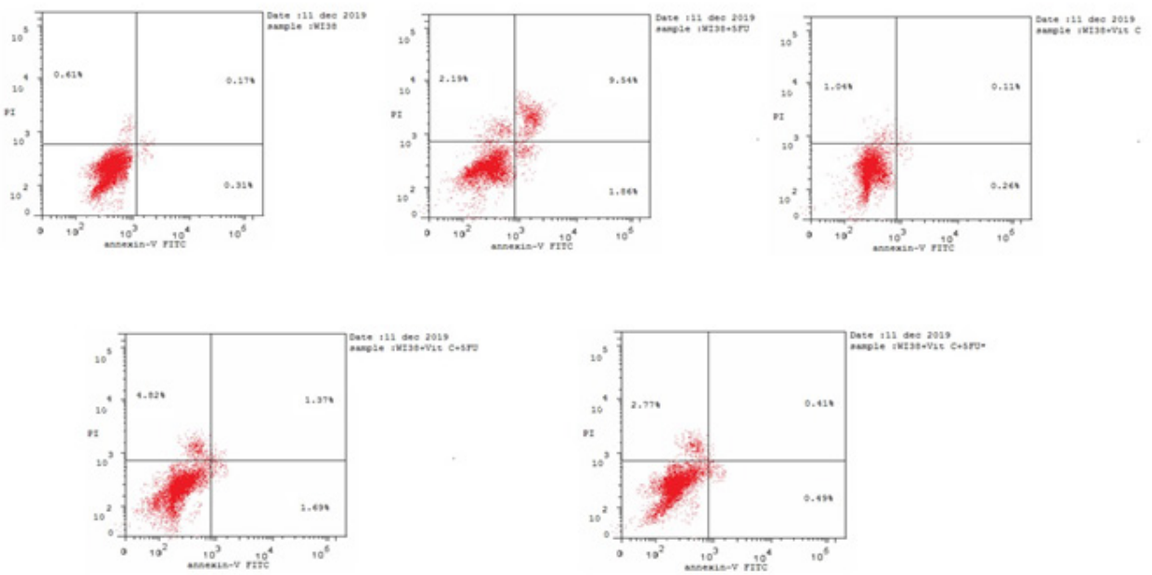

Fig. 11: Representation of Annexin V/PI staining evaluated by FCM. Numbers correspond to the percentage of Annexin V-positive cells, in each group.

Table 2: Comparison between group according to in *vitro* toxicology (MTT) assay

MTT viability	I- Control WI 38	II- WI 38_ 5FU	III- WI38+Vit C	IV- WI38+Vit C+5FU	V- WI38+Vit C+5FU*	ANOVA	*p-value*
Mean±SD	0.489±0.010	0.296±0.084	0.416±0.081	0.306±0.078	0.348±0.088		
Range	0.476-a0.502	0.195-a0.412	0.324-a0.526	0.213-a0.413	0.229-a0.452	5.906	0.003*
Post HOC test		a	b	a,c	a		

Table 3: Comparison between group regarding Lipid Peroxidation/ MDA Colorimetric Assay results

MDA results	I- Control WI 38	II- WI 38_ 5FU	III- WI38+Vit C	IV- WI38+Vit C+5FU	V- WI38+Vit C+5FU*	ANOVA	*p-value*
Mean±SD	1.512±0.062	2.908±0.051	1.345±0.094	2.433±0.098	1.937±0.065		
Range	1.445-a1.567	2.853-a2.953	1.239-a1.417	2.321-a2.507	1.881-a2.009	215.494	<0.001**
Post HOC test		a	a,b	a,b,c	a,b,c,d		

Table 4: Comparison between group according to cell cycle (DNA content%) FCM assay results

Cell Cycle (DNA content %)	I- Control WI 38	II- WI 38_ 5FU	III- WI38+Vit C	IV- WI38+Vit C+5FU	V- WI38+Vit C+5FU*	ANOVA	*p-value*
G0-G1							
Mean±SD	61.39±0.01	51.71±0.01	57.39±0.01	53.92±0.01	59.28±0.02		
Range	61.38-a61.4	51.7-a51.72	57.38-a57.4	53.91-a53.93	59.26-a59.29	330.929	<0.001**
Post HOC test		a	a,b	a,b,c	a,b,c,d		
S							
Mean±SD	27.92±0.01	25.87±0.01	33.59±0.01	28.14±0.02	34.54±0.01		
Range	27.91-a27.93	25.86-a25.88	33.58-a33.6	28.12-a28.16	34.53-a34.55	273.563	<0.001**
Post HOC test		a	a,b	a,b,c	a,b,c,d		
G2-M							
Mean±SD	10.69±0.01	22.42±0.02	9.02±0.01	17.94±0.02	6.17±0.02		
Range	10.68-a10.7	22.4-a22.43	9.01-a9.03	17.92-a17.96	6.15-a6.18	564.875	<0.001**
Post HOC test		a	a,b	a,b,c	a,b,c,d		
PRE-Restriction							
Mean±SD	1.09±0.01	13.59±0.01	1.41±0.01	7.88±0.01	3.67±0.01		
Range	1.08-a1.1	13.58-a13.6	1.4-a1.42	7.87-a7.89	3.66-a3.68	852.6	<0.001**
Post HOC test		a	a,b	a,b,c	a,b,c,d		

Table 5: Comparison between group according to Annexin V/PI FCM (cell death) results

Flow cytometry [Apoptosis FCM]	I- Control WI 38	II- WI 38_ 5FU	III- WI38+Vit C	IV- WI38+Vit C+5FU	V- WI38+Vit C+5FU*	ANOVA	*p-value*
Mean±SD	1.09±0.01	13.59±0.02	1.41±0.02	7.88±0.02	3.67±0.01		
Range	1.08-a1.1	13.58-a13.61	1.39-a1.42	7.86-a7.9	3.66-a3.68	345.25	<0.001**
Post HOC test		a	a,b	a,b,c	a,b,c,d		

DISCUSSION

5-FU is one of the most prescribed chemotherapeutics to date. Unfortunately, its use is associated with significant collateral damage to the normal non-tumor tissues in the body[19]. Thus, this study was performed in order to evaluate the potential cyto-protective effect of vitamin C on human fibroblast cell line when administered simultaneously with and after 24 hours of 5-FU.

Microscopic evaluation of the WI-38 cells in the control and the vitamin C treated groups of this study revealed that the cells were small with a regular fusiform morphology and very few signs of apoptosis. These observations were in line with the results obtained via FCM and MTT assays, where they showed the highest viability and the least cell death compared to any of the 5-FU treated groups. These groups were also found to show a correlation between the cells in the S phase of the cell cycle and apoptosis. WI-38 cell line is described to undergo doubling in 24 hours[12], and thus the presence of apoptotic cells correlating with proliferating cells in the S phase is only to be expected to maintain normal tissue homeostasis. This has been documented by Eroglu and Derry, 2016, who stated that apoptosis has non-autonomous impacts on surrounding cells like the promotion of cell proliferation in the neighboring cells. In addition, apoptosis itself may be non-autonomously controlled by neighboring cells via mechanical forces derived from cell crowding[20]. It should also be noted that the least lipid peroxidation signified by the least MDA levels was noted in the vitamin C treated groups owing to its well-known anti-oxidant effect[21].

WI-38 treatment with 5-FU alone resulted in morphological alterations that were noted microscopically in the present study. The cells were larger in size and several cells were observed to be non-adherent and to exhibit signs of apoptosis. Apoptosis is characterized by specific biochemical and morphological features in which caspase activation has a key role. Once adherent cells have initiated apoptosis, they assume a more round morphology with chromatin condensation, nuclear fragmentation and start detaching from their substrate by losing their focal adhesion sites. This is attained by caspase-mediated cleavage of the focal adhesion kinase pp125 protein, among others. This is then followed by actin reorganization into an actin-myosin II cortical ring with contractile force[22]. These findings were confirmed by the FCM and MTT assays. Cells were found to be concentrated in the G2-M and pre-restriction phases of the cell cycle to show significantly higher apoptosis than the rest of the groups. Place *et al*, 2005, stated that WI-38 cells that are arrested are larger than proliferating cells and described it as a sign of cellular senescence, which may be caused by oxidative stress[23]. This might very well be the case in the current study where there is elevated MDA levels concurrent with the presence of cells in the pre-restriction phase of the cell cycle.

Moreover, WI-38 treated with 5-FU (alone) displayed increased concentration of cells in the G2-M phase of the cell cycle, which may be due to DNA mutation caused by 5-FU mediated TS inhibition during DNA synthesis in the S phase of the cell cycle. This 5-FU induced DNA mutation subsequently results in the upregulation of p21 (a potent cell cycle inhibitor), and P 53 (the guardian of the genome), resulting in the activation of the G2-M check point and cell cycle arrest at this phase, as observed from the FCM results of this study. Moreover, p53 initiates transcription of p53-upregulated modulator of apoptosis (PUMA), a member of the Bcl2 family which activates Bax and thus promotes apoptosis[24].

Owing to the damage to the cells described above on 5-FU treatment, this study proposed the use of vitamin C along with 5-FU. This was based on the assumption that vitamin C will act as an antioxidant alleviating oxidative stress, augment autophagy thus imparting cyto-protection to normal cells[25]. And enhance epigenetic modification to restore health through DNA demethylation. Vitamin C has the added privilege of being cheap and easily available and has been repeatedly proved to act as an anticarcinogenic agent on its own and potentiate the effect of anti-cancer drugs.

Its anti-cancer effect is thought to be a result of it actually producing free radicals in cancer cells as the enzymes required for the proper metabolism of vitamin C to its reduced form are deficient in cancer cells. This results in elevated ROS levels and subsequent vitamin C cell cycle arrest and apoptosis[25].

Microscopic evaluation of the both groups treated with 5-FU and vitamin C showed fewer signs of apoptosis. The FCM and the MMT and MDA levels were also consistent with the microscopic findings. However, it was found that the administration of vitamin C 24 after 5-FU treatment conveyed more promising results with higher cell viability and cellular proliferation and lowered cell death and MDA levels. Several theories may be presented to explain these findings. Vitamin C is unstable in an alkaline medium[26], at the same time 5-FU increases the alkalinity of the surrounding tissue, hence taking them together may not allow vitamin C to work properly. Moreover, antioxidant enzymes such as catalase and glutathione peroxidase, protect normal cells from ROS. In contrast, ROS causes cell cycle arrest and cell death, in stressed cells like those treated with 5-FU, because of the exhaustion of antioxidant enzymes compared to normal cells[27]. ROS result in mitochondrial caspase-dependent and caspase-independent apoptosis, autophagy, autolysis, ATP depletion, DNA damage, and cell cycle arrest. Now even though vitamin C is an antioxidant, it can switch roles from being an antioxidant in physiologic conditions to a pro-oxidant under in stressed cells[28]. This may represent another theory to explain why administration of vitamin C after 24 hours of 5-FU treatment allowed for increased proliferation and lowered apoptosis compared to its simultaneous administration with 5-FU.

WI-38 treated with 5-FU 24 hours prior to vitamin C treatment in this study resulted in the concentration of the majority of the cells in the G0-G1 phase of the cell cycle, which is believed to signify the ability of the cells to grow (as G0-G1, is characterized by cellular growth) and hence to repair against the clastogenic effects induced by 5-FU. These results were consistent with those of Duarte *et al*, 2009, who showed that cells treated with vitamin C exhibited upregulation of gene expressions related to DNA replication and repair and that vitamin C enhanced mitogenic stimulation of quiescent fibroblasts[29].

CONCLUSIONS

Co-treatment of normal fibroblasts cell line with vitamin C along with 5-FU reduces lipid peroxidation and cell death and while slightly enhancing viability in treated fibroblasts, indicating healing and repair.

Administration of vitamin C 24 hours after 5-FU to normal fibroblasts cell line possibly further enhances viability and proliferation while reducing lipid peroxidation and cell death of 5-FU treated fibroblasts, than when vitamin C and 5-FU are administered simultaneously.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير تناول فيتامين ج بعد ٢٤ ساعة من تناول عقار ٥-فلورويوراسيل مقابل اعطائه مباشره على الانتشار الخلوي وموت الخلايا وبيروكسيد الدهون في خط الخلايا الليفية البشرية WI-38

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مقدمه: أصبح ٥-فلورويوراسيل (٥-FU) عاملًا روتينيًا مضادًا للعديد من أنواع السرطان، ويتطلب عمره القصير إعطاءه باستمرار بجرعات عالية. ينتج عن هذا آثار جانبية سامة على الأنسجة الطبيعية في الجسم. فيتامين ج هو أحد مضادات الأكسدة التي يعتقد أنها تمنح حماية الخلايا للأنسجة الطبيعية في مرضى السرطان الذين يعالجون بأدوية العلاج الكيميائي.

الهدف من الدراسة: تقييم الفائدة المحتملة لتأخير إعطاء فيتامين سي إلى ٢٤ ساعة بعد العلاج بـ ٥-FU مقارنة بمكملاته الفورية على مستويات الخلايا الليفية العادية MDA ، وحيوية الخلية ، وتوقف دورة الخلية وموت الخلايا.

المواد والطرق: تم تقسيم خط الخلايا الليفية (WI-٣٨) إلى ٥ مجموعات. المجموعة الأولى لم تعالج (المجموعة الضابطة)، المجموعة الثانية عولجت بـ ٥-FU فقط والمجموعة الثالثة تلقت فيتامين ج فقط. تلقت المجموعتان الرابعة والخامسة ٥-FU مع فيتامين ج في وقت واحد وبعد ٢٤ ساعة من إعطاء ٥- FU ، على التوالي. تم تحديد صلاحيه الخلايا في جميع المجموعات باستخدام اختبار MTT. تم استخدام اختبار MDA الكمي لتحديد بيروكسيد الدهون. تم استخدام قياس التدفق الخلوي لتقييم دورة الخلية وموت الخلية. تم تحليل جميع البيانات المسجلة باستخدام التحليل الإحصائي.

النتائج: تبدو الخلايا في المجموعة الرابعة مثل المجموعة الثانية قليله العدد تقريبا بينما في المجموعة الخامسة بعضها مغزلي ملتصق والآخر مستدير غير ملتصق. كانت مستويات MDA أقل بشكل ملحوظ في المجموعتين الثالثة والرابعة مقارنة بالمجموعة الثانية. كانت أقل قابلية للبقاء في المجموعة الثانية والثالثة والرابعة. كشف تحليل دورة الخلية عن وجود فروق ذات دلالة إحصائية بين المجموعات في مراحل مختلفة من الدورة. أظهر تحليل التدفق الخلوي أعلى موت للخلايا المبرمج في المجموعتين الثانية والثالثة.

الخلاصة: العلاج المشترك للأورمات الليفية الطبيعية بفيتامين C مع ٥-FU يقلل من بيروكسيد الدهون وموت الخلايا وعدد الخلايا في مرحلة ما قبل التقييد من دورة الخلية بينما يعزز بشكل طفيف قابلية البقاء في الخلايا الليفية المعالجة، مما يشير إلى الشفاء والإصلاح. التطبيق المتأخر لفيتامين C على الخلايا الليفية المعالجة بـ ٥-FUيحسن من الإجهاد التأكسدي ويقلل من موت الخلايا، مقارنةً بأعطاء بفيتامين C و ٥-FU في وقت واحد.